STUDY PROTOCOL

PROTOCOL TITLE: Placebo-Controlled Trial of Creatine Augmentation for Adolescent Females with Treatment-Resistant Major Depressive Disorder: a Magnetic Resonance Spectroscopy Study

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BACKGROUND AND INTRODUCTION:

Pediatric Depression: Epidemiology and Limitations of Current Drug Treatments

Pediatric major depressive disorder (MDD) is an important public health problem. With an annual incidence of about 2% in children and 4% to 8% in adolescents [1], and a cumulative lifetime prevalence of up to 20% [2], MDD in young people is associated with academic and social impairment, substance abuse, and suicide attempts [1]. Compared with healthy controls, depressed adults are more likely to have had a depressive episode in adolescence [3]. Kandel and colleagues [4] conducted a long-term study of 1,004 public high school students in the state of New York, and their study findings noted that depressive feelings in adolescence were likely to persist into adulthood, and to predict psychiatric hospitalization for women (but not men). In addition, adolescent depression was associated with a number of poor outcomes in adulthood: cigarette smoking, increased use of prescription tranquilizers in females, socially deviant activities and accidental injury [4]. Adding to the morbidity and mortality experienced by adolescent patients and their families, pediatric depression imposes a substantial economic burden on society [5]. Therefore, the discovery of novel effective treatments for patients with depression in the critical adolescent stage of development has immense public health implications [6-9].

In the United States (U.S.), selective serotonin reuptake inhibitors (SSRIs) have been the predominant method for treatment of adolescent depression for more than a decade [10]. Drug treatment of depression in youth has become standard practice, in part because of the failure of most evidence-based psychotherapy treatments to make their way into everyday practice [11]. Despite the increased attention to pediatric MDD sparked by the U.S. Food and Drug Administration's (FDA's) "Black Box" warning applied to antidepressants prescribed to children and adolescents [12], the pharmacological treatment options remain limited. In a systematic review of 13 randomized placebo-controlled trials for pediatric depression involving 2,750 participants, Usala and colleagues found a pooled response rate of 59.5% (95% C.I. 57.2–62.5) and a placebo response rate of 47.9% (95% CI, 45.5–51.1) [13]. Combined risk differences in rates of antidepressant response status favored antidepressants over placebo by just 11.0 % (95% C.I. 7.1–14.9). Similarly, a recent study that specifically examined the placebo response in pediatric MDD trials found that the mean proportion of participants per study responding to placebo was 0.46 (S.D.=0.08; Range=0.33–0.57) [14].

The number needed to treat [15] (NNT) is an evidence-based medicine statistic that represents the relative benefit of treatment compared with the control condition. The NNT is the number of patients a clinician must treat to prevent one adverse outcome (e.g. unremitted depression in one patient). Tsapakis and colleagues conducted a meta-analysis of 30 randomized, placebo-controlled trials for pediatric depression. The NNT for antidepressants in pediatric MDD is 9.35 (95% C.I. 7.1-13.7) [16]. As a measure of treatment effect, the practical implication is that a practicing clinician must treat nine patients with antidepressants in order for one pediatric patient with MDD to recover who would otherwise not have. Meanwhile, the clinician must expose all nine patients to an approximate doubling of the risk for suicidal ideation or behavior [17]. With greater than 40% of pediatric MDD patients classified as antidepressant non-responders and a narrow therapeutic margin between antidepressants and placebo, new treatment options are urgently needed.

The selective serotonin reuptake inhibitor (SSRI) antidepressants, including fluoxetine (Prozac®) have become the treatment of first choice in pediatric MDD, owing to the results of the NIH-sponsored Treatment for Adolescents with Depression Study **(**TADS) clinical trial [18]. The U.S. FDA and the U.K. Medicine and Health Care Products Regulatory Agency have concluded that an acceptable risk/benefit relationship exists for fluoxetine in pediatric MDD [19]. The European Medicines Agency initially concluded that fluoxetine should not be prescribed to minors, but later adopted a positive opinion for the use of Prozac® in children 8 years and older

with depression [20]. Fluoxetine is a recommended treatment for pediatric MDD in the Texas Children's Medication Algorithm Project [21]. In March 2009, the FDA approved the use of the SSRI escitalopram (Lexapro®) for acute and maintenance treatment of adolescent MDD. The approval was supported by two placebo-controlled studies. Emslie et al. [22] conducted a large randomized control trial evaluating the efficacy and tolerability of escitalopram in treating adolescent MDD. Significant improvements in Children's Depression Rating Scale-Revised (CDRS-R) scores from baseline to the end of treatment were found in the escitalopram group. These findings were consistent with a similar trial conducted by Wagner, et al. [23]. In addition to fluoxetine and escitalopram, the SSRI medications commonly used in the U.S. to treat adolescent depression include citalopram (Celexa®), sertraline (Zoloft®) and paroxetine (Paxil®). The NIH-sponsored $17 million TORDIA (Treatment Of Resistant Depression In Adolescents) multi-center clinical trial [125] established the accepted definition, in terms of medication dosages and durations, of treatment-resistant adolescent MDD.

Females Have an Increased Rate of Depression in Adolescence

Early-onset psychiatric disorders (e.g., conduct problems, autism, ADHD) show a marked male preponderance, whereas adolescent-onset disorders (e.g., depression, anxiety) show a marked female preponderance [24]. The prevalence of depression increases several fold following puberty, from 1% in childhood to 8% in adolescence [25]. The cumulative lifetime prevalence for depressive disorders in adolescence is estimated at 15-20%, which is comparable to the rate found in the adult population [25]. A number of epidemiologic studies have shown that the illness burden is borne primarily by **female adolescents** [26-29].

Evidence supports puberty as the transitional period in which the risk for depression rises in female adolescents. The age at menarche was found to be the transition point in one study [30]. Similarly, the elevated rate of depressive symptoms and major depressive disorder in females emerged once participants had reached Tanner Stage III [31] in the Great Smoky Mountain Study (GSMS) [32]. Further analyses of the GSMS participants' hormonal status was performed, revealing that changes in gonadal steroids were closely correlated with the rise in depressive symptoms [33]. Statistical models incorporating testosterone and 17β-estradiol levels eliminated the apparent effect of Tanner stage (physical development), leading the investigators to conclude that research on increased depression in females needs to focus on factors associated with changes in androgen and estrogen levels [33]. Available data suggest that estrogen, or its absence, is strongly implicated in the regulation of mood and behavior, as well as in the neurobiology of mood disorders [34]. The multiple effects of estrogens and their complex interactions with the brain and endocrine system have been well-documented, although the specific role of estrogen in depression has yet to be elucidated [34].

By the middle teenage years, females experience more than double the rate of depressive disorders found in males [26, 29]. This approximate 2:1 gender difference in depression continues throughout the reproductive years [35]. In addition to the increased incidence, initial episodes of depression are longer in duration and more severe in symptomatology in girls compared to boys [36]. Girls with MDD have a prolonged period of risk for recurrent depression, compared with women who have their first depressive episode as an adult [37, 38]. Women are more likely than men to have atypical symptoms of depression (e.g. hypersomnia, hyperphagia), to have co-morbid anxiety disorders, and to **attempt suicide**; Seasonal Affective Disorder is also more common in women [39]. In a global sense, 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 [40].

Metabolic Changes Associated with Depression: Findings from Magnetic Resonance Spectroscopy Brain Imaging Studies

To shed light on the neurobiology of depressive disorders requires the establishment of measurable correlates of illness and recovery. Converging lines of evidence suggest that mood disorders are associated with changes in brain energy metabolism [41-44], which normalize with resolution of a mood episode [45, 46]. These changes in brain energy metabolism can be measured with a non-invasive neuroimaging technique which uses no radiation: phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS) [47]. At magnetic field strengths of 1.5 Tesla or higher, proton magnetic resonance spectroscopy (^{1}H-MRS) allows the ascertainment of values of myo-inositol, choline-containing compounds, creatine, glutamate, glutamine, and N-acetyl aspartate [48]. At similar field strengths, cerebral ^{31}P-MRS can measure levels of phosphomonoesters, inorganic phosphate, phosphodiesters, phosphocreatine (PCr), and the gamma, alpha and beta nucleotide triphosphate (mainly adenosine triphosphate (ATP)) resonances [48]. PCr represents a high-energy reservoir linked to ATP in a bidirectional pH-dependent chemical reaction in which ATP is formed by PCr and vice versa with a 1:1 (PCr:ATP) molar ratio [49]. The equilibrium for this reaction favors ATP formation so that energy demands in excess of the cellular capacity for ATP synthesis are met initially through a shift in this equilibrium, which maintains ATP concentration constant through PCr hydrolysis [50]. Previous ^{31}P-MRS studies support a model of mitochondrial dysfunction in MDD involving impaired oxidative phosphorylation and a shift toward glycolysis, which results in decreased regional brain pH [51].

In 1992, Kato et al. were the first to report ^{31}P-MRS findings in MDD [52]. The investigators measured brain phosphorus metabolism in 22 patients with depressive disorders. PCr (a high-energy phosphate storage compound) was significantly decreased in severely depressed patients compared to patients with mild depression. Moore and colleagues [53] noted that beta nucleoside triphosphate (β-NTP) resonance, which arises primarily from ATP, was 16% lower in adults with major depressive disorder (MDD) than in healthy controls. Similarly, in a study evaluating abnormalities in cerebral purine metabolism in MDD, researchers found that baseline β-NTP was lower, by 21%, in adult females with MDD who responded to SSRI treatment relative to non-SSRI responders [47]. Forester and colleagues [54] detected reduced β-NTP and total NTP in depressed geriatric subjects relative to a comparison group. Finally, Kondo et al. [55] found lower PCr/β-NTP levels in depressed female adolescents compared to healthy controls. These findings suggest that high-energy phosphate metabolism, intracellular pH and membrane phospholipid metabolism may be altered in depression [52].

^{31}P-MRS findings in adults with MDD have consistently shown a characteristic pattern of metabolic change: reduced β-NTP and increased PCr [46, 53, 56]. This pattern, which is **more common in females** than males [47], suggests that some depressed patients have increased brain energy stores they cannot access to support cerebral activity. This specific pattern of altered energy metabolism is associated with an increased likelihood of treatment response to both SSRI antidepressants and thyroid hormone [46]. In healthy adults, administration of the dietary supplement creatine can induce the equivalent changes in resting brain chemistry [57], suggesting the possibility of using creatine supplementation to modify brain high-energy phosphate metabolism in patients with depression.

