PROTOCOL TITLE: UREA FOR CHRONIC HYPONATREMIA: A PILOT STUDY

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PROTOCOL SYNOPSIS

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Study Rationale:	Hyponatremia, defined as a plasma sodium concentration (PNa) <135 mmol/L, is the most common electrolyte disorder encountered clinically. Hyponatremia is categorized as mild (i.e., PNa 130-134 mmol/L), moderate (i.e., PNa 120-129 mmol/L), or severe (i.e., PNa<120 mmol/L) and as acute (i.e., duration <48 hours), or chronic (i.e., duration ≥48 hours). The small proportion of patients with this disorder who present with severe and/or acute hyponatremia frequently have overt neurological symptoms and require hospitalization and urgent treatment. Much more commonly, patients with this condition have chronic non-severe hyponatremia that does not typically require hospitalization or urgent therapy. While such patients are seemingly asymptomatic, a growing body of evidence demonstrates that even mild chronic hyponatremia is associated with subtle neurocognitive deficits, gait and postural disturbances, development of osteoporosis, heightened risk for

	falls and fractures, and increased mortality. As a result, there has been substantial interest in identifying treatments that can be used for the long-term management of patients with chronic hyponatremia that are safe, well tolerated, and that mitigate the morbidity and mortality associated with this condition. The most common etiology of chronic hyponatremia is the syndrome of inappropriate antidiuretic hormone secretion (SIADH), and interventions currently used to treat this condition are based on our understanding of its pathophysiology. However, some of these treatments, including loop diuretics, oral sodium chloride tablets, and fluid restriction lack evidence of efficacy from clinical trials, and in the case of fluid restriction, pose significant challenges to long-term patient compliance. Other therapies such as vasopressin receptor antagonists (i.e., vaptans) have been shown to improve PNa in clinical trials, yet their widespread use is limited by the notable risk for serious side effects, including liver injury, as well as very high costs. Consequently, at present, there are no treatment interventions available that have been shown in clinical trials to be efficacious, safe, easy for patients to adhere to, and affordable for long-term use. Small case series conducted in Europe have investigated the efficacy of increasing urinary solute excretion through the administration of oral urea, and found this agent to be safe and effective for the treatment of chronic hyponatremia. However, these studies were not designed or powered to examine the effect of long-term urea therapy on key patient-centered clinical outcomes. Moreover, urea has not been available for clinical use in the United States until recently when a novel commercial formulation was introduced. In the first published study of this novel formulation of urea, our group reported it to be effective, safe, and well tolerated for the treatment of inpatient hyponatremia. However, these findings were derived from a retrospective cohort study that focused exclusively on short-term inpatient use of urea in a small number of patients. To date, there have been no clinical trials that have investigated the efficacy and safety of oral urea for the treatment of chronic hyponatremia.
Study Objectives:	**Primary** 1. To determine the number and proportion of patients with chronic hyponatremia due to SIADH who met inclusion/exclusion criteria and were enrolled in the study. 2. To determine the number and proportion of enrolled patients with chronic hyponatremia due to SIADH who completed the study. 3. To determine the monthly enrollment rate of patients with chronic hyponatremia due to SIADH who met inclusion/exclusion criteria.

	4. To determine the number of prescribed urea doses taken by patients with chronic hyponatremia due to SIADH at completion of the study. 5. To determine the reasons for non-adherence to urea therapy by patients with chronic hyponatremia due to SIADH. 6. To assess the change in percentage accuracy action boundary selection in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy 7. To assess the change in overall score of sensorimotor ability battery in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy. 8. To assess the change in the sample entropy of the center of pressure data from the force plate in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy. 9. To assess the change in percentage angular deviation of vestibular control system using dynamic representation of upright stance in patients with chronic hyponatremia from SIADH from baseline to day 42 of urea therapy. 10. To assess the change in percentage angular deviation of somatosensory control system using dynamic representation of upright stance in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy. 11. To assess the change in percentage angular deviation of visual control system using dynamic representation of upright stance in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy. 12. To assess the change in percentage weight symmetry using dynamic representation of upright stance in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy. 13. To assess the change in movement latency of posture control and stability using dynamic representation of upright stance in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy. 14. To assess the change in amplitude scaling of posture control and stability using dynamic representation of upright stance in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy 15. To determine the number and proportion of patients with chronic hyponatremia due to SIADH enrolled in the study with adverse events related to the use of urea 16. To determine the presence and nature of adverse events in patients with chronic hyponatremia due to SIADH related to urea therapy. **Secondary**

	1. To determine the number of patients screened for the study. 2. To determine the number and proportion of patient screened who met inclusion/exclusion criteria for the study. 3. To determine the number and proportion of patients enrolled in the study who took more than 80% of prescribed urea doses. 4. To determine the number and proportion of patients enrolled in the study who thought urea was acceptable. 5. To determine the patients' average ratings for urea acceptability. 6. To assess the change in SF-12 (Health Survey) Mental Component Summary (MCS) in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy. 7. To assess the change in SF-12 (Health Survey) Physical Component Summary (PCS) in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy.
Study Hypothesis:	1. We hypothesize that we will be able to recruit at least 10% of eligible patients with chronic hyponatremia due to SIADH. 2. We hypothesize that we will be able to document at least 80% compliance with prescribed urea doses in patients with chronic hyponatremia due to SIADH. 3. We hypothesize that urea will significantly increase the plasma sodium concentration at day 42 compared with the baseline level in patients with chronic hyponatremia due to SIADH. 4. We hypothesize that urea will significantly improve the scores on neurocognitive and posture control and stability assessments at day 42 compared with the baseline scores in patients with chronic hyponatremia due to SIADH. 5. We hypothesize that adverse events related to the use of urea in patients with chronic hyponatremia due to SIADH will be very uncommon.
Study Aims:	1. To assess the feasibility of recruiting patients with chronic hyponatremia due to SIADH into a study of oral urea and to evaluate their adherence to oral urea. 2. To assess the effect of oral urea on plasma sodium concentration and on neurocognitive function and postural control and stability in patients with chronic hyponatremia due to SIADH. 3. To explore the safety of oral urea in patients with chronic hyponatremia due to SIADH.
Study Design:	The proposed pilot study is designed as a prospective, cross-over trial. Over a 13.5-month period, we will recruit 30

	outpatients with chronic non-severe hyponatremia (PNa 125 to 132 mmol/L) confirmed to be due to SIADH. Following enrollment, patients will be randomly assigned on 1:1 ratio to one of two sequence groups. Patients assigned to group "Urea ON, then Urea OFF" will receive oral urea for 42 ± 3 days (period 1), followed by a 10 ± 4-day period in which patients will be off urea (washout period). Patients then will be off urea for 42 ± 3 days (period 2). Patients assigned to group "Urea OFF, then Urea ON" will be off urea therapy for 42 ± 3 days (period 1), and following a 10 ± 4-day washout period then they will initiate urea for 42 ± 3 days (period 2). Patients will have PNa assessed at baseline and on days 7 ± 2, 14 ± 2, and 42 ± 3 during periods 1 and 2 of the study and will undergo neurocognitive and postural control and stability testing on days 0 and 42 of each of the study periods. All patients will be advised to restrict their fluid intake to ≤1.2 liters during periods 1 and 2.
Planned Sample Size:	30 patients
Duration of Treatment:	42 ± 3 days
Major Inclusion Criteria:	1. Age ≥18 years 2. Attended ≥1 visit at a UPMC outpatient clinic within the prior 12 months 3. Chronic hyponatremia with a history of ≥ 2 sequential plasma sodium concentration (PNa) between 125 mmol/L and 132 mmol/L performed ≥ 14 days apart within the last 18 months with most recent PNa ≤ 132 mmol/L prior to screening 4. Patients are ambulatory without the need for any assist device (e.g., cane, walker) 5. Mini-mental state examination (MMSE) score ≥ 25 6. Diagnosis of SIADH established by the Bartter and Schwartz criteria as follows: a. Hyponatremia with a PNa between 125 mmol/L and 132 mmol/L b. Plasma osmolality < 275 mOsm/kg c. Clinical euvolemia d. Urine osmolality > 100 mosm/kg e. Urine Na ≥ 20 mmol/L f. Intact adrenal function (i.e., morning plasma cortisol value ≥15 µg/dL, or negative corticotropin stimulation test) g. Normal thyroid stimulating hormone level (i.e., TSH between 0.3 to 5 µIU/mL) h. eGFR ≥ 45 ml/min/1.73 m2) ***7.*** Nephrology patients who meet eligibility for the study including a nadir serum sodium of no less than125 mmol/L and have already started urea and treating physician agrees to discontinuing prescribed urea.