Creatine Supplementation

A French scientist, Michel Eugene Chevreul, first described creatine as a nitrogenous organic acid that occurs naturally in vertebrate animals in the 1830s. The capacity for creatine biosynthesis via the creatine-kinase-phosphocreatine energy buffering system was established early in the history of life on Earth [58]. Acting as a substrate for hydrogen ions, creatine facilitates the production of adenosine triphosphate (ATP) from adenosine diphosphate (ADP), thus increasing the amount of free energy available within cells [59].

Creatine has a role in high-energy phosphoryl group transfer during skeletal muscle contraction via the creatine kinase reaction. The enzyme creatine kinase catalyzes the reversible reaction of creatine and ATP, forming PCr and ADP [60]. The intracellular creatine/PCr ratio plays an important role in maintaining an adequate supply of energy, in the form of cellular ATP [61]. PCr may be viewed as a reservoir of "high-energy phosphate," which is able to supply to 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 [60]. In humans, **brain** and **muscle** are two such tissue types.

Creatine plays a role in transferring energy from the mitochondria to the cytosol in tissues with high energy requirement such as brain and skeletal muscle [62]. Creatine was also recently identified as a potent natural survival- and neuroprotective-factor for developing nigral dopaminergic neurons [63]. Creatine supplementation improves the function of creatine kinase/PCr system by increasing cellular creatine and PCr levels, improving the rate of ATP re-synthesis, and maintaining cellular-energy homeostasis [64].

Clinical trials in humans provide evidence for creatine's effect on brain function. A study published by Rae et al. [65] showed that increasing oral creatine intake resulted in a significant positive effect on both working memory and intelligence. These results were in agreement with the observations that brain creatine levels correlate positively with recognition memory [66], and that creatine supplementation reduces mental fatigue on a serial calculation task [67]. The latter study also showed reduced activation-stimulated oxyhemoglobin delivery to the activated area following creatine supplementation [67]. This suggests that creatine supplementation is acting to smooth fluctuations in the blood oxygen level-dependent response curve, which results from brain activation [68, 69], possibly by altering rates of ATP synthesis in the mitochondrion through the mitochondrial creatine kinase-adenine nucleotide translocase-porin complex [61, 70]. Lyoo and colleagues [57] administered oral creatine to a sample of healthy volunteers, and the results of their study indicated that PCr concentrations increased over time in the creatine group relative to the placebo group.

Creatine is currently being investigated as a treatment for neuromuscular and neurodegenerative diseases. Walter et al. [71] administered creatine to treat Muscular Dystrophy in a double-blind placebo-controlled trial. The study enrolled 32 participants and 5 grams/day of creatine was administered [71]. The researchers noted a significant improvement in muscle strength and daily activities in the creatine group and there were no reported side effects from creatine use [71].

Creatine supplementation has also been used for the treatment of Huntington's Disease (HD). Study findings suggest that PCr and inorganic phosphate are reduced in the cortex and basal ganglia of HD patients [72]. Creatine supplementation in animal models of HD has been shown to reduce neuronal atrophy, extend survival, attenuate the loss in brain weight, lower the number of Huntington-positive aggregates, and improve motor performance [73-75]. Moreover, creatine supplementation has been shown to be neuroprotective, and to reduce lesion volume in mitochondrial toxin models of HD [76]. Hersch and colleagues [77] conducted a double-blind placebo-controlled trial testing the safety and tolerability of creatine administration in HD patients. In addition, they evaluated brain and serum biomarkers of creatine availability and activity [77]. Participants took 8 grams of creatine daily for 16 weeks, which was well tolerated with no treatment-emergent safety issues [77]. The researchers noted increased brain and serum creatine concentrations in the creatine-treated group, which returned to baseline after a washout period [77].

There is strong evidence that altered energy metabolism due to mitochondrial dysfunction plays a role in the pathogenesis of Parkinson's Disease (PD) [78]. Due to this bioenergetic impairment, creatine has been explored as a PD treatment option. Matthews et al. [76] noted that oral creatine supplementation resulted in protection against an electron transport chain of mitochondria called 3-nitropropionic acid, 1-methyl-4-phenyl-2,3,6-tetrahydropyridine (MPTP)-

induced dopamine depletion in mice. In a randomized double-blind clinical trial of creatine in early PD patients, creatine supplementation delayed increases in the Unifed Parkinson's Disease Scale by as much as 50% [79]. Creatine is currently under investigation in a Phase III trial examining 1,720 patients with PD (http://clinicaltrials.gov/ct2/show/NCT00449865).

Creatine has Potential Antidepressant Effects in Females

Data from preclinical animal studies suggest that creatine has sex-dependent antidepressant properties. The Porsolt Forced Swim Test (FST) is the most widely employed experimental animal model of depression. Fed to rats, diets enriched with 4% creatine for six weeks confer a longer latency to immobility in female rats compared with rats fed 0% creatine. In male rats, however, creatine has the opposite effect [80]. Moreover, supplementation with 4% creatine in female rats was of greater benefit for reducing depression-like behavior than 10mg/kg of fluoxetine (Allen, 2011, unpublished findings). The gender-specific nature of these findings may be due to the fact that estrogen receptors are quite common on mitochondria [47]. Estrogen has potent effects on mitochondria, particularly in times of mitochondrial stress [81]. Estrogen has cell-specific effects on a variety of physiologic endpoints, including regulation of mitochondrial biogenesis and activity [82].

In terms of data from human subjects trials, here at the **University of Utah**, we have completed two studies of creatine augmentation in female adolescents with SSRI-resistant major depression. The first study was an eight-week open label study, which was conducted under U.S. Food and Drug Administration (FDA) Investigational New Drug Application (IND) #104,586. The second study was a placebo-controlled dose-ranging trial conducted under the same IND, which sought to determine which of four daily doses of adjunctive creatine (0g/placebo, 2g, 4g, or 10g), when added to participants' current antidepressant treatment, offers the best combination of efficacy, tolerability and safety. The National Institute of Mental health (NIMH) funded the dose-ranging trial as an R21 study, and contingently awarded funds for a 3-year R33 study based on the R21's success.

In the open-label study, participants' Children's Depression Rating Scale (CDRS-R) scores were reduced by 44% [55]. Moreover, utilizing pre- and post-treatment brain scans we found that brain PCr concentrations increased with eight weeks of creatine supplementation when compared to a comparison group of non-depressed adolescent controls [55]. In the dose-ranging study, participants were randomly assigned to treatment from one of the four study drug groups for 8 weeks. Participants randomized to all creatine treatment groups showed greater decreases in CDRS-R scores, with reductions between 28-42%, compared to the CDRS-R scores of participants randomized to placebo, who showed a decrease of 24% [unpublished data]. Participants treated with 10g daily creatine showed the highest increases in frontal lobe PCr, and were the only participants showing post-treatment increases in frontal lobe beta-NTP. In both studies, creatine was well-tolerated, and participants experienced no suicide attempts or psychiatric hospitalizations over the 8-week trial. We observed no persistent or clinically significant abnormalities on serum or urine laboratory tests.

The neuroimaging and clinical results from the R21 study suggest that daily adjunctive treatment of 10g creatine is associated with the greatest change in PCr, and higher PCr is associated with lower depression scores. Therefore, the dose of creatine our data suggests is most likely to engage the neurochemical target is the 10g dose. At the same time, our data do not suggest that the 10g dose poses significantly greater risks to subjects compared with placebo, other than weight gain attributable to intracellular water.

The current protocol seeks to expand upon our previous work by opening recruitment on a pilot study of creatine 10g daily vs. placebo as a treatment for female adolescents with SSRI-resistant MDD. The purpose of the pilot study is twofold: A) to evaluate several aspects of the feasibility of creatine supplementation as a treatment for this population; and B) to estimate the

effect size of adjunctive creatine, to inform the design and implementation of a potential future efficacy trial.

In addition, a large-scale trial of SSRI+Creatine vs. SSRI+Placebo for adult females with MDD was recently completed. (See: http://clinicaltrials.gov/ct2/show/NCT00729755) The daily dose of creatine was 5 gm. The data suggest that adjunctive creatine was safe and well-tolerated, and that the creatine experienced an earlier response and an increased likelihood of achieving remission.

Safety Profile and Toxicity of Creatine in Adults

The Cochrane Database of Systematic Reviews is the gold standard for evidence-based medicine meta-analyses. In 2007, Kley and colleagues published a review on behalf of the Cochrane Collaboration that pooled results of 12 randomized controlled trials (RCTs) of creatine which enrolled 266 patients with muscle disorders [83]. The authors concluded that there were no significant side effects associated with creatine at recommended doses. High-dose creatine (150mg/kg/day, or 6.8 grams per day for a participant weighing 100 lbs., 13.6 grams per day for a participant weighing 200 lbs.) increased patients' muscle pain when it was used to treat Glycogen Storage Disease Type V. The same authors published a summary of their findings in the *Journal of Neurology, Neurosurgery and Psychiatry* [84], stating that: "No trial reported any clinically relevant adverse event."

Prospective and retrospective studies in humans have found no evidence for long-term or short-term significant side effects from creatine supplementation taken at recommended doses [85-88]. Most controlled studies of creatine report an absence of side effects or report no difference in the incidence of side effects between creatine and placebo [89]. Mihic and colleagues have demonstrated that creatine loading increases fat-free mass, but does not affect blood pressure, plasma creatinine, or creatine kinase activity in adult men and women [88].

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 [89]. Studies conducted in athletes and military personnel indicate a substantial level safety of both short- and long-term creatine supplementation in healthy adults [86, 90-94]. 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 [95, 96]. Literature reviews and expert consensus panels have concluded there is no evidence supporting an association between creatine and renal disease [86, 97-100].

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 [101]. 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 [85], and that chronic supplementation of up to five years' duration did not impair renal function in healthy athletes [86]. In a letter published in *The Lancet*, Poortmans reported that his laboratory's results show that oral creatine supplementation has no adverse effects on the renal responses of healthy individuals [102].

Gualano et al. conducted a randomized, double-blind, placebo-controlled trial of the renal effects of high-dose (10 grams/day) creatine supplementation for 3 months [103]. The outcome measures included serum creatinine, serum and urinary sodium and potassium at baseline and at the end of the study. The authors concluded that high-dose creatine supplementation daily for 90 days does not provoke renal dysfunction [103].