Major Exclusion Criteria:	1. Cirrhosis and/or end-stage liver disease 2. Heart failure on diuretics and/or with recorded left ventricular ejection fraction <40% 3. Chronic kidney disease with most recent estimated glomerular filtration rate < 45 ml/min/1.73m2 4. Adrenal insufficiency 5. Untreated hypothyroidism 6. Urinary tract obstruction within the prior 2 months 7. Uncontrolled hyperglycemia (most recent random plasma glucose ≥ 200 mg/dL) 8. Ongoing drug treatment for hyponatremia with vaptans or combination of loop diuretics and salt tablets. 9. Active malignancy (not on remission) 10. Active infection 11. Neurological disorders with impairment of ambulation and/or cognition 12. End-stage lung disease with marked impairment in ambulatory capacity 13. Chronic pain with impairment of ambulation and/or cognition 14. Chronic nausea 15. Hypersensitivity to urea 16. Women who are pregnant, breast feeding, or of childbearing potential who are not using contraception 17. Patient is unable to consent for himself/herself
Study Endpoints:	Primary Endpoints: 1. Number and proportion of patients who met inclusion/exclusion criteria and were enrolled in the study 2. Number and proportion of patients enrolled who completed the study 3. Monthly enrollment rate 4. Number of prescribed urea doses taken by patients 5. Reasons for non-adherence to urea therapy 6. Change in plasma sodium concentration 7. Change in percentage accuracy action boundary selection 8. Change in overall score of sensorimotor ability battery 9. Change in the sample entropy of the center of pressure data from the force plate 10. Change in percentage angular deviation of vestibular control system using dynamic representation of upright stance 11. Change in percentage angular deviation of somatosensory control system using dynamic representation of upright stance 12. Change in percentage angular deviation of visual control system using dynamic representation of upright stance 13. Change in percentage weight symmetry using dynamic representation of upright stance

	14. Change in movement latency of posture control and stability using dynamic representation of upright stance 15. Change in amplitude scaling of posture control and stability using dynamic representation of upright stance 16. Number and proportion of patients enrolled in the study with adverse events related to the use of urea 17. Adverse events related to urea Secondary Endpoints: 1. Number of patients screened 2. Number and proportion of patient screened who met inclusion/exclusion criteria for the study 3. Number and proportion of patients who took more than 80% of prescribed urea doses 4. Number and proportion of patients who thought the medication was acceptable 5. Average ratings for medication acceptability 6. Change in SF-12 (Health Survey) Mental Component Summary (MCS) 7. Change in SF-12 (Health Survey) Physical Component Summary (PCS)

1. OBJECTIVE, SPECIFIC AIMS, BACKGROUND, AND SIGNIFICANCE

1.1 OBJECTIVE

Primary:

a. To determine the number and proportion of patients with chronic hyponatremia due to SIADH who met inclusion/exclusion criteria and were enrolled in the study.
b. To determine the number and proportion of enrolled patients with chronic hyponatremia due to SIADH who completed the study.
c. To determine the monthly enrollment rate of patients with chronic hyponatremia due to SIADH who met inclusion/exclusion criteria.
d. To determine the number of prescribed urea doses taken by patients with chronic hyponatremia due to SIADH at completion of the study.
e. To determine the reasons for non-adherence to urea therapy by patients with chronic hyponatremia due to SIADH.
f. To assess the change in percentage accuracy action boundary selection in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy
g. To assess the change in overall score of sensorimotor ability battery in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy.
h. To assess the change in the sample entropy of the center of pressure data from the force plate in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy.
i. To assess the change in percentage angular deviation of vestibular control system using dynamic representation of upright stance in patients with chronic hyponatremia from SIADH from baseline to day 42 of urea therapy.
j. To assess the change in percentage angular deviation of somatosensory control system using dynamic representation of upright stance in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy.

k. To assess the change in percentage angular deviation of visual control system using dynamic representation of upright stance in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy.
l. To assess the change in percentage weight symmetry using dynamic representation of upright stance in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy.
m. To assess the change in movement latency of posture control and stability using dynamic representation of upright stance in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy.
n. To assess the change in amplitude scaling of posture control and stability using dynamic representation of upright stance in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy
o. To determine the number and proportion of patients with chronic hyponatremia due to SIADH enrolled in the study with adverse events related to the use of urea
p. To determine the presence and nature of adverse events in patients with chronic hyponatremia due to SIADH related to urea therapy.

Secondary:

a. To determine the number of patients screened for the study.
b. To determine the number and proportion of patient screened who met inclusion/exclusion criteria for the study.
c. To determine the number and proportion of patients enrolled in the study who took more than 80% of prescribed urea doses.
d. To determine the number and proportion of patients enrolled in the study who thought urea was acceptable.
e. To determine the patients' average ratings for urea acceptability.
f. To assess the change in SF-12 (Health Survey) Mental Component Summary (MCS) in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy.
g. To assess the change in SF-12 (Health Survey) Physical Component Summary (PCS) in patients with chronic hyponatremia due to SIADH from baseline to day 42 of urea therapy.

1.2 SPECIFIC AIMS

Hypotheses:

a. We hypothesize that we will be able to recruit at least 10% of eligible patients with chronic hyponatremia due to SIADH.
b. We hypothesize that we will be able to document at least 80% compliance with prescribed urea doses in patients with chronic hyponatremia due to SIADH.
c. We hypothesize that urea will significantly increase the plasma sodium concentration at day 42 compared with the baseline level in patients with chronic hyponatremia due to SIADH.
d. We hypothesize that urea will significantly improve the scores on neurocognitive and posture control and stability assessments at day 42 compared with the baseline scores in patients with chronic hyponatremia due to SIADH.
e. We hypothesize that adverse events related to the use of urea in patients with chronic hyponatremia due to SIADH will be very uncommon.

Specific Aims:

Primary:

1. To assess the feasibility of recruiting patients with chronic hyponatremia due to SIADH into a study of oral urea and to evaluate their adherence to oral urea.
2. To assess the effect of oral urea on plasma sodium concentration and on neurocognitive function and postural control and stability in patients with chronic hyponatremia due to SIADH.
3. To explore the safety of oral urea in patients with chronic hyponatremia due to SIADH.

1.3. BACKGROUND and RATIONALE

Introduction

Hyponatremia, defined as a plasma sodium concentration (PNa) <135 mmol/L, is the most common electrolyte disorder encountered clinically. Hyponatremia is categorized as mild (i.e., PNa 130-134 mmol/L), moderate (i.e., PNa 120-129 mmol/L), or severe (i.e., PNa<120 mmol/L) and as acute (i.e., duration <48 hours), or chronic (i.e., duration ≥48 hours). The small proportion of patients with this disorder who present with severe and/or acute hyponatremia frequently have overt neurological symptoms and require hospitalization and urgent treatment. Much more commonly, patients with this condition have chronic non-severe hyponatremia that does not typically require hospitalization or urgent therapy. While such patients are seemingly asymptomatic, a growing body of evidence demonstrates that even mild chronic hyponatremia is associated with subtle neurocognitive deficits, gait and postural disturbances, development of osteoporosis, heightened risk for falls and fractures, and increased mortality.(1) As a result, there has been substantial interest in identifying treatments that can be used for the long-term management of patients with chronic hyponatremia that are safe, well-tolerated, and that mitigate the morbidity and mortality associated with this condition. The most common etiology of chronic hyponatremia is the syndrome of inappropriate antidiuretic hormone secretion (SIADH), and interventions currently used to treat this condition are based on our understanding of its pathophysiology. However, some of these treatments, including loop diuretics, oral sodium chloride tablets, and fluid restriction lack evidence of efficacy from clinical trials, and in the case of fluid restriction, pose significant challenges to long-term patient compliance. Other therapies such as vasopressin receptor antagonists (i.e., vaptans) have been shown to improve PNa in clinical trials (2), yet their widespread use is limited by the notable risk for serious side effects, including liver injury, as well as very high costs. Consequently, at present, there are no treatment interventions available that have been shown in clinical trials to be efficacious, safe, easy for patients to adhere to, and affordable for long-term use. Small case series conducted in Europe have investigated the efficacy of increasing urinary solute excretion through the administration of oral urea, and found this agent to be safe and effective for the treatment of chronic hyponatremia.(3) However, these studies were not designed or powered to examine the effect of long-term urea therapy on key patient-centered clinical outcomes. Moreover, urea has not been available for clinical use in the United States until recently when Ure-Na™, a novel commercial formulation was introduced. In the first published study of this novel formulation of urea, our group reported it to be effective, safe, and well tolerated for the treatment of inpatient hyponatremia.(4) However, these findings were derived from a retrospective cohort study that focused exclusively on short-term inpatient use of urea in a small number of patients. To date, there have been no clinical trials that have investigated the efficacy and safety of oral urea for the treatment of chronic hyponatremia.

Prevalence and costs of chronic non-severe hyponatremia in the ambulatory setting

While the prevalence of chronic non-severe hyponatremia has not been extensively investigated, existing data suggest this condition is quite common. Gankam-Kengne et al. (5) demonstrated that 6.3% of ambulatory patients in Dallas, Texas had hyponatremia (median PNa=133 mmol/L). Using data from the University of Pittsburgh Medical Center (UPMC), our group found that among 413,000 adult patients seen at UPMC outpatient clinics over a 1-year period, 4,941 (1.2%) had a PNa between 125 and 132 mmol/L. While this represents a small proportion of the overall population, extrapolating 1.2% to all American adults suggests that approximately 3 million Americans have chronic non-severe hyponatremia. In fact, a study from Boscoe et al.(6) suggested that the overall treatment costs associated with hyponatremia in the United States exceed $3.5 billion annually.

Clinical significance of chronic non-severe hyponatremia

Chronic non-severe hyponatremia, even if seemingly asymptomatic, is associated with serious adverse outcomes.(7) Renneboog et al.(8) performed neurocognitive testing in 16 patients with chronic hyponatremia and found that patients with PNa 123-132 mmol/L manifested deficits in attention and gait (postural stability). Chronic non-severe hyponatremia is also associated with an increased risk of osteoporosis. Using NHANES III data, Verbalis et al.(9) found that mild hyponatremia (mean PNa=133 mmol/L) was independently associated with an increased risk of osteoporosis (OR, 2.85; 95%CI, 1.03-7.86; p<0.01). Kruse et al.(10) demonstrated that mild hyponatremia (mean PNa=132 mmol/L) was associated with decreased bone mineral density, bone mineral content, and T-score on DEXA scan. The clinical significance of impaired attention, postural instability, and osteoporosis in patients with chronic non-severe hyponatremia relates to the increased risk for falls and fractures in these patients. Renneboog et al.(8) found that 26 of 122 patients (21.3%) with apparently asymptomatic, chronic hyponatremia (mean PNa 126 mmol/L), seen in an Emergency Department had been evaluated for falls compared with 13 of 244 (5.3%) age-matched normonatremic controls (OR, 67.43; 95%CI, 7.48-607.42; p<0.001). The threshold PNa below which fall risk significantly increased was 134 mmol/L. Most recently, Jamal et al.(11) demonstrated in a study of 5,122 men that mild hyponatremia (mean PNa=132 mmol/L) was associated with an increased risk of hip fractures (HR, 3.48; 95%CI, 1.76-6.87), as well as a higher risk for prevalent (HR, 2.78; 95%CI, 1.46-5.30) and incident (HR, 3.36; 95%CI, 1.36-8.27) morphometric fractures (i.e., fractures identified by x-ray rather than from symptoms) compared with patients with normal PNa. Chronic non-severe hyponatremia is also associated with increased mortality. In a prospective cohort study of 5,208 elderly patients, Hoorn et al.(12) demonstrated that mild hyponatremia (mean PNa=133 mmol/L) was associated with an increased risk of death after adjusting for demographic characteristics and clinical comorbidities (HR, 1.21; 95%CI, 1.03-1.43; p=0.02). Similarly, in a study that used the NHANES database, Mohan et al.(13) found that mild hyponatremia (mean PNa=132 mmol/L) was independently associated with an increased risk of death over 7 years of follow up (HR, 3.61; 95%CI, 2.31-5.63; p<0.001). While the precise mechanism(s) linking hyponatremia with mortality has not been definitively established, these and other studies underscore the potential seriousness of chronic non-severe hyponatremia. In summary, chronic non-severe hyponatremia is associated with impairments in attention, gait disturbances, and increased risk for osteoporosis, falls, fractures, and death; findings that highlight the clear need to identify treatments that not only increase PNa, but mitigate risk for these serious, adverse outcomes.

Paucity of effective, safe, well-tolerated, and affordable therapies.

At present, there are several interventions that are available for the treatment of chronic non-severe hyponatremia. However, each has important limitations that preclude its routine use. A key pathophysiological process underlying the development of hyponatremia is water intake in excess of urinary free water excretory capacity. Hence, one treatment approach is restriction of

oral fluid intake. However, data documenting the effectiveness of and patient adherence to this treatment are lacking. In a recent study of over 3,000 subjects with hyponatremia, the increase in PNa observed with fluid restriction in the first 24 hours was not significantly different from that observed in untreated patients.(14) It is estimated that as many as 70% of patients with SIADH will not respond to fluid restriction alone.(15) Furthermore, widespread and long-term use of fluid restriction is limited by patient non-compliance, particularly in the outpatient setting.
Another available treatment is oral loop diuretics in combination with NaCl tablets. Loop diuretics impair the formation of an osmotic gradient necessary for water reabsorption in the kidneys, while oral NaCl helps prevent negative sodium balance from the diuretic.
This treatment combination has been shown to raise PNa in small case series, yet its effect on patient-centered outcomes and tolerability to patients are unknown.(16-18)
Vasopressin antagonists (vaptans) block the action of vasopressin at the V2 receptor in the collecting duct resulting in increased renal free water excretion. Randomized clinical trials have demonstrated the efficacy of vaptans to treat hyponatremia. The SALT-1 and SALT-2 trials (2) enrolled 448 hyponatremic patients (i.e., PNa<135 mmol/L) to receive tolvaptan or placebo and demonstrated that patients who received tolvaptan had a larger increase in PNa at 30 days (6.2±4.1 mmol/L vs. 1.7±3.6 mmol/L in SALT1; and 6.2±3.9 mmol/L vs. 1.8±3.8 mmol/L in SALT2). Subsequently, 5 meta-analyses that included between 11 and 18 studies comprising 1,000 to 5,000 patients confirmed the efficacy of vaptans in raising PNa.(19-23) In 2009, the Food and Drug Administration (FDA) approved tolvaptan for the treatment of hyponatremia. However, the use of this agent has been greatly limited due to safety concerns related to liver damage in a study of polycystic kidney disease(24), which led the FDA to issue a drug safety communication restricting the use of tolvaptan to 30 days and avoiding its use in patients with underlying liver disease.(25) In addition to these safety issues, the cost of tolvaptan (i.e., $438 per single 15 mg tablet) is an important barrier to its routine use. In fact, certain clinical practice guidelines on the management of hyponatremia recommend against the use of tolvaptan for this indication.(26) In summary, currently available treatments for chronic hyponatremia lack data on efficacy and/or have important limitations related to patient non-adherence, adverse side effects, and/or significant costs. Consequently, there is a clear need for investigation of alternative treatments for this common condition.

Preliminary studies of urea

Urea, also known as Carbamide, it is an organic compound with formula CH_4N_2O. This amide has two amino groups (-NH2) joined by a carbonyl (C=O) functional group. Urea is an endogenous product of protein and amino acid catabolism. Oral urea works as an osmotic diuretic that increases urinary water excretion and has been available for use in Europe for the treatment of hyponatremia for many years. Preliminary evidence of the potential efficacy of urea derives from small European case series.(1) Decaux et al.(3) studied 7 patients with chronic hyponatremia and found that treatment with urea over a period of up to 9 months resulted in an increase in mean PNa from 116 to 136 mmol/L and was not associated with any major side effects. Another study by Decaux et al.(27) of 50 patients with mild to moderate hyponatremia (PNa 120 to 134 mmol/L) found that 2 days of treatment with urea increased PNa by 7±4 mmol/L. Collectively, these and other small studies demonstrated that urea increases PNa. However, these studies were retrospective, lacked a control group, included small numbers of patients, used a formulation of urea that is not available in the United States, and did not examine whether the effect of urea on raising PNa translates into a reduction in morbidity and/or mortality. In 2016, Ure-Na™, a novel formulation of oral urea became available in the United States. The FDA considers urea to be a medical food, and therefore, does not require a medical prescription for its use. Our group published the first study on the efficacy of this agent for the treatment of hyponatremia in the United States.(4) We identified patients hospitalized at UPMC with PNa <135 mmol/L who received urea, including a subgroup with SIADH who received urea

as the sole drug therapy for hyponatremia ('urea-only' patients). Overall, 58 patients received urea (7.5-90 g/day) over a median of 4.5 days and demonstrated an increase in PNa from 124 to 131 mmol/L (p<0.001). Among 12 'urea-only' treated patients, PNa increased from 125 to 131 mmol/L (p=0.001) with a larger increase in PNa by 24 h (2.5 mmol/L [IQR 0-4.5] vs. -0.5 mmol/L [IQR -2.5 to 1.5], p=0.04) and more patients normalized PNa (33% vs. 8%, p=0.08) compared to a control group. No patients experienced overly rapid correction of PNa and no serious adverse events were reported. While our study was retrospective and limited to hospitalized patients, the findings support the potential efficacy and safety of this agent. After the publication of our study, two other observational studies were published supporting the efficacy, safety, and tolerability of oral urea in hyponatremia.(28, 29) Just one past study compared urea to other therapies for hyponatremia. Soupart et al. compared urea to vasopressin antagonists in 13 patients with chronic SIADH.(30) Patients were treated with vaptans (satavaptan and tolvaptan) for 1 year, during which PNa increased from 125±3 mmol/L to 135±3 mmol/L. Vaptans were then discontinued, leading to recurrent hyponatremia. After an 8-day washout period, oral urea was prescribed for 1 year with normalization of PNa in all patients (mean PNa 135±2 mmol/L). Patients tolerated urea well with no significant side effects. To date, there have been no studies comparing urea to fluid restriction.

Effect of therapy for chronic hyponatremia on patient-centered outcomes

Prior studies suggest that treatment of chronic hyponatremia may improve key patient-centered outcomes. A meta-analysis by Corona et al comprising 15 studies and 13,186 patients found that treatment of hyponatremia was associated with reduced mortality (OR=0.57 [0.40-0.81], p=0.002).(31) Vandergheynst et al. prospectively studied 11 elderly patients with mild hyponatremia (PNa 127.7±2.5 mmol/L) due to SIADH and found a significant improvement in mobility measured by 'Up and Go' test times after PNa normalization with fluid restriction and urea.(32) Peripheral nerve conduction velocities also improved significantly with correction of hyponatremia, but muscle strength did not change. Finally, in a study of 19 patients with hyponatremia, treatment that included salt tablets, fluid restriction, and/or discontinuation of the offending medication was associated with correction in PNa from 128.8±3.9 mmol/L to 133.5±3.5 mmol/L and improved clinical symptoms and neurocognition.(33) Collectively, these studies suggest that correction of chronic hyponatremia results in improvements in patient centered outcomes; yet they underscore the clear need for further investigation of this issue.

Summary

Chronic non-severe hyponatremia is prevalent, and while seemingly asymptomatic, is associated with significant morbidity and mortality. Current therapies have important limitations that preclude their widespread use. Our group demonstrated that a novel formulation of urea with a cost of $3.70 per 15-g dose appears to be effective in raising PNa. However, evidence of its efficacy for the prevention of serious, adverse events related to hyponatremia is lacking. Past studies suggest that correcting chronic hyponatremia could potentially have beneficial effects on patient-centered outcomes. This pilot proposal seeks to assess the feasibility of recruitment, acceptability of urea to ambulatory patients, and proof of concept on the efficacy and safety of urea that are necessary to justify and inform the design of an adequately powered, randomized, placebo-controlled clinical trial of urea for the prevention of serious, patient-centered outcomes related to chronic non-severe hyponatremia.

1.4 SIGNIFICANCE

By demonstrating the feasibility of recruiting patients, documenting patient adherence, and establishing a signal of efficacy to increase PNa and potentially improve neurocognitive function and postural control and stability, this pilot study will generate the data needed to justify and

inform the design of an adequately powered, multi-center, randomized, placebo-controlled clinical trial to investigate whether therapy with urea decreases morbidity, mortality, and costs. This proposal will bring together a multi-disciplinary group of investigators with expertise in the diagnosis and treatment of hyponatremia (Drs. Rondon and Sterns), assessment of neurocognition and posture control/stability (Dr. Connaboy and Dr. Flanagan), clinical trial design and implementation (Drs. Weisbord and Palevsky), and biostatistics (Dr. Yabes). Establishing a track record of collaboration among this group of investigators will greatly strengthen future efforts to extend this line of investigation to a much larger numbers of patients. Assuming that as many as 1.2% of the overall adult population has chronic hyponatremia and that half or more of these patients could be treated with urea, as many as 2 million patients nationwide could
ultimately benefit from this novel and inexpensive intervention.