In 2010, to address concerns about creatine and renal toxicity, the prestigious British Medical Journal published a *"Lesson of the Week"* demonstrating how -- and why -- **non-nephrologists misdiagnose kidney disease in patients taking creatine [104]**. Previously, it was also suggested that oral creatine had the potential to give rise to carcinogen formation

[105], due to the low pH and presence of nitrite in the human digestive tract – conditions which could theoretically result in formation of *N*-nitrososarcosine (NSAR) [106]. To address this concern, Derave and colleagues conducted a placebo-controlled trial of daily creatine at both high (20 grams/day) and low (5 grams/day) doses [107]. The average age of participants was 18.8 years, and the study lasted 20 weeks. Systemic NSAR was measured acutely and after 20 weeks, and the investigators found no difference in NSAR in the creatine group compared with the placebo group [107]. The study found that that creatine supplementation does not result in the formation of NSAR in the human gastrointestinal tract, as had been previously hypothesized (but not verified via the scientific method). The authors concluded that there is no evidence that creatine is carcinogenic through formation of NSAR [107].

Another hypothesis regarding creatine's potential for adverse effects was put forth by Yu, who posited that because creatine can be metabolized to methylamine, the downstream effect of creatine supplementation might be increased formaldehyde [108], which is a known human carcinogen. Other health factors believed to increase levels of formaldehyde include exposure to nicotine [109], and elevated levels of stress-related adrenaline [110].

To examine the creatine-formaldehyde hypothesis, Candow and colleagues administered creatine at a dose of 8 grams/day together with a protein supplement to elderly men aged between 59 and 77 years [111]. Urine specimens were collected before-and-after creatine supplementation, and formaldehyde levels were measured. The authors concluded that creatine supplementation in elderly men reduces muscle breakdown and bone resorption, but does not result in an increase in systemic formaldehyde [111].

Schilling and colleagues 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 [112]. 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 [112].

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 Disease who had received either placebo or 4 grams/day of creatine for two years. They found no differences between the creatine and placebo groups in laboratory markers of renal dysfunction [113]. Interestingly, the participants who received creatine performed better on the depression subscale of the Unified Parkinson Disease Rating Scale [114].

In a placebo-controlled trial of 104 patients with Amyotrophic Lateral Sclerosis lasting 6 months at a dose of 5 grams/day, the authors reported that no serious or non-serious adverse events could be attributed to study drug [115]. They further reported that creatine-treated subjects' serum creatinine and urea levels remained within normal limits throughout the study [115].

Shewmon and colleagues have completed a study of creatine supplementation for prophylaxis of muscle aches associated with lipid-lowering HMG-CoA reductase inhibitor drugs (a.k.a. "statins") [http://clinicaltrials.gov/ct2/show/NCT00797407]. The investigators monitored kidney function tests, liver function tests and creatine kinase. Analyzing their safety data with a generalized linear model, the investigators found no significant effect of creatine administration in terms of adverse events [116]. In 2004, Brudnak authored a literature review of the risk-benefit ratio of creatine supplementation, noting that blinded studies do not support a difference in side effects between creatine and placebo [117].

Safety Profile and Toxicity of Creatine in Children & Adolescents

A recent review in *Adolescent Medicine Clinics* included a discussion of creatine dosing [118]. Recommended dosing regimens have two main formats. The first features "loading doses" of 5 grams each four times daily (20 grams total per day) for 5 to 10 days, followed by

doses of 2 to 5 grams/day to maintain intramuscular creatine stores; other studies have shown that creatine stores can be maintained with between 2 and 5 grams/day, which eliminates the need for a loading phase [118].

Smith and Dahm were among the first to report systematic research on the use of creatine among adolescents [119]. Creatine users reported mild gastrointestinal side effects, including diarrhea, cramps, and decreased appetite. In a study of twenty adolescent soccer players (average age 16.6 years; STD 1.9 years), Ostojic reported no adverse events at a dose of **30 grams per day** [120].

Cancela et al. conducted a placebo-controlled trial of creatine that was designed to prospectively identify changes in markers of health caused by creatine supplementation. The average age of participants was 19.6 $\pm$ 3.5 years [121]. Creatine was administered at a dose of 15 grams/day for 7 days, followed by 3 grams/day for 49 days. Blood and urine were collected from all participants prior to ingestion of study supplements, then again after supplementation with creatine (or placebo) for 8 weeks. The investigators were unable to find adverse effects on participants' serum and urinary clinical health markers of liver, renal, muscular and metabolic function [121]. The authors reported that no gastrointestinal distress, musculoskeletal problems or other clinical problems were reported by participants in the creatine-treated group [121].

Bourgeois and colleagues treated eleven children between the ages of 3 and 17 (mean 7.6 years) with Acute Lymphoblastic Leukemia with creatine for 32 weeks, in an attempt to attenuate body fat accumulation caused by steroid administration [122]. The authors reported no compliance issues arose due to side effects, that no participants or parents reported adverse events, and no changes in laboratory measures including serum creatinine [122].

In a study of creatine for Mitochondrial Encephalopathy, Komura et al. treated five patients between the age of 7 and 19 years with a dose of 0.35 gram/kg of body weight/day [123]. The investigators reported normal laboratory tests, including complete blood count and liver and kidney function in all patients [123]. Parents and patients were queried at each visit for side effects, and none were reported [123].

Sakellaris has studied creatine supplementation to prevent complications of Traumatic Brain Injury (TBI) in children [124, 125]. The study randomized 39 patients between 1 and 18 years of age with TBI to treatment with 0.4 gm/kg of body weight per day, or to treatment as usual for six months. The authors found no evidence of hepatic, renal, or cardiac toxicity in the patients who received creatine [124]. There was no increased incidence of intracranial events or medical complications observed in the patients receiving creatine [124]. The investigators concluded that no side effects were observed due to creatine administration [125]. Interestingly, there was a significant difference between the creatine-treated group and the control group on the TBI sequela of "Fatigue" ($p < 0.001$). Specifically, the proportion of children who reported experiencing "No Fatigue" 6 months after their TBI was significantly higher in creatine-treated participants than in controls (88.9% vs. 17.6%). This is notable because "Fatigue or loss of energy" is one of the diagnostic criteria for Major Depressive Disorder [126].

The Cooperative International Neuromuscular Research Group conducted a randomized placebo-controlled trial that enrolled 50 children between the ages of 4 and 10 years with Duchenne Muscular Dystrophy. The study was a 3-arm trial: placebo vs. creatine vs. glutamine [127]. Participants in the creatine group received 5 gm/day for six months. The research group reported that there were no significant differences in side effect profile among the three groups, and that creatine and glutamine were safe and well-tolerated over 6 months at the doses prescribed [127].

In summary, SSRI-resistant adolescent MDD is a public health problem that represents a significant unmet need. The dietary supplement creatine is a hypothesis-driven SSRI augmentation strategy, and measurement of PCr with ^{31}P-MRS has the potential to serve as a translational biomarker of juvenile MDD pathophysiology and treatment response. Creatine has been studied as a treatment for numerous pediatric central nervous system disorders, but its

use in child psychiatry is novel. Creatine's gender-specific effects in animal models mandate that studies in youth be initially limited to female participants. Expanding upon our group's recent completion of a multidose study to establish which of four daily creatine doses offers the best combination of efficacy, safety and tolerability in adolescent females with SSRI-resistant MDD, we propose to conduct a pilot, placebo-controlled study of 10g daily adjunctive creatine, the dose selected from the dose-ranging trial, for adolescent females with SSRI-resistant MDD. We propose to examine the relationship between depression severity and cerebral PCr and to measure the effect size of adjunctive creatine vs. placebo on brain chemistry and clinical outcome in adolescent females with SSRI-resistant MDD.

OBJECTIVES:

The primary hypothesis is that compared to placebo, 10g of daily creatine monohydrate for eight weeks will be associated with significant increases in frontal lobe PCr and β-NTP concentrations. A secondary hypothesis is that decreased depressive symptoms measured with the CDRS-R and Montgomery Asberg Depression Rating Scale (MADRS) will be reciprocally correlated with increased β-NTP concentrations.

PARTICIPANT SELECTION CRITERIA:

Thirty-three female adolescents with MDD will be randomly assigned in a 1:1 ratio to either treatment for eight weeks, for a total of 66 MDD participants. Additionally, 40 healthy adolescents will be recruited as comparison subjects for the study. Key inclusion, exclusion, and study withdrawal criteria for each subject group are summarized below.

MDD SUBJECTS
Inclusion Criteria:

- Participants must be female.
- Participants must be able to grant informed consent (age ≥18), or parent/guardian permission plus participant assent (age <18).
- Participants must meet DSM-IV criteria for MDD, with current mood state depressed for ≥2 weeks.
- Participants must be between the ages of 12 and 21.
- Current CDRS-R raw score of ≥40 or MADRS score ≥25; and CGI-S score ≥4.
- Participants may be enrolled in individual and/or group psychotherapy, if it has been ongoing for at least 8 weeks.
- Participants must have been in treatment with an SSRI for at least 8 weeks, the last 4 of which were at a dosage of ≥20 mg per day of fluoxetine or its equivalent, e.g. 20 mg per day of paroxetine, 20 mg citalopram, 10 mg escitalopram, or 100 mg sertraline. If the participant attempted, but could not tolerate, a dose comparable to 20 mg fluoxetine, they will be considered eligible. (This definition of "Adolescent SSRI Resistant Depression" is modified from the NIH-sponsored, $17 million TORDIA Randomized Controlled Trial [http://clinicaltrials.gov/ct2/show/NCT00018902] [128].

Exclusion Criteria:

- Unstable co-morbid medical, neurological, or psychiatric disorder.
- Current DSM-IV criteria for substance abuse or dependence (excepting nicotine/cigarettes).
- Clinically significant suicidal or homicidal risk.
- Pre-existing renal disease.