2. RESEARCH DESIGN AND METHODS

2.1 CLASSIFICATION AND METHODOLOGICAL DESIGNS

The proposed pilot study is designed as a prospective, cross-over trial. Over a 13.5-month period, we will recruit 30 outpatients with chronic non-severe hyponatremia (PNa 125 to 132 mmol/L) confirmed to be due to SIADH. Subjects will not be withdrawn from any known effective therapy for chronic hyponatremia for the purpose of participating in this study. Following enrollment, patients will be randomly assigned on 1:1 ratio to one of two sequence groups. Patients assigned to group “Urea ON, then Urea OFF” will receive oral urea for 42 ± 3 days (period 1), followed by a 10 ± 4-day period in which all patients will be off urea (washout period). Patients then will be off urea for 42 ± 3 days (period 2). Patients assigned to group “Urea OFF, then Urea ON” will be off urea therapy for 42 ± 3 days (period 1), and following a 10 ± 4-day washout period then they will initiate urea for 42 ± 3 days (period 2). Patients will have PNa assessed at baseline and on days 7 ± 2, 14 ± 2, and 42 ± 3 during periods 1 and 2 of the study and will undergo neurocognitive and postural control and stability testing on days 0 and 42 of each of the study periods. All patients will be advised to restrict their fluid intake to ≤1.2 liters during periods 1 and 2.

2.2 DETAILED DESCRIPTION OF STUDY DESIGN

Table 1 – Study assessments

Assessment	Day 0	Day 7	Day 14	Day 42
Phone interview		X	X	
In person clinic assessment:	X			X
SF-12	X			X
Neurocognitive assessment	X			X
Postural stability assessment	X			X
BMP	X	X	X	X
Plasma osmolality, uric acid	X			X
24h urine Cr, volume, urea nitrogen, uric acid, osmolality, Na, K	X			X

Baseline (day 0) assessments:

The baseline study visit will take place at the University of Pittsburgh Neuromuscular Research Laboratory. All patients will provide a blood sample to measure a basic metabolic panel, plasma osmolality, and plasma uric acid. The 24h urine collection (started the day before) will be sent for measurement of volume, creatinine, urea nitrogen, uric acid, osmolality, sodium, and potassium. Patients will complete the short form 12 (SF-12) to assess health-related quality of life. They will then undergo assessments of neurocognition and postural control and stability (See Appendix). Specifically, the neurocognition assessment will involve the Perception-Action Coupling Task (PACT), an affordance-based assessment conducted on an iPad, which uses matched pairs of 'virtual' balls and 'virtual' holes to assess patients' ability to accurately assess their action boundaries. Additional neurocognitive testing will involve administration of six measures from the Senaptec Sensory Station™ test battery to examine separate sensorimotor elements including; multiple object tracking, reaction time, perception span, go/no go, depth perception and dynamic visual acuity. Postural control and stability will be determined using both static and dynamic representations of upright stance. Employing non-linear analysis (sample entropy), the complexity and control of the static representation of postural stability in quiet upright stance will be determined. In addition, dynamic postural stability will be assessed using the NeuroCom™ Sensory Organization and Motor Control Tests. These tests enable both the examination of postural control and stability in response to a direct perturbation of the control systems underlying the maintenance of upright posture (vestibular, somatosensory, and visual), giving insight into the relative contributions and/or any deficits in the sensorimotor systems involved in maintaining upright stance in dynamic situations.

Randomization:

Patients will be randomized to either group "Urea ON, then Urea OFF" or "Urea OFF, then Urea ON" in a 1:1 allocation scheme. We will generate a randomization list using permuted blocks of random block sizes of 4 and 6. Our systems analyst will load the list into the web-based data management structure to preserve allocation concealment for consecutively enrolled participants.

Intervention (oral urea) and study period 1:
Patients randomized to study group "Urea ON, then Urea OFF" (N=15) will begin 42 ± 3 days of urea therapy, while those randomized to study group "Urea OFF, then Urea ON" (N=15) will not receive urea during this period. All patients will be asked to restrict their fluid intake to ≤1.2 L/day during period 1. We will provide participants with a protocol for recording their daily fluid intake which includes carefully measuring all ingested fluids with a measuring cup and recording them on a study diary during the entire study period. We will use the new American formulation of oral urea (i.e., Ure-Na™), which is packaged as a powder that is mixed with 4 oz. of water for oral consumption. Group "Urea ON, then Urea OFF" patients will be given 48 powder pouches of Ure-Na (15 g of urea per pouch) and will be instructed to start at a dose of 15 g once daily. Dose titration will be based on the absolute increase in PNa on days 7 ± 2 and 14 ± 2 as follows:

- Day 7: If PNa increases ≥3 mmol/L from baseline, then continue same urea dose. If PNa increases <3 mmol/L from baseline, then increase urea to next dose.
- Day 14: If PNa increases ≥ 6 mmol/L from baseline, or PNa increases <6 mmol/L from baseline and PNa ≥135 mmol/ L, then continue same urea dose. If PNa increases <6 mmol/L from baseline and PNa <135 mmo/L, then increase urea to next dose.

The dosing scheme for urea will involve increasing when appropriate, from the starting dose of 15 g/day, to 30 g/day in 2 divided doses, and subsequently, when appropriate, from 30 g/day to 60 g/day (in 2 divided doses). The maximal dose of urea administered will be 60 g/day. Patients will undergo a PNa check approximately 7 ± 2 days after a urea dose adjustment to less than the maximal dose of urea allowed. Patients will record the doses of urea they consume each day during treatment period 1 in the study diary.

Follow-up research assessments:

All patients in group "Urea ON, then Urea OFF" will have scheduled follow up assessments on days 7, 14, and 42 as follows:

- Day 7: Phone interview and basic metabolic panel (BMP).
- Day 14: Phone interview and BMP. More boxes of Ure-Na will be mailed if needed to complete the study.
- Day 42: same assessment as in the baseline study visit

The phone interviews visits will focus on assessing side effects, tolerability, and adherence to urea and as appropriate, dose titration of urea. Side effects and tolerability will be assessed with questionnaires (See Medication Side Effects Questionnaire and Medication Acceptability Questionnaire). Patient also will be asked to bring their study diary to the in-person clinic visits to track their adherence to fluid restriction and urea. Visits at day 0 and day 42 will take place in the Neuromuscular Research Laboratory at the University of Pittsburgh. BMP assessment on days 7, 14 will take place at the UPMC clinical laboratory of patient's choice. At the 42-day visit, patients will be asked to return their study diary and any urea doses they did not consume. During period 1 of the study, patients randomized to group "Urea OFF, then Urea ON" will undergo same assessments as outlined for group "Urea ON, then Urea OFF", although they will not be asked about tolerability or adherence to urea as they will not be on this treatment during this period.

Crossover and study period 2:

Upon completion of period 1, all patients (groups "Urea ON, then Urea OFF" and "Urea OFF, then Urea ON") will enter a 10 ± 4-day washout period during which they will not take urea. Patients will be advised that they do not need to restrict their fluid intake during this 10 ± 4-day period. Subsequently, all patients will start the second 42 ± 3-day phase of the study (period 2). Patients in group "Urea OFF, then Urea ON" will be started on urea for 42 ± 3 days using the same titration protocol as described for group "Urea ON, then Urea OFF" during period 1. Patients in group "Urea ON, then Urea OFF" will continue off urea for the duration of period 2 and will complete the same plasma and urine assessments and clinic visits as group "Urea OFF, then Urea ON". All patients will be instructed to limit fluid intake to ≤1.2 liters per day during this final study period. At the conclusion of the study, all patients will be offered the opportunity to visit the UPMC Kidney Clinic for continuation of urea therapy.

Research activities

Screening visit

- Patient will come in person to the UPMC Kidney Clinic.

- For Nephrology inpatients who meet eligibility for the study and have not started urea in the hospital, we will obtain informed consent during their hospitalization and schedule them for a screening visit within approximately 5-7 days after discharge. For Nephrology inpatients who meet eligibility for the study including a nadir serum sodium of no less than 125 mmol/L and have already started urea in the hospital, we will obtain informed consent during hospitalization, discuss with the nephrologist and/or hospitalist, we will ask them if the patient can come off urea and if so, to discontinue urea. We will then schedule these patient for a screening visit within 5-7 days after discharge. Screening visit for subjects enrolled from inpatient will take place at UPMC Kidney Clinic.
- For Nephrology outpatients who meet eligibility for the study including a nadir serum sodium of no less than 125 mmol/L and are already on urea, we will obtain informed consent, discuss with the nephrologist, we will ask them if the patient can come off urea and if so, to discontinue urea. We will then schedule these patients for a screening visit within 5-7 days after urea discontinuation. Screening visit for subjects enrolled from the outpatient setting will take place at UPMC Kidney Clinic.
- The principal investigator will obtain informed consent from the patient to be enrolled in the study.
- Patient will undergo a physical examination by principal investigator which involves: assessment of patient's ability to walk normally without help, assessment of patient's cognition with the use of the Mini-Mental State Examination test, and assessment of volume status
- The research coordinator will draw approximately 14 mL of patient's blood and send the specimen promptly to the UPMC Presbyterian clinical laboratory for processing and analysis of the following: basic metabolic panel, plasma cortisol, thyroid stimulating hormone, and plasma osmolality..
- The research coordinator will ask the patient to provide a urine sample. The research coordinator will send the specimen promptly to the UPMC Presbyterian clinical laboratory for processing and analysis of the following: urine osmolality, urine sodium, and urine pregnancy test when indicated.
- After the principal investigator analyzes the results of the physical exam and laboratory test results and determines patient eligibility for the study, the research coordinator will call the patient to inform him/her whether he/she is eligible to participate in the study.
- For patients with serum cortisol level less than 15 mcg/dL in the screening visit then we will proceed to schedule a corticotropin stimulation test (cosyntropin stimulation test) at the UPMC Montefiore Clinical and Translational Research Center.
- If patient is eligible to participate in the study then the research coordinator will give the patient instructions about the first study visit as well as mail him/her a container with instructions to collect urine for 24 hours prior to first study visit.
- If patient is not eligible to participate in the study then the principal investigator will give the patient the choice to follow up in the UPMC Kidney Clinic for the management of his/her hyponatremia.
- This visit will last approximately 1.5 hours.

First study visit (Day 0 of Period 1)

- Patient will come in person to the University of Pittsburgh Neuromuscular Research Laboratory located at 3860 S Water St, Pittsburgh, PA 15203.

- Patient will bring the container with the 24-hour urine collection he/she started the day prior. The research coordinator will send the specimen promptly to the UPMC Presbyterian clinical laboratory for processing and analysis of the following: creatinine, urea nitrogen, uric acid, osmolality, sodium, and potassium.
- The research coordinator will draw approximately 14 mL of patient's blood and send the specimen promptly to the UPMC Presbyterian clinical laboratory for processing and analysis of the following: basic metabolic panel, plasma osmolality, and plasma uric acid.
- Patient will complete SF-12 survey with pen and paper.
- A co-investigator will test patients' reactions and decision making using specialized software on a tablet computer.
- A co-investigator will test patients' balance while standing on a specialized piece of equipment. Patient will be asked to stand on both legs on top of a platform in a dynamic moving environment. Patient will go through six conditions, with three trials per condition, lasting about 20 seconds each. Patient will be asked to look forward, and stand as motionless as possible with your arms at your sides. The six conditions include the possibility of moving surroundings or moving base of support. If patient move or lose his/her balance during the procedures, patient will be asked to repeat that part again. Patient will be supported by means of a harness in the equipment to make sure he/she cannot fall over while performing the tests.
- The research coordinator will instruct the patient to restrict his/her fluid intake to no more 1200 mL/day for the next 42 ± 3 days. The research coordinator will provide the patient with a diary to record daily fluid intake.
- Patients will be randomized prior to or at the first study visit (Period 1 Day 0) following completion of baseline assessments. Our biostatistician will generate the randomization list using permuted blocks of random block sizes of 4 and 6 and our systems analyst will load the list into RedCAP. To preserve allocation concealment for consecutively enrolled participants, we will use the randomization module in RedCAP to assign each participant 1:1 to either On-to-Off Urea sequence or Off-to-On Urea sequence.
- If patient is randomized to take On-to-Off Urea sequence then the research coordinator will give the patient 48 urea powder pouches with instructions on how to take the urea every day. The research coordinator will provide the patient with a diary to record patient's daily urea intake.
- This visit will last approximately 2.5 hours.

Second study visit (Day 7 of Period 1)

- The research coordinator will call the patient on the phone to ask some questions about any new symptoms patient might have experienced since his/her last visit.
- The research coordinator will direct the patient to go the UPMC clinical laboratory of the patient's choice that day to have his blood drawn for analysis of a basic metabolic panel.
- If patient is taking urea, we might call the participant on the phone again to ask to change the dose of urea depending on the sodium level results.
- This visit will last approximately 20 minutes.

Third study visit (Day 14 of Period 1)

- The research coordinator will call the patient on the phone to ask some questions about any new symptoms patient might have experienced since his/her last visit.
- The research coordinator will direct the patient to go the UPMC clinical laboratory of the patient's choice that day to have his blood drawn for analysis of a basic metabolic panel.
- The research coordinator will instruct the patient to bring his fluid and urea intake diaries to the next study visit.
- The research coordinator will give or mail the patient a container with instructions to collect urine for 24 hours prior to next study visit.
- If patient is taking urea then research coordinator will give and/or mail the patient enough urea powder pouches to complete his/her participation in this period of the study.
- If patient is taking urea, we might call the participant on the phone to ask to change the dose of urea depending on the sodium level results.
- If urea dose is adjusted to less than the maximum allowed dose, research coordinator will direct the patient to go the UPMC clinical laboratory of the patient's choice in approximately 7 days to have his/her blood drawn for analysis of a basic metabolic panel.
- This visit will last approximately 30 minutes.

Reminder telephone call

- The research coordinator will call the patient on the phone 48-72 hours before the next study visit to remind him/her of the upcoming study visit.

Fourth study visit (Day 42 of Period 1)

- Patient will come in person to the University of Pittsburgh Neuromuscular Research Laboratory located at 3860 S Water St, Pittsburgh, PA 15203.
- The research coordinator will collect the patient's fluid and urea intake diaries.
- The research coordinator will ask the patient some questions about any new symptoms patient might have experienced since his/her last visit.
- If patient is taking urea then the research coordinator will ask patient some questions related to acceptability of urea.
- Patient will bring the container with the 24-hour urine collection he/she started the day prior. The research coordinator will send the specimen promptly to the UPMC Presbyterian clinical laboratory for processing and analysis of the following: creatinine, urea nitrogen, uric acid, osmolality, sodium, and potassium
- The research coordinator will draw approximately 14 mL of patient's blood and send the specimen promptly to the UPMC Presbyterian clinical laboratory for processing and analysis of the following: basic metabolic panel, plasma osmolality, and plasma uric acid.
- Patient will complete the SF-12 survey with pen and paper.
- A co-investigator will test patients' reactions and decision making using specialized software on a tablet computer.
- A co-investigator will test patients' balance while standing on a specialized piece of equipment. Patient will be asked to stand on both legs on top of a platform in a dynamic moving environment. Patient will go through six conditions, with three trials per condition, lasting about 20 seconds each. Patient will be asked to look forward, and stand as motionless as possible with your arms at your sides. The six conditions

include the possibility of moving surroundings or moving base of support. If patient move or lose his/her balance during the procedures, patient will be asked to repeat that part again. Patient will be supported by means of a harness in the equipment to make sure he/she cannot fall over while performing the tests.

- The research coordinator will ask patient to stop fluid restriction.
- If patient is taking urea then the research coordinator will instruct the patient to stop taking urea and return all the urea pouches he/she did not use to the research coordinator.
- The research coordinator will provide the patient with a container with instructions to collect urine for 24 hours prior to next study visit.
- This visit will last approximately 2.5 hours.

Reminder telephone call

- The research coordinator will call the patient on the phone 48-72 hours before the next study visit to remind him/her of the upcoming study visit.

Fifth study visit (Day 0 of Period 2)

- Patient will come in person to the University of Pittsburgh Neuromuscular Research Laboratory located at 3860 S Water St, Pittsburgh, PA 15203.
- Patient will bring the container with the 24-hour urine collection he/she started the day prior. The research coordinator will send the specimen promptly to the UPMC Presbyterian clinical laboratory for processing and analysis of the following: creatinine, urea nitrogen, uric acid, osmolality, sodium, and potassium.
- The research coordinator will draw approximately 14 mL of patient's blood and send the specimen promptly to the UPMC Presbyterian clinical laboratory for processing and analysis of the following: basic metabolic panel, plasma osmolality, and plasma uric acid.
- Patient will be given the SF-12 survey to complete with pen and paper.
- A co-investigator will test patients' reactions and decision making using specialized software on a tablet computer.
- A co-investigator will test patients' balance while standing on a specialized piece of equipment. Patient will be asked to stand on both legs on top of a platform in a dynamic moving environment. Patient will go through six conditions, with three trials per condition, lasting about 20 seconds each. Patient will be asked to look forward, and stand as motionless as possible with your arms at your sides. The six conditions include the possibility of moving surroundings or moving base of support. If patient move or lose his/her balance during the procedures, patient will be asked to repeat that part again. Patient will be supported by means of a harness in the equipment to make sure he/she cannot fall over while performing the tests.
- The research coordinator will instruct the patient again to restrict his/her fluid intake to no more 1200 mL/day for the next 42 ± 3 days. The research coordinator will provide the patient with a new diary to record daily fluid intake.
- If patient was randomized to take Off-to-On Urea sequence then the research coordinator will give the patient 48 urea powder pouches with instructions on how to take the urea every day. The research coordinator will provide the patient with a new diary to record patient's daily urea intake.
- This visit will last approximately 2.5 hours.

Sixth study visit (Day 7 of Period 2)

- The research coordinator will call the patient on the phone to ask some questions about any new symptoms patient might have experienced since his/her last visit.
- The research coordinator will direct the patient to go the UPMC clinical laboratory of the patient's choice that day to have his/her blood drawn for analysis of a basic metabolic panel.
- If patient is taking urea, we might call the participant on the phone again to ask to change the dose of urea depending on the sodium level results.
- This visit will last approximately 20 min.

Seventh study visit (Day 14 of Period 2)

- The research coordinator will call the patient on the phone to ask some questions about any new symptoms patient might have experienced since his/her last visit.
- The research coordinator will direct the patient to go the UPMC clinical laboratory of the patient's choice that day to have his blood drawn for analysis of a basic metabolic panel.
- The research coordinator will instruct the patient to bring his fluid and urea intake diaries to the next study visit.
- The research coordinator will give or mail the patient a container with instructions to collect urine for 24 hours prior to next study visit.
- If patient is taking urea then research coordinator will give and/or mail the patient enough urea powder pouches to complete his/her participation in this period of the study.
- If patient is taking urea, we might call the participant on the phone to ask to change the dose of urea depending on the sodium level results.
- If urea dose is adjusted to less than the maximum allowed dose, research coordinator will direct the patient to go the UPMC clinical laboratory of the patient's choice in approximately 7 days to have his/her blood drawn for analysis of a basic metabolic panel.
- This visit will last approximately 30 minutes.

Reminder telephone call

- The research coordinator will call the patient on the phone 48-72 hours before the next study visit to remind him/her of the upcoming study visit.