- Proteinuria on baseline urinalysis testing.
- Pregnancy or breastfeeding.
- Sexually active and unwilling to practice contraception during the study.
- Contraindication to magnetic resonance imaging (e.g. ferromagnetic implant or claustrophobia)
- History of hypersensitivity to creatine.
- History of a previous failed therapeutic trial of creatine.
- Participants may be outpatients or inpatients, but incarcerated persons will be excluded because this study is not approved for "Research Involving Prisoners."
- More than 2 suicide attempts prior to the screening visit
- Participants who made a suicide attempt within 6 months prior to the screening visit

Study Withdrawal Criteria:

- Withdrawal of parental permission, participant informed consent, or participant assent.
- Onset of a psychotic disorder or bipolar disorder.
- Intolerable or clinically significant side effects to creatine.
- Worsening depression, as demonstrated by an increase in CDRS-R or MADRS score $\geq$25% from baseline.
- Positive pregnancy test.
- A significant change to the participant's medication or psychotherapy treatment from the regimen reported at their baseline/screening visit (e.g. the SSRI is discontinued).
- Incarceration, as the study is not approved to conduct "Research Involving Prisoners."
- If a clinically significant intracranial lesion is found by the Radiologist on a participant's baseline brain scan, they will be withdrawn from the study and referred for appropriate medical care.
- 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.

HEALTHY COMPARISON SUBJECTS

Inclusion Criteria:

- Participants must be able to grant informed consent (age $\geq$18), or parent/guardian permission plus participant assent (age <18).
- Participants must be female.
- Participants must be between the ages of 12 and 21 years.
- Participants must not meet DSM-IV-TR criteria for a current psychiatric illness or substance use disorder.
- Participants must have a CDRS-R score $\leq$30.

Exclusion Criteria:

- Unstable medical or neurological illness.
- Clinically significant psychiatric or substance use disorder.
- Pregnant subjects, due to the unknown effects of MRI/MRS scans on a fetus. In addition, women of childbearing potential who are unable or unwilling to practice contraception during the study will be excluded. Female participants who are of childbearing potential must have a negative urine pregnancy test before the MRI/MRS scan.
- Participants with a contraindication to MRI/MRS scanning, such as a metallic implant.

Study Withdrawal Criteria:

- Withdrawal of parental permission or participant assent.
- Onset of a psychotic disorder or depression.
- Positive pregnancy test.
- A significant change to the participant's medication or psychotherapy treatment from the regimen reported at their baseline/screening visit, unless directed by the principal investigator.

DESIGN:

The proposed study will use a randomized double-blind placebo-controlled design. Sixty-six participants will be randomly assigned in a 1:1 ratio to placebo or 10g of oral daily creatine monohydrate augmentation for eight weeks.

The study's design is consistent with the acceptable ethical justifications for the use of placebo controls outlined in the University of Utah Institutional Review Board's (IRB's) Investigator Guidance on Placebo Comparators (Version B0608). Specifically, this study fits situations #3, #4 and #5 of the Guidance:

- "*Participants are refractory to the available therapies by virtue of their past treatment history*" (#3): by definition, the proposed study will recruit female adolescents with SSRI-resistant a.k.a. treatment-refractory depression. To meet the study's inclusion criteria, participants must have failed an adequate trial of the standard-of-care intervention.
- "*The study involves adding a new investigational therapy to an established effective therapy*" (#4): The FDA has approved the SSRI medicines fluoxetine and escitalopram to treat adolescent depression. The guidelines of the American Academy of Pediatrics, and the American Academy of Child & Adolescent Psychiatry advocate cautious use of SSRIs to treat adolescents with MDD. This is an **augmentation** study, meaning this protocol does not remove an established treatment from any participant. All participants will **not** have a "washout period." All participants must continue on an adequate dose of a prescription SSRI antidepressant throughout the time they are enrolled in the study.
    - This is similar to the Placebo Justification listed on the ERICA website, which says: "Any placebo use in this trial is in addition to usual and standard therapy." The proposed study conforms to this criterion, as only participants whose depression has proven resistant to standard antidepressant treatment are eligible. Furthermore, participants in the placebo group will **not** discontinue their SSRI prescription during the study. On the contrary, they must **continue** the usual and standard therapy throughout their participation in the trial.
- "*Participants have determined that the response to the established effective therapies for their condition is unsatisfactory to them*" (#5): the proposed study will only enroll adolescents who have had an adequate trial of a standard-of-care SSRI antidepressant treatment, and who continue to meet diagnostic criteria for major depression with at least moderate residual symptoms. Persons who have never taken an SSRI antidepressant, or who consider their response to SSRI(s) to be satisfactory, will be excluded from participation in this study.

Participants will undergo neuroimaging at baseline, prior to administration of investigational drug. The neuroimaging will then be repeated following 8 weeks of study drug. If the study treatment is tolerated well, after the initial 8 weeks, open-label continuation treatment with

creatine monohydrate 10g/day will be offered for 6 months. The open-label extension offers participants initially randomized to placebo an opportunity for a 6-month therapeutic trial of adjunctive creatine at no cost, with monitoring by the research team and 24/7 cellphone access to a board-certified child psychiatrist. Participants who elect to take 10g/day of creatine will be scheduled for monthly visits over the course of 6 months. At the sixth visit, repeat labs will be obtained. Additionally, per Dr. Kondo's discretion, supplementary labs may be obtained throughout the open-label follow-up phase.

STUDY 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 1** outlines the schedule of study procedures.

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 and parents/guardians 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.

Randomization of participants to the two treatment conditions will take place according to a randomization list generated prior to the start of recruitment, by an individual (Dr. Sung) who will have no contact with participants or with study drug. Thirty-three participants (50%) will be randomized to placebo. 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 with investigational drug. There is no "washout period" for any participant in this study, and participants randomized to placebo will continue treatment with a prescription SSRI antidepressant throughout their time in the study.

If during the MRI/MRS scans we unexpectedly discover something that warrants further inspection, we will refer the participant to her primary care provider for follow-up, along with the MRI/MRS report that shows the findings. Any costs related to following up on unexpected findings will be the responsibility of her and/or her insurance provider.

Participants will be compensated for their participation. The overall expected amount of compensation per MDD participant will be 245 dollars and participants will be paid at each visit. Payment of compensation will vary from visit to visit depending on the length of time needed for that session. Sessions that are scheduled to take longer (e.g., initial screenings, MRI's, and end of study visits) will be compensated accordingly. In order to avoid study participation costing participants money due to travel expenses, participants traveling more than 100 miles round trip per study visit will be given an additional $50 at the week 10 visit, making their total

compensation for the study $295. Participants that are screened but not eligible to enroll in the study will be compensated $25.

HC participants will be paid $25 for the screening visit and $50 for the scan visit, totaling $75.

Table 1: Schedule of Procedures for Major Depression (MDD) & Healthy Control (HC) Participants

Study Week #	- 1	0	1	2	3	4	6	8	10
Parent / Guardian Consent and Participant Assent OR Participant Consent	MDD HC								
Medical History & Physical Exam	MDD								
Electrocardiogram, Phlebotomy for Serum Labs (CBC & CMP), Urinalysis*†	MDD							MDD	
Urine HCG Test & Drug Screen*	MDD HC	MDD HC						MDD	
Randomization		MDD							
Dispense Study Medication**		MDD	MDD	MDD	MDD	MDD	MDD		
KSADS-PL (< 18 years old) or SCID (> 18 years old)	MDD HC								
CDRS-R, CGI, C-SSRS, MADRS, Safety Plan	MDD HC	MDD HC	MDD	MDD	MDD	MDD	MDD	MDD	MDD
DAS	MDD							MDD	
Vital Signs, Concomitant Meds, Adverse Events	MDD HC	MDD HC	MDD	MDD	MDD	MDD	MDD	MDD	MDD
3-Tesla MRI / ^{31}P-MRSI Brain Scan		MDD HC						MDD	

CBC = complete blood count
CMP = comprehensive metabolic panel
CDRS-R = Children's Depression Rating Scale-Revised
CGI = Clinical Global Impression Scale
C-SSRS = Columbia-Suicide Severity Scale
KSADS-PL = Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version
SCID = Structured Clinical Interview for DSM-IV Disorders
MADRS = Montgomery-Asberg Depression Rating Scale
DAS= Dysfunctional Attitude Scale
MRI = Magnetic Resonance Imaging
^{31}P-MRSI = 31-Phosphorus Magnetic Resonance Spectroscopy Imaging

*May be collected at Study Week #0 only if time does not permit for the collection of these during Study Week #-1.

**Participants will not begin taking study medication until serum lab and urinalysis results have been reviewed by Dr. Kondo.

† Per Dr. Kondo's discretion, additional lab tests may be ordered throughout the open-label follow-up phase.

Table 2: Schedule of 6-Month Follow-Up Procedures for MDD Participants

Month #	1	2	3	4	5	6
Phlebotomy for Serum Labs (CBC & CMP) and Urinalysis †						X
Dispense Study Medication	X	X	X	X	X	X
CDRS-R, CGI, C-SSRS, MADRS, Safety Plan	X	X	X	X	X	X
DAS						X
Vital Signs, Concomitant Meds, Adverse Events*	X	X	X	X	X	X

CBC = complete blood count
CMP = comprehensive metabolic panel
CDRS-R = Children's Depression Rating Scale-Revised
CGI = Clinical Global Impression Scale
C-SSRS = Columbia-Suicide Severity Scale
MADRS = Montgomery-Asberg Depression Rating Scale
DAS= Dysfunctional Attitude Scale

*Adverse events will not be evaluated after the follow-up portion of the study

† Per Dr. Kondo's discretion, additional lab tests may be ordered

RISKS AND BENEFITS TO STUDY PARTICIPANTS

- During the intake and assessment interview, participants may become emotionally upset when asked about their psychiatric history including suicide attempts, or physical and sexual abuse.
- There is a 50% chance that the participant will be assigned to placebo, in which they will not receive creatine.
- Participants may experience discomfort or swelling when blood is drawn for laboratory tests. Rarely, infection can result from blood draws.
- It is possible that the participant's illness could worsen during the study. This could be related or unrelated to the study. Adolescents with Major Depressive Disorder are at risk for depression, suicidal ideation, and suicide attempts as part of their illness. Physicians working on the study will be available 24 hours per day. If the participant's illness worsens to the point that the study doctor considers them a danger to themselves or others, they will be hospitalized. If the participant is hospitalized they will be withdrawn from the study. In the event of hospitalization, the participant or the participant's insurance company will be responsible for the associated costs.
- It is possible that treatment with creatine will not be effective for the participant's depression, and that study participation will therefore delay the start of effective treatment.
- The researchers will take precautions to safeguard the participant's confidentiality, but it is possible that a breach of confidentiality could occur. A Certificate of Confidentiality has been obtained for this study.
- The participants may experience gastrointestinal discomfort as a result of taking study medication. We recommend taking the study medication with food to reduce possible stomach discomfort.
- 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 (please tell us if your child has a tattoo)
- Certain transdermal (skin) patches such as NicoDerm (nicotine for tobacco dependence),
- Transderm Scop (scopolamine for motion sickness), 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 3.0T scanner for routine clinical studies in children. 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 that your child 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.

We cannot promise any benefits to the participants from being in the study. However, there are some possible benefits to the participants if they participate in this study:

- Participants will receive a thorough medical and psychiatric evaluation, and will be followed more closely than in routine clinical care.
- We hope that the participant will benefit, but this cannot be guaranteed.

Other than direct benefits to the participants, there are possible indirect benefits:

- Results from the study will help doctors understand the way creatine affects young people with Major Depressive Disorder. This could help us improve treatment for female adolescents with depression.

- The brain scans may improve our understanding of the biology of depression in female adolescents. However, that would not directly benefit the participants.
- The participants will receive a copy of the clinical MRI of their brain, if they wish. A copy of the MRI scan report will also go to the research team.

DATA SAFETY AND MONITORING:

The principal investigator and study coordinator will perform monitoring of the study records on a continuous basis. Unanticipated problems and adverse events that are related to the research, or which place participants at greater-than-expected risk, will be reported to the IRB within 10 working days of learning 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 subjects 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.

Dr. Kondo will make the initial determination if an unexpected, adverse event is related or unrelated to the investigational drug or a clinical or research study procedure. An adverse event is “related to the research” if in the opinion of the principal investigator or co-investigator, it was more likely than not related to the investigational agent or intervention.

Study Premature Termination / Withdrawal Criteria:

Participants may withdraw from the study at any time without giving a reason. Dr. Kondo or co-investigators may also make study withdrawal determinations, in coordination with participants, their families (when appropriate), and their non-study treating clinician(s). A subject’s participation in the trial will also end if any of the following criteria are met:

a) If intolerable adverse effects or toxicity from the investigational drug occurs. Any treatment-emergent and medically serious symptoms will be grounds for an immediate evaluation by a primary care or emergency department provider, depending on severity. After the medical evaluation, a decision will be made by the study team regarding the patient’s continued participation in the study.

b) If clinically significant suicidal or homicidal ideation develops, study withdrawal will be at the discretion of the study team, in consultation with participants and their treating

clinician(s). Generally, a current or recent suicide plan with a degree of intention to carry out the plan will be deemed clinically significant. The investigators will work closely with participants' families and community clinician(s) to maintain safety, and to arrange for hospitalization if it is necessary. It is the research team's experience, however, that suicidal ideation and attempts are not rare in the medical history of depressed adolescents. Rather, they are frequently a clinical feature of the condition. Therefore, withdrawal decisions will be exercised on a case-by-base basis, with the guiding principle that participants must be withdrawn when there is reason to believe that study participation is causing harm, or is no longer in their best interest.

c) If a participant is unable or unwilling to adhere to the study protocol, the principal investigator retains the right to withdraw them from the study without their permission. For example, study medication noncompliance for more than two study visits would be unacceptable and the participant would be subject to withdrawal by the principal investigator.

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

a) Terms and Definitions:

1) *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.
2) *Disability.* A substantial disruption of a person's ability to conduct normal life functions and activities of daily living (ADLs).
3) *Life-threatening adverse drug experience:* Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred.
4) *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.
5) *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 shall 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:

1) *Written reports*

i) The principal investigator and study coordinator shall notify the FDA and all participating investigators 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, the study team 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.* The principal investigator 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 7 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 the principal investigator submit IND safety reports in a format or at a frequency different than that required under this paragraph.

d) Follow-Up:

1) The research team 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 the team's investigation show that an adverse drug experience not initially determined to be reportable is so reportable, the researchers 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 the team's investigation of other safety information shall be submitted, as appropriate, in an information amendment or annual report to the FDA.

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.

Protocol Amendments:

Any amendments or administrative changes in the research protocol during the period, for which the IRB approval has already been given, will not be initiated without submission of an amendment for IRB review and approval.

All amendments will be submitted to the FDA for review.

THE DATA SAFETY MONITORING BOARD & 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*.

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

1. THE DATA AND 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. Douglas Gray. Dr. Gray is a national expert on adolescent suicidology, and serves as Medical Director of the Utah Youth Suicide Study, a multifaceted program of research with the goal of reducing the public health burden of youth suicide in the State of Utah. Because suicidal ideation and behavior is the most serious of the anticipated adverse events for this trial of adolescent MDD, Dr. Gray will serve as Chairman of 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

Range of Safety Reporting by the study team to the DSMB. 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.

Anticipated Adverse Events

Anticipated Adverse Events for this study include the following:

- Emotional upset due to questions during the assessment about participants' psychiatric and social histories (including current suicidal ideation, history of suicide attempts, and history of physical or sexual abuse);
- Pain, swelling or infection due to phlebotomy for blood laboratory testing;

- Nausea, diarrhea, vomiting, abdominal gas or bloating, appetite changes, headache, edema or weight gain associated with administration of the investigational drug creatine;
- Injury due to mobilization of metallic objects inadvertently brought or worn by the participant into the MRI scanner. (The MRI technicians will make every effort to avoid this by reminding the participant and their parent or guardian to take the appropriate precautions prior to entering the scanning suite. An MRI safety questionnaire must be completed by participants immediately prior to each scanning session.);
- Claustrophobia (fear of being in small spaces), dizziness, headache, metallic taste in the mouth, double vision, visions of flashing lights, or tingling in the back during the MRI scans. All of these events are rare, temporary and not medically dangerous.
- Worsening depression, suicidal ideation, and/or suicide attempts as part of the illness of MDD.

The effects of creatine and MRI scans on a developing fetus are unknown, so participants will undergo pregnancy testing, and will be withdrawn from the study if they have a positive pregnancy test. Sexually active persons unwilling to practice contraception during the study will be excluded from participation.

Unanticipated Adverse Events are defined as events associated with study participation not listed above. All SAEs will be reported to the FDA, regardless of whether they are "anticipated" or "unanticipated."

Other Safety-Related Reports – The DSMB will also receive an annual summary report of treatment retention and reasons for withdrawal.

Reporting of Letters or Notices Issued by the FDA – In accordance with NIH Notice OD-00-053 and the applicable Federal law [45 CFR 74.51 (f)], the principal investigator will report to the NIMH (the grant awarding agency) any communication from the FDA regarding the safety of the investigational drug, within 72 hours of receiving it. Immediate notification will be made by telephone, and a copy of the FDA correspondence will be faxed and mailed to the NIMH. The FDA communications covered by 45 CFR 74.51 (f) include the following: Warning Letters, Notice of Initiation of Disqualification Proceedings and Opportunity to Explain Letter (NIDPOE), Notice of Opportunity for Hearing (NOOH), Notice of Disqualification, Consent Agreements, and Clinical Hold Letters. Simultaneous transmission will be made locally to the IRB.

The study will adopt the following **Stopping Rules**:

- *At any time* during the study, if the DSMB unanimously agrees that the risks to participants outweigh the potential benefits, the DSMB will recommend to the IRB and the principal investigator that the study be stopped. The DSMB chairman will communicate this to the IRB and the principal investigator.
- If a unanimous recommendation to stop the study is made by the DSMB, the study will be halted. Recruitment will be closed, and the study team will contact all active and inactive study participants. The FDA, NIMH, and investigational drug manufacturer will also be notified.
- If the study is stopped, active study participants will be instructed to discontinue the investigational drug, and follow-up safety visits will be scheduled for all participants.

1.3 Monitoring of Data Quality by the DSMB

The DSMB will receive annual reports prepared by the principal investigator and the study coordinator on data quality and completeness. This will include an overview of recruitment and retention, a summary report describing participants' adherence to the protocol's procedures and investigational drug, and a summary of the completeness and quality of the data elements needed to characterize the participants and their primary and secondary outcomes. These reports will be used by the DSMB to evaluate the adequacy of the research team's data capture and management to support a scientifically valid analysis at the study's conclusion. The DSMB will make recommendations to improve data management as needed. After reviewing the report, the DMSB will send a letter to the principal investigator recommending that the study continue, or that the study be halted due to safety concerns. All DSMB letters will be submitted to the IRB.

METHODS, DATA ANALYSIS AND INTERPRETATION:

1. Neuroimaging Protocol

i. Magnetic Resonance Imaging (Siemens Trio 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 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 (MPRAGE), 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 a quadrature radio-frequency 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 2100/3.97/1100 ms, matrix 256x256, FOV 256x256 mm, flip angle 12 degree, slice thickness 1.5 mm, slab 192 mm, bandwidth 190 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 Radiologist to screen for structural abnormalities.

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

a. Phosphorus 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.

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

Spectroscopy will be analyzed using Liner Combination of Model Spectra (LCModel) [130], 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, phosphoethanolomine, scyllo-inositol, 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% [131].

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, The 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.

Statistical Analyses

We hypothesize that at baseline, CDRS-R scores are negatively correlated with cerebral PCr in female adolescents with SSRI-resistant MDD. We also hypotehesize that for participants whose MDD responds to creatine, change CDRS-R score will be inversely correlated with change in brain beta-NTP levels (i.e. participants who have a significant decrease intheir CDRS-R score will show an increase in beta-NTP).

Both intent-to-treat (ITT; encompasses all participants who receive >1 dose of study drug) and protocol completer analyses will be performed. A participant completing 8 weeks of treatment and both 31P-MRS scans will be considered a "completer." Data will be expressed as mean + standard deviation (SD). Group differences in demographic variables involving continuous data will be calculated using one-way analysis of variance. Between-group comparisons involving categorical variables will be assessed using Fisher's exact test for n x k contingency tables. A linear mixed model (LMM) will be used to account for the association between measurements (baseline and post-treatment) for each individual for the repeated measures analysis. Exploratory data analysis using non-parametric statistical tests (e.g., Wilcoxon Rank-Sum test and the Mann-Whitney U-test) will also be performed. Statistical significance will be defined at the two-tailed, 0.05 level. Sata 11 (StataCorp LP, College Station, TX) and SAS 9.2 (SAS Institute, Cary, NC) software will be used for statistical calculations. Confidence intervals for all estimated parameters will be constructed using a 95% level.