Eighth study visit (Day 42 of Period 2)

- Patient will come in person to the University of Pittsburgh Neuromuscular Research Laboratory located at 3860 S Water St, Pittsburgh, PA 15203.
- The research coordinator will collect the patient's fluid and urea intake diaries.
- The research coordinator will ask the patient some questions about any new symptoms patient might have experienced since his/her last visit.
- If patient is taking urea then the research coordinator will ask patient some questions related to acceptability of urea.
- Patient will bring the container with the 24-hour urine collection he/she started the day prior. The research coordinator will send the specimen promptly to the UPMC

Presbyterian clinical laboratory for processing and analysis of the following: creatinine, urea nitrogen, uric acid, osmolality, sodium, and potassium.

- The research coordinator will draw approximately 14 mL of patient's blood and send the specimen promptly to the UPMC Presbyterian clinical laboratory for processing and analysis of the following: basic metabolic panel, plasma osmolality, and plasma uric acid.
- Patient will be given the SF-12 survey to complete with pen and paper.
- A co-investigator will test patients' reactions and decision making using specialized software on a tablet computer.
- A co-investigator will test patients' balance while standing on a specialized piece of equipment. Patient will be asked to stand on both legs on top of a platform in a dynamic moving environment. Patient will go through six conditions, with three trials per condition, lasting about 20 seconds each. Patient will be asked to look forward, and stand as motionless as possible with your arms at your sides. The six conditions include the possibility of moving surroundings or moving base of support. If patient move or lose his/her balance during the procedures, patient will be asked to repeat that part again. Patient will be supported by means of a harness in the equipment to make sure he/she cannot fall over while performing the tests.
- The research coordinator will instruct patient to stop fluid restriction.
- If patient is taking urea then the research coordinator will instruct the patient to stop taking urea and return all the urea pouches he/she did not use to the research coordinator.
- The principal investigator will give the patient the choice to follow up in the UPMC Kidney Clinic to continue the management of his/her hyponatremia.
- This visit will last approximately 2.5 hours.

2.3 STUDY DRUG

The drug to be administered is urea (brand name Ure-Na™). Urea is a medical food or dietary supplement considered by the FDA under the GRAS (Generally Regarded As Safe) category. Patients will be instructed to start urea at a dose of 15 g once daily. Dose titration then will occur based on the absolute increase in PNa on days 7 and 14 as follows:

- Day 7: If PNa increases ≥3 mmol/L from baseline, then continue same urea dose. If PNa increases <3 mmol/L from baseline, then increase urea to next dose.
- Day 14: If PNa increases ≥ 6 mmol/L from baseline, or PNa increases <6 mmol/L from baseline and PNa ≥135 mmol/ L, then continue same urea dose. If PNa increases <6 mmol/L from baseline and PNa <135 mmo/L, then increase urea to next dose.

The dosing scheme for urea will involve increasing when appropriate, from the starting dose of 15 g/day, to 30 g/day in 2 divided doses, and subsequently, when appropriate, from 30 g/day to 60 g/day (in 2 divided doses). The maximal dose of urea administered will be 60 g/day. The route of administration will be oral and the total duration of administration will be 42 ± 3 days.

Oral urea use is occasionally associated with the onset of nausea, vomiting, diarrhea and headaches. If these symptoms appear then they will be initially managed by asking patients to monitor their symptoms and report to the research coordinator if symptoms persist after

24h. If symptoms persist, urea dose will be reduced by 50%. If symptoms persist 72h after reducing the dose of urea, urea will be discontinued.

Subjects will be seen at the UPMC kidney clinic immediately after the completion of their participation in the study for consideration of continuation of urea therapy.

2.3.1. Study Drug Preparation and Dispensing

The study drug will be donated by Nephcentric, the manufacturer of Ure-Na™. Ure-Na™ is formulated as a powder packaged in a 21-g pouch or sachet. Each pouch of Ure-Na™ contains 15 g of pharmaceutical grade urea and 6 g of other ingredients including natural flavors, citric acid, maltodextrin, calcium silicate, and sucralose. The powder is then mixed with 3 to 4 oz. of water or juice for oral consumption. No on-site preparation of the study drug will be performed. Patients will be given 6 boxes of Ure-Na™ each containing eight pouches (total of 48 pouches) and instructed to take the study drug at home as indicated. Some more pouches of Ure-Na™ will be given at day 14 as necessary to complete their participation in the study.

2.3.2. Drug Administration

During the study visit at day 0, patients will be given six boxes of Ure-Na™ each containing eight pouches (total of 48 pouches) to take home. Some more pouches of Ure-Na™ will be given at day 14 as necessary to complete their participation in the study. Subjects will be instructed to take 1 pouch (15 g of urea) per mouth once daily. Subjects will be instructed to carefully open the pouch, pour all the content of the pouch into a glass or cup, add 3 to 4 oz of water, stir, and drink after a meal to decrease gastrointestinal intolerance. Subjects will be told that the dose of urea will be titrated throughout the study based on plasma sodium levels and/or the appearance of side effects and this will be communicated during the study assessments by the research coordinator. In addition, subjects will be instructed to store the study drug at home at room temperature, in a dry place, out of direct sun-light.

2.3.3. Dose Selection

Urea, also known as Carbamide, it is an organic compound with formula CH_4N_2O. This amide has two amino groups (-NH2) joined by a carbonyl (C=O) functional group. Urea is an endogenous product of protein and amino acid catabolism. Oral urea works as an osmotic diuretic that increases urinary water excretion and has been available for use in Europe for the treatment of hyponatremia for many years. Preliminary evidence of the potential efficacy of urea derives from small European case series.(1) Decaux et al.(3) studied 7 patients with chronic hyponatremia and found that treatment with urea over a period of up to 9 months resulted in an increase in mean PNa from 116 to 136 mmol/L and was not associated with any major side effects. Another study by Decaux et al.(27) of 50 patients with mild to moderate hyponatremia (PNa 120 to 134 mmol/L) found that 2 days of treatment with urea increased PNa by 7±4 mmol/L. Collectively, these and other small studies demonstrated that urea increases PNa. However, these studies were retrospective, lacked a control group, included small numbers of patients, used a formulation of urea that is not available in the United States, and did not examine whether the effect of urea on raising PNa translates into a reduction in morbidity and/or mortality. In 2016, Ure-Na™, a novel formulation of oral urea became available in the United States. The FDA considers urea to be a medical food, and therefore, does not

require a medical prescription for its use. Our group published the first study on the efficacy of this agent for the treatment of hyponatremia in the United States.(4) We identified patients hospitalized at UPMC with PNa <135 mmol/L who received urea, including a subgroup with SIADH who received urea as the sole drug therapy for hyponatremia ('urea-only' patients). Overall, 58 patients received urea (7.5-90 g/day) over a median of 4.5 days and demonstrated an increase in PNa from 124 to 131 mmol/L ($p<0.001$). Among 12 'urea-only' treated patients, PNa increased from 125 to 131 mmol/L ($p=0.001$) with a larger increase in PNa by 24 h (2.5 mmol/L [IQR 0-4.5] vs. -0.5 mmol/L [IQR -2.5 to 1.5], $p=0.04$) and more patients normalized PNa (33% vs. 8%, $p=0.08$) compared to a control group. No patients experienced overly rapid correction of PNa and no serious adverse events were reported. While our study was retrospective and limited to hospitalized patients, the findings support the potential efficacy and safety of this agent. After the publication of our study, two other observational studies were published supporting the efficacy, safety, and tolerability of oral urea in hyponatremia.(28, 29) Just one past study compared urea to other therapies for hyponatremia. Soupart et al. compared urea to vasopressin antagonists in 13 patients with chronic SIADH.(30) Patients were treated with vaptans (satavaptan and tolvaptan) for 1 year, during which PNa increased from 125±3 mmol/L to 135±3 mmol/L. Vaptans were then discontinued, leading to recurrent hyponatremia. After an 8-day washout period, oral urea was prescribed for 1 year with normalization of PNa in all patients (mean PNa 135±2 mmol/L). Patients tolerated urea well with no significant side effects. To date, there have been no studies comparing urea to fluid restriction.

Our study propose to use the oral route for urea administration. Pharmacological urea currently exist in topical and oral formulations. The oral formulation allows for systemic absorption and renal excretion causing the desired pharmacological effect (i.e. osmotic diuresis). The initial dose of urea to be administered during our study will be 15 g/day but this can be titrated in subsequent study visits based on efficacy and safety. The next dose in our titration algorithm is 30 g/day (in 2 divided doses), and the next and maximal dose allowed will be 60 g day (in 2 divided doses). The European Clinical practice guideline on diagnosis and treatment of hyponatraemia(26) recommended a urea dose of 0.25 – 0.5 g/kg/day which for a 60 kg person constitutes between 15 and 30 g of urea per day. Our study using Ure-Na™ at the University of Pittsburgh Medical Center(4) showed that patients were treated with doses ranging from 7.5 g/day up to 90 g/day, however most patients required doses between 15 and 30 g. The rationale behind the divided doses with doses of 30 or 60 g/day of urea is double. Urea's half-life is 2.5 h and a single dose of urea is completely eliminated via the kidneys within 12 h. Also, higher doses of urea are more likely to cause gastrointestinal side effects. Therefore, to achieve a sustained effect while minimizing adverse reactions a twice daily dose of urea will be more appropriate. While past observational studies demonstrated a potential benefit of increasing plasma sodium on neurocognitive outcomes within 1 week (8, 32, 33), we have increased the treatment duration to 42 ± 3 days to ensure a longer period of plasma sodium normalization that captures the potential beneficial effects of urea on these outcomes.

For a summary of the information related to the human pharmacokinetics and human safety profile of urea, as well as findings from non-clinical studies that support the evaluation of urea in humans, please, refer to: *Toxicological Review of Urea by the United Stated Environmental Protection Agency (EPA).*

2.3.4. Treatment Period

42 ± 3 days

2.3.5. Breaking the Blind

The proposed clinical study will not be blinded.