Power Analysis and Sample Size Calculations: Calculations are based on the open-label female adolescent SSRI-resistant MDD creatine data obtained at our site, and our adult creatine studies. All power calculations are two-tailed with an alpha level of 0.05, and were performed in the specialized software PASS (NCSS, Kaysville, UT). The minimum required sample size

satisfying our hypothesis is 30 creatine-treated, 30 placebo-treated, and 30 age-matched healthy controls. Assuming a 10% attrition rate for MDD subjects and a 30% attrition rate for healthy controls, we propose to enroll a total of 66 female adolescents with SSRI-resistant MDD and 40 healthy controls. The central hypothesis and power analysis for the R33 clinical trial are described below.

The hypothesis that PCr and beta-NTP levels measured with 31P-MRS will show significant change associated with 8 weeks of 10g daily creatine compared with placebo in female adolescents with SSRI-resistant MDD evaluates the significance of treatment-related recovery. In our open-label creatine data, we found that female adolescents wtih SSRI-resistant MDD showed increased PCr and decreased beta-NTP following creatine augmentation. The effect size of these changes were estimated to be approximately 1.0 (Cohen's d) with 10% SD. Therefore, when we conservatively set the effect size to be 0.9; sample size of 30 per each group; and common SD within a group of 10%, the changes of metabolite levels between creatine and placebo groups will be significantly (alpha level of 0.05) detectable with 86% of power.

ADMINISTRATIVE RESPONSIBILITIES

Resources:

The Brain Institute

The facilities at the University of Utah that are available for the proposed research include the following: the Brain Institute and UNI. The Brain Institute currently occupies 5,900 square feet of office and laboratory space in a building in the University of Utah Research Park adjacent to the Health Sciences campus. It also occupies clinical space in UNI, the site of the Brain Institute's Siemens 3T MRI research scanner.

Recruitment of Participants:

- No "cold calls" will be made by members of the study team.
- An informational flyer will be placed (with permission) on public bulletin boards. The flyer will be submitted to the IRB for approval prior to posting. Examples of public bulletin boards may include but are not limited to: University of Utah bulletin boards (for example, the Health Science Education Building, the College of Nursing, the Department of Psychology, etc.), the Salt Lake Community College, and business frequented by adolescents and their parents.
- An informational brochure has been created, to be displayed (with permission) in appropriate public areas, and to be provided to practicing mental health clinicians, pediatricians and family practitioners in the Salt Lake City area. The clinicians, at their sole discretion and without compensation, will pass the brochure to the parent(s) or guardian(s) of adolescent clients in cases where they deem it appropriate. The brochure will be submitted to the IRB for approval, prior to distribution to clinicians.
- The recruitment flyer and brochure will be posted on the Brain Institute and Department of Psychiatry Internet websites, following approval by the IRB.
- The principal investigator will give presentations to advocacy groups such as NAMI, in which the study is described and parent(s) or guardian(s) are invited to contact the principal investigator to learn more about the study.
- An informational Internet website will be created and submitted to the IRB for approval.
- An IRB-approved paragraph informing University of Utah faculty of the study will be posted in the faculty newsletter (FYI newsletter). The FYI newsletter is published once a month, and we will submit our IRB-approved paragraph to appear in the newsletter up to 12 times per year.

- A webpage describing the study and providing contact information will be uploaded onto the Brain Institute's website at utahbrain.org following IRB approval.
- Study information will be provided to the UNI receiving center to encourage clinician referrals. If a participant calls the Brain Institute regarding study participation and reports that they were referred by a UNI clinician, that clinician will receive a $5 meal card to the UNI cafeteria.

CONTROL OF INVESTIGATIONAL DRUG

The University of Utah pharmacy's Investigational Drug Service (IDS) will prepare the doses of placebo and 10g of creatine required for the study. IDS will send the study medication in a blinded fashion to the University of Utah Neuropsychiatric Institute (UNI). The study medication will be stored securely at the UNI pharmacy, where it will be stored in a climate-controlled environment and accessible only to pharmacy staff and research personnel. The study will be conducted in compliance with all applicable FDA regulations for IND studies.

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. IDS will be contacted in order to break the blind.

DATA COLLECTION, MANAGEMENT AND PROTECTED HEALTH INFORMATION

The study team will create case report forms (CRFs) for data collection. The consent, permission and assent forms, CRFs, laboratory results, radiology reports, MRI registration forms, and other necessary documents will be filed in each study participant's binder. Study participant binders will contain protected health information such as name, date of birth, age, address, and phone number. Binders will be stored in locked cabinets located in locked offices. Only essential study team members will have access to participant binders.

Using a password-protected computer with access limited to study personnel, the research team will maintain a computerized screening and enrollment log. Participant screening, eligibility status, and consent for participation status will be recorded in this log. Before the performance of any study procedures, potential participants (and where applicable their parent(s) or guardians(s)), verbal and written informed consent will be obtained. When a participant is screened for study eligibility, they will be assigned a screening ID number. The individual will maintain the screening ID until they have completed the screening visit. If a subject is determined to be ineligible during screening, the reason for the screen failure will be recorded on the computerized screening and enrollment log. Once a subject has completed screening enrolled for study participation, they will be assigned a subject ID number, which will be used to identify all of the data collected from them during the study.

Data from CRFs will be entered into REDCap. REDCap is a secure, web-based application for building and managing online surveys and databases. No personal identifiers from study participants will be entered into REDCap. Forms that have missing or inconsistent data will be recorded in the database; however a "missing data" code will be entered in place of each piece of missing or inconsistent data. Two study team members -- the individual who enters the data, and a second individual monitoring data entry -- will check each CRF form entered into REDCap for accuracy and completeness.

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.

REFERENCES:

1. Birmaher, B., et al., *Practice parameter for the assessment and treatment of children and adolescents with depressive disorders.* J Am Acad Child Adolesc Psychiatry, 2007. **46**(11): p. 1503-26.
2. Lewinsohn, P.M., et al., *Adolescent psychopathology: I. Prevalence and incidence of depression and other DSM-III-R disorders in high school students.* J Abnorm Psychol, 1993. **102**(1): p. 133-44.
3. Fergusson, D.M., et al., *Subthreshold depression in adolescence and mental health outcomes in adulthood.* Arch Gen Psychiatry, 2005. **62**(1): p. 66-72.
4. Kandel, D.B. and M. Davies, *Epidemiology of depressive mood in adolescents: an empirical study.* Arch Gen Psychiatry, 1982. **39**(10): p. 1205-12.
5. Lynch, F.L. and G.N. Clarke, *Estimating the economic burden of depression in children and adolescents.* Am J Prev Med, 2006. **31**(6 Suppl 1): p. S143-51.
6. Fombonne, E., et al., *The Maudsley long-term follow-up of child and adolescent depression. 2. Suicidality, criminality and social dysfunction in adulthood.* Br J Psychiatry, 2001. **179**: p. 218-23.
7. Fombonne, E., et al., *The Maudsley long-term follow-up of child and adolescent depression. 1. Psychiatric outcomes in adulthood.* Br J Psychiatry, 2001. **179**: p. 210-7.
8. Vitiello, B., et al., *Functioning and quality of life in the Treatment for Adolescents with Depression Study (TADS).* J Am Acad Child Adolesc Psychiatry, 2006. **45**(12): p. 1419-26.
9. Weissman, M.M., et al., *Depressed adolescents grown up.* JAMA, 1999. **281**(18): p. 1707-13.
10. Ma, J., K.V. Lee, and R.S. Stafford, *Depression treatment during outpatient visits by U.S. children and adolescents.* J Adolesc Health, 2005. **37**(6): p. 434-42.
11. Gillham, J.E., et al., *School-based prevention of depressive symptoms: A randomized controlled study of the effectiveness and specificity of the Penn Resiliency Program.* J Consult Clin Psychol, 2007. **75**(1): p. 9-19.
12. Newman, T.B., *A black-box warning for antidepressants in children?* N Engl J Med, 2004. **351**(16): p. 1595-8.
13. Usala, T., et al., *Randomised controlled trials of selective serotonin reuptake inhibitors in treating depression in children and adolescents: A systematic review and meta-analysis.* European Neuropsychopharmacology, 2008. **18**(1): p. 62-73.
14. Bridge, J.A., et al., *Placebo Response in Randomized Controlled Trials of Antidepressants for Pediatric Major Depressive Disorder.* Am J Psychiatry, 2008: p. appi.ajp.2008.08020247.
15. Barratt, A., et al., *Tips for learners of evidence-based medicine: 1. Relative risk reduction, absolute risk reduction and number needed to treat.* CMAJ, 2004. **171**(4): p. 353-8.
16. Tsapakis, E.M., et al., *Efficacy of antidepressants in juvenile depression: meta-analysis.* The British Journal of Psychiatry, 2008. **193**(1): p. 10-17.
17. Hammad, T.A., T. Laughren, and J. Racoosin, *Suicidality in Pediatric Patients Treated With Antidepressant Drugs.* Arch Gen Psychiatry, 2006. **63**(3): p. 332-339.
18. March, J., et al., *Fluoxetine, cognitive-behavioral therapy, and their combination for adolescents with depression: Treatment for Adolescents With Depression Study (TADS) randomized controlled trial.* JAMA, 2004. **292**(7): p. 807-20.
19. Kratochvil, C.J., et al., *Selective Serotonin Reuptake Inhibitors in Pediatric Depression: Is the Balance Between Benefits and Risks Favorable?* Journal of Child and Adolescent Psychopharmacology, 2006. **16**(1-2): p. 11-24.
20. Allchurch, M.H., *PRESS RELEASE: European Medicines Agency adopts a positive opinion for the use of Prozac in the treatment of children and adolescents suffering from depression*, P. Office, Editor 2006, European Medicines Agency: London.