2.3.6. Medication Compliance

Patients will record the doses of urea they consume each day during treatment period in a study diary which will be reviewed during study visits.

We will be assessing patient compliance with urea as described above so patients will not be withdrawn due to non-compliance with the study drug. If patients are non-compliant with serum sodium checks such that the PIs believe the patient should not continue to take urea, we will formally discuss the case with the study team and if it is agreed by all members of the study team that the patient be withdrawn, they will be asked to stop study drug, return remaining doses, and will be formally withdrawn from the study

Subjects withdrawn from the study will be replaced. We will continue recruitment until the target number of patients are enrolled.

2.3.7. Medication Storage and Accountability

The UPMC Investigational Drug Services (IDS) will be utilized in this study. They are a separate pharmacy who only handles investigational drugs. Each drug is stored in a separately labeled bin in a temperature controlled environment. All files and drug are kept in a locked pharmacy and only IDS staff has access. Only PI's and Co-I's that we list on the order form are able to prescribe the investigational product.

IDS creates a study binder to maintain accountability. The IDS staff will log every time there is a dispense or they receive medication. The IDS staff will maintain patient accountability keeping a separate tab in the binder for each patient enrolled. The IDS staff will file all invoices for the investigational product.

At the end of the study, all the unused investigational product shall be transferred to the IDS accountability and destruction. The investigational product returns will be accounted for by both the study team and pharmacy. The study team will provide the first count followed by a second count performed by the IDS staff. The IDS staff will reconcile the count returned with the research coordinator and document in the accountability log. After reconciliation the investigational product will be immediately discarded into the appropriate waste stream containers.

2.3.8 Concomitant Medications

During their participation in the study, subjects will not be allowed to take the following medications:

- Vasopressin antagonists: tolvaptan, conivaptan

- Thiazide and thiazide-like diuretics: hydrochlorothiazide, chlorothiazide, bendroflumethiazide, chlorthalidone, indapamide, metolazone
- Osmotic diuretics: mannitol
- Carbonic anhydrase inhibitors: acetazolamide, topiramate
- Desmopressin
- Demeclocycline

Subjects' medical history will be reviewed during every subject's study visit looking for changes in health status, hospitalizations, and new prescription drugs. In addition, subjects will be asked about the use of any new medication (prescription or non-prescription) during every study visit including the ones listed above.

2.3.9 Rescue Medications

No rescue medications will be used during this study.

2.4 STUDY ENDPOINTS

2.5 STATISTICAL ANALYSIS

2.5.1 Sample Size Determination

This pilot study aims to assess the feasibility of conducting a future clinical trial. Thus, the sample size of 30 patients was determined based on clinical and logistical reasons rather than statistical requirements. For Aim 1, we anticipate identifying approximately 300 potentially eligible patients during the 13.5 months of recruitment (~22 per month). A 95% confidence interval (CI) for the randomization rate provides a 3% margin of error (MoE) assuming the true rate was 10% (~3 per month). If the underlying dropout and adherence rates were 80%, a sample size of 30 provides 14% MoE for 95% CIs. For Aim 2, our sample size provides 83% power to detect a standardized mean difference of 0.65 for our efficacy outcomes. We are not powered to detect smaller effect sizes. However, since this is a proof-of-concept study, detecting a moderate-to-large effect size increases our confidence in the potential efficacy of urea. For Aim 3, underlying adverse events rates of 5% to 10%, 95% CIs will provide 8% to 11% MoE, respectively.

2.5.2 Study Conduct Analysis

At the conclusion of the study, we will assess the achievement of our aims including the target number of patients.

2.5.3 Efficacy Analysis

This study is not powered to establish the efficacy of urea on increasing PNa or improving neurocognition and/or postural control/stability. However, to justify a future large, randomized clinical trial of urea, we need proof of concept data that urea has potential benefits for these outcomes. We will summarize patient demographics and clinical characteristics by study group. For the primary outcomes (PNa, neurocognition scores, postural control/stability scores) and secondary exploratory outcome (SF-12 scores), we will calculate change from baseline to day 42 in each treatment period. We will analyze these data using a linear mixed effects model with patient-specific random effects to account for within-patient correlations. Model predictors will include treatment,

period and treatment period interaction. Coefficients and 95% CIs will be used to quantify the treatment effect. We will also use this model to assess period effect and carryover effects (treatment period interaction).

2.5.4 Safety Analysis

This pilot study is not designed to comprehensively identify low frequency adverse events. Nonetheless, we will report all adverse events/side effects during urea therapy. We will report to the sponsor the seriousness and severity of each event and whether the event was potentially related to urea. We recognize that osmotic demyelination syndrome is a complication of overly rapid correction of PNa (increase in PNa >8 mmol/L/24h) in patients with severe hyponatremia (PNa ≤120 mmol/L). However, we do not believe this is a concern in our study as patients with PNa ≥125 mmol/L who do not have serious liver disease (an exclusion criterion for our study) have a negligible risk of osmotic demyelination syndrome if overly rapid correction of hyponatremia occurs.

2.5.5 Handling Missing Data

The extent and reasons that data are missing will be described. A comparison of baseline characteristics and intermediate outcomes between those who complete the study and those who do not will be conducted. We will investigate the randomness of missing data using available information on patient characteristics to help discern patterns in the missing data and to identify the possible covert missing data mechanisms. The primary efficacy analytic approach (mixed models) can handle data that are ignorable missing (i.e., either missing completely at random or missing at random). However, strategies to handle missing data (single/multiple imputation, selection models, pattern-mixture models, etc.) and sensitivity analyses to encompass different scenarios of assumptions will be considered as appropriate

2.5.6 Data Management

Standardized clinical data collection protocols will be in place for the clinical trial as documented in a study manual of procedures (MOP). The Principal Investigator (PI) will ensure that the study is conducted according to the protocol and will be responsible for carrying out the Data Safety and Monitoring Plan (DSMP). Regular study team meetings will be used to ensure that all data quality and IRB policies and procedures are being followed. The CRHC-DC will help the PIs develop the forms and setting up the study options in REDCap (Research Electronic Data Capture) then support it for the life of the study. Access will be restricted to certain study staff members.

<u>Data collection and form development</u>
Each case report form (CRF) will be developed by CRHC-DC in conjunction with the clinical study team. In order to make sure that all data elements are collected, CRFs will be considered across the following categories: 1) screening & baseline information; 2) follow up visits, tests, and procedures; 3) adherence to study treatment; 4) adverse experiences; 5) clinical endpoints; and 6) subject treatment and follow up. This allows discussions on CRF development to center around each of the categories and prevents particular forms from being missed. To minimize missing data, all of the study forms will have certain key fields that are required before form submission. Pop-up messages will also be used to remind the study coordinator or data entry personnel that particular fields

are empty upon form submission and give reasons as to why incomplete data was submitted.

Data entry
Data will be entered electronically via The REDCap password-protected web-based data entry system, but paper versions of forms will be provided for manual entry in case of technical issues. The data will be stored on University of Pittsburgh Clinical and Translational Science Institute (CTSI) REDCap servers.

Participant eligibility, & randomization
The data entry process will begin during the online participant enrollment into the clinical trial. The study coordinator will have the ability to generate a participant ID upon initiation. The data capture system will utilize an "eligibility checklist" which is pre-populated with information from all questions that directly relate to inclusion and exclusion criteria. By not having a separate form with checkboxes for each criterion, this will prevent any data entry errors which may result in ineligible randomizations. Once the eligibility criteria are met and confirmed, the study coordinator will submit the randomization form for the subject, and the REDCap randomization module will return the participant's study group assignment ("Urea ON, then Urea OFF" versus "Urea OFF, then Urea ON" sequence).

Data quality control
The CHRC-DC has several systems programmers and data managers who will design and maintain the data entry/checking in REDCap, generate reports, and provide technical support for data entry personnel. REDCap validation rules such as setting acceptable range of values will be used to ensure data entry quality. Audit trails for tracking data manipulation will be used to ensure data integrity.

Data management, security, and confidentiality
Study data will be collected and managed using REDCap electronic data capture tools hosted at The University of Pittsburgh. REDCap is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability.

Access to data will be controlled through password and authentication policies. Only approved individuals will have access to data. The CRHC-DC biostatistical group uses current versions of SAS, R, and Stata for all data manipulation, statistical analyses, and reporting with set guidelines for code documentation and reproducibility. In addition, the statisticians use M-Plus and StatXact for specific types of analyses or simulations.

3. HUMAN SUBJECTS

3.1 SUBJECT POPULATION

3.1.1 Inclusion of Women and Minorities

For the proposed study, there are no exclusions based on gender, race, ethnicity, socioeconomic status, religion or sexual orientation. All patients with chronic non-severe hyponatremia who are seen at UPMC ambulatory clinics will be eligible for enrollment.

We will include both men and women, as well as all minority populations in this pilot study as they present to ambulatory clinics. However, it is expected that among these patients, we will have higher participation among women given the slightly higher prevalence of hyponatremia among women. By virtue of recruiting from an urban/suburban population in/around Pittsburgh, we anticipate that our study population will mirror the demographic population of the greater Pittsburgh area. Overall, it is anticipated that approximately 17 subjects will be female, based on prior studies of hyponatremia. Likewise, based on prior studies and considering the population of the greater Pittsburgh area, we predict that approximately 20 subjects will be White, 5 will be African American, and 5 will be of Hispanic ethnicity.

3.1.2 Inclusion of Children

This pilot study will provide preliminary data on the effectiveness, safety and tolerability of urea for the treatment of chronic hyponatremia in patients' age 18 years old and older. No patients less than age 18 will be studied for several reasons. First, there is very limited data available on clinical outcomes of pediatric hyponatremia or the safety of urea in children with hyponatremia. Second, no one under the age of 18 receives care at the sites of enrollment. Third, the waiver of HIPPA needed to screen adult patients would not be approved by the Institutional Review Boards at the University of Pittsburgh due to the special status of minors as a vulnerable population, deemed to require a higher level of protection than adults in similar circumstances. Finally, the Principal Investigators and co-Investigator Dr. Palevsky are adult nephrologists and do not care for patients less than age 18. For these reasons, the use of urea in pediatric hyponatremia should be studied separately and is beyond the scope of the current proposal.