21. Hughes, C.W.P.D., et al., *Texas Children's Medication Algorithm Project: Update From Texas Consensus Conference Panel on Medication Treatment of Childhood Major Depressive Disorder.* Journal of Amer Academy of Child & Adolescent Psychiatry, 2007. **46**(6): p. 667-686.
22. Emslie, G.J., et al., *Escitalopram in the treatment of adolescent depression: a randomized placebo-controlled multisite trial.* J Am Acad Child Adolesc Psychiatry, 2009. **48**(7): p. 721-9.
23. Wagner, K.D., et al., *A double-blind, randomized, placebo-controlled trial of escitalopram in the treatment of pediatric depression.* J Am Acad Child Adolesc Psychiatry, 2006. **45**(3): p. 280-8.
24. Zahn-Waxler, C., E.A. Shirtcliff, and K. Marceau, *Disorders of childhood and adolescence: gender and psychopathology.* Annu Rev Clin Psychol, 2008. **4**: p. 275-303.
25. Birmaher, B., et al., *Childhood and adolescent depression: a review of the past 10 years. Part I.* J Am Acad Child Adolesc Psychiatry, 1996. **35**(11): p. 1427-39.
26. Garrison, C.Z., et al., *Incidence of major depressive disorder and dysthymia in young adolescents.* J Am Acad Child Adolesc Psychiatry, 1997. **36**(4): p. 458-65.
27. Hankin, B.L., et al., *Development of depression from preadolescence to young adulthood: emerging gender differences in a 10-year longitudinal study.* J Abnorm Psychol, 1998. **107**(1): p. 128-40.
28. Wichstrom, L., *The emergence of gender difference in depressed mood during adolescence: the role of intensified gender socialization.* Dev Psychol, 1999. **35**(1): p. 232-45.
29. Wade, T.J., J. Cairney, and D.J. Pevalin, *Emergence of gender differences in depression during adolescence: national panel results from three countries.* J Am Acad Child Adolesc Psychiatry, 2002. **41**(2): p. 190-8.
30. Patton, G.C., et al., *Menarche and the onset of depression and anxiety in Victoria, Australia.* J Epidemiol Community Health, 1996. **50**(6): p. 661-6.
31. Tanner, J.M. and G.R. Tanner, *Growth*. Life Science Library. Vol. 13. 1965, New York: Time-Life Books. 200.
32. Angold, A., E.J. Costello, and C.M. Worthman, *Puberty and depression: the roles of age, pubertal status and pubertal timing.* Psychol Med, 1998. **28**(1): p. 51-61.
33. Angold, A., et al., *Pubertal changes in hormone levels and depression in girls.* Psychol Med, 1999. **29**(5): p. 1043-53.
34. Halbreich, U. and L.S. Kahn, *Role of estrogen in the aetiology and treatment of mood disorders.* CNS Drugs, 2001. **15**(10): p. 797-817.
35. Kessler, R.C., et al., *Sex and depression in the National Comorbidity Survey. I: Lifetime prevalence, chronicity and recurrence.* J Affect Disord, 1993. **29**(2-3): p. 85-96.
36. McCauley, E., et al., *Depression in young people: initial presentation and clinical course.* J Am Acad Child Adolesc Psychiatry, 1993. **32**(4): p. 714-22.
37. Kovacs, M., *The Emanuel Miller Memorial Lecture 1994. Depressive disorders in childhood: an impressionistic landscape.* J Child Psychol Psychiatry, 1997. **38**(3): p. 287-98.
38. Kovacs, M., *Presentation and course of major depressive disorder during childhood and later years of the life span.* J Am Acad Child Adolesc Psychiatry, 1996. **35**(6): p. 705-15.
39. Gorman, J.M., *Gender differences in depression and response to psychotropic medication.* Gend Med, 2006. **3**(2): p. 93-109.
40. Murray, C.J.L. and A.D. Lopez, *The Global Burden of Disease*1996, Cambridge, MA: Harvard University Press. 117-120.
41. Rezin, G.T., et al., *Mitochondrial Dysfunction and Psychiatric Disorders.* Neurochem Res, 2008.
42. Shao, L., et al., *Mitochondrial involvement in psychiatric disorders.* Ann Med, 2008. **40**(4): p. 281-95.

43. Kato, T., *Mitochondrial dysfunction as the molecular basis of bipolar disorder: therapeutic implications.* CNS Drugs, 2007. **21**(1): p. 1-11.
44. Moretti, A., A. Gorini, and R.F. Villa, *Affective disorders, antidepressant drugs and brain metabolism.* Mol Psychiatry, 2003. **8**(9): p. 773-85.
45. Iosifescu, D.V. and P.E. Renshaw, *31P-magnetic resonance spectroscopy and thyroid hormones in major depressive disorder: toward a bioenergetic mechanism in depression?* Harv Rev Psychiatry, 2003. **11**(2): p. 51-63.
46. Iosifescu, D.V., et al., *Brain bioenergetics and response to triiodothyronine augmentation in major depressive disorder.* Biol Psychiatry, 2008. **63**(12): p. 1127-34.
47. Renshaw, P.F., et al., *Multinuclear magnetic resonance spectroscopy studies of brain purines in major depression.* Am J Psychiatry, 2001. **158**(12): p. 2048-55.
48. Puri, B.K., *Proton and 31-phosphorus neurospectroscopy in the study of membrane phospholipids and fatty acid intervention in schizophrenia, depression, chronic fatigue syndrome (myalgic encephalomyelitis) and dyslexia.* Int Rev Psychiatry, 2006. **18**(2): p. 145-7.
49. Brosnan, J.T. and M.E. Brosnan, *Creatine: endogenous metabolite, dietary, and therapeutic supplement.* Annu Rev Nutr, 2007. **27**: p. 241-61.
50. Schlattner, U., M. Tokarska-Schlattner, and T. Wallimann, *Mitochondrial creatine kinase in human health and disease.* Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 2006. **1762**(2): p. 164-180.
51. Stork, C. and P.F. Renshaw, *Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research.* Mol Psychiatry, 2005. **10**(10): p. 900-19.
52. Kato, T., et al., *Brain phosphorous metabolism in depressive disorders detected by phosphorus-31 magnetic resonance spectroscopy.* J Affect Disord, 1992. **26**(4): p. 223-30.
53. Moore, C.M., et al., *Lower levels of nucleoside triphosphate in the basal ganglia of depressed subjects: a phosphorous-31 magnetic resonance spectroscopy study.* Am J Psychiatry, 1997. **154**(1): p. 116-8.
54. Forester, B.P., et al., *31Phosphorus magnetic resonance spectroscopy study of tissue specific changes in high energy phosphates before and after sertraline treatment of geriatric depression.* Int J Geriatr Psychiatry, 2009. **24**(8): p. 788-97.
55. Kondo, D.G., et al., *Open-label adjunctive creatine for female adolescents with SSRI-resistant major depressive disorder: A 31-phosphorus magnetic resonance spectroscopy study.* J Affect Disord, 2011.
56. Volz, H.P., et al., *31P magnetic resonance spectroscopy in the frontal lobe of major depressed patients.* Eur Arch Psychiatry Clin Neurosci, 1998. **248**(6): p. 289-95.
57. Lyoo, I.K., et al., *Multinuclear magnetic resonance spectroscopy of high-energy phosphate metabolites in human brain following oral supplementation of creatine-monohydrate.* Psychiatry Res, 2003. **123**(2): p. 87-100.
58. Ellington, W.R. and T. Suzuki, *Early evolution of the creatine kinase gene family and the capacity for creatine biosynthesis and membrane transport.* Subcell Biochem, 2007. **46**: p. 17-26.
59. Kamber, M., et al., *Creatine supplementation--part I: performance, clinical chemistry, and muscle volume.* Med Sci Sports Exerc, 1999. **31**(12): p. 1763-9.
60. McLeish, M.J. and G.L. Kenyon, *Relating structure to mechanism in creatine kinase.* Crit Rev Biochem Mol Biol, 2005. **40**(1): p. 1-20.
61. Wallimann, T., et al., *Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis.* Biochem J, 1992. **281 (Pt 1)**: p. 21-40.
62. Wyss, M., et al., *The therapeutic potential of oral creatine supplementation in muscle disease.* Med Hypotheses, 1998. **51**(4): p. 333-6.
63. Andres, R.H., et al., *Effects of creatine treatment on the survival of dopaminergic neurons in cultured fetal ventral mesencephalic tissue.* Neuroscience, 2005. **133**(3): p. 701-13.

64. Jost, C.R., et al., *Creatine kinase B-driven energy transfer in the brain is important for habituation and spatial learning behaviour, mossy fibre field size and determination of seizure susceptibility.* Eur J Neurosci, 2002. **15**(10): p. 1692-706.
65. Rae, C., et al., *Oral creatine monohydrate supplementation improves brain performance: a double-blind, placebo-controlled, cross-over trial.* Proc Biol Sci, 2003. **270**(1529): p. 2147-50.
66. Ferrier, C.H., et al., *N-Acetylaspartate and creatine levels measured by (1)H MRS relate to recognition memory.* Neurology, 2000. **55**(12): p. 1874-83.
67. Watanabe, A., N. Kato, and T. Kato, *Effects of creatine on mental fatigue and cerebral hemoglobin oxygenation.* Neurosci Res, 2002. **42**(4): p. 279-85.
68. Gjedde, A., P.H. Poulsen, and L. Ostergaard, *On the oxygenation of hemoglobin in the human brain.* Adv Exp Med Biol, 1999. **471**: p. 67-81.
69. Madsen, P.L., et al., *Cerebral oxygen/glucose ratio is low during sensory stimulation and rises above normal during recovery: excess glucose consumption during stimulation is not accounted for by lactate efflux from or accumulation in brain tissue.* J Cereb Blood Flow Metab, 1999. **19**(4): p. 393-400.
70. Saks, V.A., et al., *Role of the creatine/phosphocreatine system in the regulation of mitochondrial respiration.* Acta Physiol Scand, 2000. **168**(4): p. 635-41.
71. Walter, M.C., et al., *Creatine monohydrate in muscular dystrophies: A double-blind, placebo-controlled clinical study.* Neurology, 2000. **54**(9): p. 1848-50.
72. Jenkins, B.G., et al., *Evidence for impairment of energy metabolism in vivo in Huntington's disease using localized 1H NMR spectroscopy.* Neurology, 1993. **43**(12): p. 2689-95.
73. Ferrante, R.J., et al., *Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease.* J Neurosci, 2000. **20**(12): p. 4389-97.
74. Andreassen, O.A., et al., *Creatine increase survival and delays motor symptoms in a transgenic animal model of Huntington's disease.* Neurobiol Dis, 2001. **8**(3): p. 479-91.
75. Dedeoglu, A., et al., *Creatine therapy provides neuroprotection after onset of clinical symptoms in Huntington's disease transgenic mice.* J Neurochem, 2003. **85**(6): p. 1359-67.
76. Matthews, R.T., et al., *Creatine and cyclocreatine attenuate MPTP neurotoxicity.* Exp Neurol, 1999. **157**(1): p. 142-9.
77. Hersch, S.M., et al., *Creatine in Huntington disease is safe, tolerable, bioavailable in brain and reduces serum 8OH2'dG.* Neurology, 2006. **66**(2): p. 250-2.
78. Beal, M.F., *Therapeutic approaches to mitochondrial dysfunction in Parkinson's disease.* Parkinsonism Relat Disord, 2009. **15 Suppl 3**: p. S189-94.
79. Investigators, N.N.-P., *A randomized, double-blind, futility trial of creatine and minocucline in early Parkinson disease.* Neurology, 2006. **66**: p. 664-671.
80. Allen, P.J., et al., *Chronic creatine supplementation alters depression-like behavior in rodents in a sex-dependent manner.* Neuropsychopharmacology, 2010. **35**(2): p. 534-46.
81. Simpkins, J.W., et al., *Estrogen actions on mitochondria--physiological and pathological implications.* Mol Cell Endocrinol, 2008. **290**(1-2): p. 51-9.
82. Klinge, C.M., *Estrogenic control of mitochondrial function and biogenesis.* J Cell Biochem, 2008. **105**(6): p. 1342-51.
83. Kley, R.A., M. Vorgerd, and M.A. Tarnopolsky, *Creatine for treating muscle disorders.* Cochrane Database of Systematic Reviews, 2007(1): p. 1-25.
84. Kley, R.A., M.A. Tarnopolsky, and M. Vorgerd, *Creatine treatment in muscle disorders: a meta-analysis of randomised controlled trials.* J Neurol Neurosurg Psychiatry, 2008. **79**(4): p. 366-7.

85. Poortmans, J.R., et al., *Effect of short-term creatine supplementation on renal responses in men.* Eur J Appl Physiol Occup Physiol, 1997. **76**(6): p. 566-7.
86. Poortmans, J.R. and M. Francaux, *Long-term oral creatine supplementation does not impair renal function in healthy athletes.* Med Sci Sports Exerc, 1999. **31**(8): p. 1108-10.
87. Poortmans, J.R. and M. Francaux, *Adverse effects of creatine supplementation: fact or fiction?* Sports Med, 2000. **30**(3): p. 155-70.
88. Mihic, S., et al., *Acute creatine loading increases fat-free mass, but does not affect blood pressure, plasma creatinine, or CK activity in men and women.* Med Sci Sports Exerc, 2000. **32**(2): p. 291-6.
89. Shao, A. and J.N. Hathcock, *Risk assessment for creatine monohydrate.* Regul Toxicol Pharmacol, 2006. **45**(3): p. 242-51.
90. Robinson, T.M., et al., *Dietary creatine supplementation does not affect some haematological indices, or indices of muscle damage and hepatic and renal function.* Br J Sports Med, 2000. **34**(4): p. 284-8.
91. Bennett, T., et al., *Effect of creatine on performance of militarily relevant tasks and soldier health.* Mil Med, 2001. **166**(11): p. 996-1002.
92. Greenwood, M., et al., *Cramping and Injury Incidence in Collegiate Football Players Are Reduced by Creatine Supplementation.* J Athl Train, 2003. **38**(3): p. 216-219.
93. Greenwood, M., et al., *Creatine supplementation during college football training does not increase the incidence of cramping or injury.* Mol Cell Biochem, 2003. **244**(1-2): p. 83-8.
94. Kreider, R.B., et al., *Long-term creatine supplementation does not significantly affect clinical markers of health in athletes.* Mol Cell Biochem, 2003. **244**(1-2): p. 95-104.
95. Pritchard, N.R. and P.A. Kalra, *Renal dysfunction accompanying oral creatine supplements.* Lancet, 1998. **351**(9111): p. 1252-3.
96. Koshy, K.M., E. Griswold, and E.E. Schneeberger, *Interstitial nephritis in a patient taking creatine.* N Engl J Med, 1999. **340**(10): p. 814-5.
97. Terjung, R.L., et al., *American College of Sports Medicine roundtable. The physiological and health effects of oral creatine supplementation.* Med Sci Sports Exerc, 2000. **32**(3): p. 706-17.
98. Farquhar, W.B. and E.J. Zambraski, *Effects of creatine use on the athlete's kidney.* Curr Sports Med Rep, 2002. **1**(2): p. 103-6.
99. Yoshizumi, W.M. and C. Tsourounis, *Effects of creatine supplementation on renal function.* J Herb Pharmacother, 2004. **4**(1): p. 1-7.
100. Pline, K.A. and C.L. Smith, *The effect of creatine intake on renal function.* Ann Pharmacother, 2005. **39**(6): p. 1093-6.
101. Harris, R.C., K. Saderlund, and E. Hultman, *Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation.* Clin. Sci., 1992. **83**(3): p. 367-374.
102. Poortmans, J.R. and M. Francaux, *Renal dysfunction accompanying oral creatine supplements.* Lancet, 1998. **352**(9123): p. 234.
103. Gualano, B., et al., *Effects of creatine supplementation on renal function: a randomized, double-blind, placebo-controlled clinical trial.* Eur J Appl Physiol, 2008. **103**(1): p. 33-40.
104. Willis, J., et al., *Protein and creatine supplements and misdiagnosis of kidney disease.* BMJ, 2010. **340**: p. b5027.
105. Archer, M.C., *The use of creatine to enhance athletic performance.* Nutrition, 2004. **20**(9): p. 841.
106. Archer, M.C., et al., *Environmental nitroso compounds: reaction of nitrite with creatine and creatinine.* Science, 1971. **174**(16): p. 1341-3.
107. Derave, W., et al., *Oral creatine supplementation in humans does not elevate urinary excretion of the carcinogen N-nitrososarcosine.* Nutrition, 2006. **22**(3): p. 332-3.

108. Yu, P.H. and Y. Deng, *Potential cytotoxic effect of chronic administration of creatine, a nutrition supplement to augment athletic performance.* Med Hypotheses, 2000. **54**(5): p. 726-8.
109. Yu, P.H., *Increase of formation of methylamine and formaldehyde in vivo after administration of nicotine and the potential cytotoxicity.* Neurochem Res, 1998. **23**(9): p. 1205-10.
110. Yu, P.H., C.T. Lai, and D.M. Zuo, *Formation of formaldehyde from adrenaline in vivo; a potential risk factor for stress-related angiopathy.* Neurochem Res, 1997. **22**(5): p. 615-20.
111. Candow, D.G., et al., *Low-Dose Creatine Combined with Protein during Resistance Training in Older Men.* Med Sci Sports Exerc, 2008.
112. Schilling, B.K., et al., *Creatine supplementation and health variables: a retrospective study.* Med Sci Sports Exerc, 2001. **33**(2): p. 183-8.
113. Bender, A., et al., *Long-term creatine supplementation is safe in aged patients with Parkinson disease.* Nutrition Research, 2008. **28**(3): p. 172-178.
114. Bender, A., et al., *Creatine supplementation in Parkinson disease: a placebo-controlled randomized pilot trial.* Neurology, 2006. **67**(7): p. 1262-4.
115. Shefner, J.M., et al., *A clinical trial of creatine in ALS.* Neurology, 2004. **63**(9): p. 1656-61.
116. Shewmon, D., *Safety data for creatinine*, D.G. Kondo, Editor 2008: Salt Lake City, UT. p. Report of adverse events.
117. Brudnak, M.A., *Creatine: are the benefits worth the risk?* Toxicol Lett, 2004. **150**(1): p. 123-30.
118. Laos, C. and J.D. Metzl, *Performance-enhancing drug use in young athletes.* Adolesc Med Clin, 2006. **17**(3): p. 719-31; abstract xii.
119. Smith, J. and D.L. Dahm, *Creatine use among a select population of high school athletes.* Mayo Clin Proc, 2000. **75**(12): p. 1257-63.
120. Ostojic, S.M., *Creatine supplementation in young soccer players.* Int J Sport Nutr Exerc Metab, 2004. **14**(1): p. 95-103.
121. Cancela, P., et al., *Creatine supplementation does not affect clinical health markers in football players.* Br J Sports Med, 2008. **42**(9): p. 731-5.
122. Bourgeois, J.M., et al., *Creatine monohydrate attenuates body fat accumulation in children with acute lymphoblastic leukemia during maintenance chemotherapy.* Pediatr Blood Cancer, 2008. **51**(2): p. 183-7.
123. Komura, K., et al., *Effectiveness of creatine monohydrate in mitochondrial encephalomyopathies.* Pediatr Neurol, 2003. **28**(1): p. 53-8.
124. Sakellaris, G., et al., *Prevention of complications related to traumatic brain injury in children and adolescents with creatine administration: an open label randomized pilot study.* J Trauma, 2006. **61**(2): p. 322-9.
125. Sakellaris, G., et al., *Prevention of traumatic headache, dizziness and fatigue with creatine administration. A pilot study.* Acta Paediatr, 2008. **97**(1): p. 31-4.
126. Zimmerman, M., et al., *Diagnosing major depressive disorder introduction: an examination of the DSM-IV diagnostic criteria.* J Nerv Ment Dis, 2006. **194**(3): p. 151-4.
127. Escolar, D.M., et al., *CINRG randomized controlled trial of creatine and glutamine in Duchenne muscular dystrophy.* Ann Neurol, 2005. **58**(1): p. 151-5.
128. Brent, D., et al., *Switching to another SSRI or to venlafaxine with or without cognitive behavioral therapy for adolescents with SSRI-resistant depression: the TORDIA randomized controlled trial.* JAMA, 2008. **299**(8): p. 901-13.
129. Kondo, D.G., et al., *Open-label adjunctive creatine for female adolescents with SSRI-resistant major depressive disorder: a 31-phosphorus magnetic resonance spectroscopy study.* J Affect Disord, 2011. **135**(1-3): p. 354-61.

130. Provencher, S.W., *Estimation of metabolite concentrations from localized in vivo proton NMR spectra.* Magn Reson Med, 1993. **30**(6): p. 672-9.
131. Provencher, S.W., *Automatic quantitation of localized in vivo 1H spectra with LCModel.* NMR Biomed, 2001. **14**(4): p. 260-4.