3.1.3 Inclusion of Prisoners

No prisoners will be included in this study.

3.2 INCLUSION CRITERIA

a. Age ≥18 years
b. Attended ≥1 visit at a UPMC outpatient clinic within the prior 12 months
c. Chronic hyponatremia with a history of ≥ 2 sequential plasma sodium concentration (PNa) between 125 mmol/L and 132 mmol/L performed ≥ 14 days apart within the last 18 months with most recent PNa ≤ 132 mmol/L prior to screening
d. Patients are ambulatory without the need for any assist device (e.g., cane, walker)
e. Mini-mental state examination (MMSE) score ≥ 25
f. Diagnosis of SIADH established by the Bartter and Schwartz criteria as follows:
   - Hyponatremia with a PNa between 125 mmol/L and 132 mmol/L
   - Plasma osmolality < 275 mOsm/kg
   - Clinical euvolemia
   - Urine osmolality > 100 mosm/kg
   - Urine Na ≥ 20 mmol/L
   - Intact adrenal function (i.e., morning plasma cortisol value ≥15 µg/dL, or negative corticotropin stimulation test)
   - Normal thyroid stimulating hormone level (i.e., TSH between 0.3 to 5 µIU/mL)
   - eGFR ≥ 45 ml/min/1.73 m2)

g. Nephrology patients who meet eligibility for the study including a nadir serum sodium of no less than125 mmol/L and have already started urea and treating physician agrees to discontinuing prescribed urea.

3.3 EXCLUSION CRITERIA

a. Cirrhosis and/or end-stage liver disease
b. Heart failure on diuretics and/or with recorded left ventricular ejection fraction <40%
c. Chronic kidney disease with most recent estimated glomerular filtration rate < 45 ml/min/1.73m2
d. Adrenal insufficiency
e. Untreated hypothyroidism
f. Urinary tract obstruction within the prior 2 months
g. Uncontrolled hyperglycemia (most recent random plasma glucose ≥ 200 mg/dL)
h. Ongoing drug treatment for hyponatremia with vaptans or combination of loop diuretics and salt tablets.
i. Active malignancy (not on remission)
j. Active infection
k. Neurological disorders with impairment of ambulation and/or cognition
l. End-stage lung disease with marked impairment in ambulatory capacity
m. Chronic pain with impairment of ambulation and/or cognition
n. Chronic nausea
o. Hypersensitivity to urea
p. Women who are pregnant, breast feeding, or of childbearing potential who are not using contraception
q. Patient is unable to consent for himself/herself

4. IRB APPROVAL AND FDA AMENDMENTS

The Investigator will obtain, from the University of Pittsburgh Institutional Review Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the Investigator will promptly notify the University of Pittsburgh IRB of the deviation. The Investigator should also notify the sponsor of this event.

The University of Pittsburgh IRB operates in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (CGP).

In the event that the University of Pittsburgh IRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of the Investigator's decision to modify the previously accepted clinical protocol:

- for a Phase 1 clinical study: The Sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to the Phase 1

clinical protocol that significantly affects the safety of the subjects. For changes that do not affect critical safety assessments, the revisions to the clinical protocol will be addressed in the Annual Report to the IND.

- for Phase 2 and 3 clinical studies: The Sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to a Phase 2 or Phase 3 protocol that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study. Examples of Phase 2 and 3 clinical protocol changes requiring the submission of a Protocol Amendment include:

    - Any increase in drug dosage or duration of exposure of individual subjects to the investigational drug beyond that described in the current protocol, or any significant increase in the number of subjects under study.

    - Any significant change in the design of the protocol (such as the addition or deletion of a control group).

    - The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or adverse event; or the dropping of a test intended to monitor the safety of the investigational drug.

5. RECRUITMENT AND INFORMED CONSENT PROCEDURES

5.1 RECRUITMENT METHODS:

To identify study patients, we will conduct three sequential processes.

First stage: after obtaining IRB approval, we will use a University of Pittsburgh Health Records Research Request service patient-generated list, the Pitt+Me University of Pittsburgh research registry, self-referrals, direct patient referrals from other physicians, and review of medical records of Nephrology outpatients at the UPMC Kidney Clinic and Nephrology inpatients at UPMC Presbyterian hospital, UPMC Shadyside hospital, UPMC Magee-Womens hospital, and UPMC Mercy hospital to identify patients who meet the following criteria:

- Age ≥18 years
- Attended ≥1 visit at a UPMC outpatient clinic within the prior 12 months
- Evidence of chronic hyponatremia defined by ≥2 sequential PNa measurement between 125 mmol/L and 132 mmol/L performed ≥ 14 days apart within the prior 18 months.
- Most recent PNa measurement ≤132 mmol/L

Second stage: this will involve electronic medical record reviews of patients identified above to confirm that the aforementioned inclusion criteria were met and to exclude those patients with any of the following:

- Cirrhosis and/or end-stage liver disease
- Heart failure on diuretics and/or with recorded left ventricular ejection fraction <40%
- Chronic kidney disease with most recent estimated glomerular filtration rate < 45 ml/min/1.73m2
- Adrenal insufficiency

- Untreated hypothyroidism
- Urinary tract obstruction within the prior 2 months
- Uncontrolled hyperglycemia (most recent random plasma glucose≥200 mg/dL)
- Ongoing drug treatment for hyponatremia with vaptans or combination of loop diuretics and salt tablets
- Active malignancy (not on remission)
- Active infection
- Neurological disorders with impairment of ambulation and/or cognition
- End-stage lung disease with marked impairment in ambulatory capacity
- Chronic pain with impairment of ambulation and/or cognition
- Chronic nausea
- Hypersensitivity to urea
- Women who are pregnant, breast feeding, or of childbearing potential who are not using contraception
- Patient is unable to consent for himself/herself

For all patients meeting these inclusion and exclusion criteria, we will contact their primary care provider (PCP) to discuss the study and their patients' potential eligibility. We will ask PCPs for permission to mail a letter to their patient on the PCP's behalf that introduces the study and describes how the patient can contact our study team if they are interested in participating. The letter will also state that if patients do not contact our team within 2 weeks, we will call them to inquire about their interest in participating in the study. This process of identifying and contacting potential study subjects has been deemed acceptable by the UPMC Institutional Review Board.

For Nephrology outpatients and inpatients, if the patient is not known to a study team clinician, the study team will request a clinician known to the subject ask the subject if a research team member may approach. Upon confirmation from the clinician that the subject is interested, the study team will approach.

Third stage: For patients who are interested in potentially participating, we will proceed with the third step. This will involve an in-person visit to obtain written informed consent from the patient to perform a physical examination and laboratory testing to confirm the presence of hyponatremia and to establish the etiology as SIADH. This informed consent will also cover patient participation in the study (patients who fail this screening testing will have their consent to participate withdrawn).

For Nephrology inpatients who meet eligibility for the study and have not started urea in the hospital, we will obtain informed consent during their hospitalization and schedule them for a screening visit within approximately 5-7 days after discharge. For Nephrology inpatients who meet eligibility for the study including a nadir serum sodium of no less than 125 mmol/L and have already started urea in the hospital, we will obtain informed consent during hospitalization, discuss with the nephrologist and/or hospitalist, we will ask them if the patient can come off urea and if so, to discontinue urea. We will then schedule these patient for a screening visit within 5-7 days after discharge. This will ensure that if urea is discontinued, that the process is approved and implemented by the treating physician and that the patient will have a follow up plasma sodium checked within approximately 5-7 days after urea discontinuation. We are specifically limiting the inclusion of Nephrology inpatients, including those who are on urea in the hospital, to those with plasma sodium levels no less than 125 mmol/L to avoid enrolling patients with plasma sodium levels that are more severely reduced.

For Nephrology outpatients who meet eligibility for the study including a nadir serum sodium of no less than 125 mmol/L and are already on urea, we will obtain informed consent, discuss with the nephrologist, we will ask them if the patient can come off urea and if so, to discontinue urea. We will then schedule these patients for a screening visit within 5-7 days after urea discontinuation. Screening visit for subjects enrolled from the outpatient setting will take place at UPMC Kidney Clinic.

The screening physical examination will confirm that patients: 1) are ambulatory without the need for any assist device (e.g., cane, walker) in order to comply with study postural control and stability assessments; 2) have intact cognition based on a mini-mental state examination (MMSE) score ≥25 in order to comply with neurocognitive assessment and; 3) are euvolemic in order to meet diagnostic criteria for SIADH. For ambulatory, cognitively intact, euvolemic patients, we will then check a basic metabolic panel, plasma osmolality, plasma uric acid, thyroid stimulating hormone, morning plasma cortisol; and urine osmolality and urine sodium. Patients with a morning plasma cortisol value <15 µg/dL will be considered to have an equivocal result and will need to undergo a standard-dose (250 µg) corticotropin stimulation test to rule out adrenal insufficiency in the UPMC Montefiore Clinical and Translational Research Center. Patients determined to have hyponatremia due to SIADH based on the following laboratory criteria will be eligible to participate:

- PNa between 125 mmol/L and 132 mmol/L
- Plasma osmolality <275 mOsm/kg
- Urine osmolality >100 mOsm/kg
- Urine Na ≥ 20 mmol/L
- Intact adrenal function (i.e., morning plasma cortisol value ≥15 µg/dL, or negative corticotropin stimulation test)
- Normal thyroid stimulating hormone level (i.e.,TSH 0.3-5 µIU/mL)
- Normal kidney function based on eGFR ≥ 45 ml/min/1.73 m2

Patients deemed eligible to participate based on this testing will be notified by the study team and sent a 24-hour urine collection container with instructions to return the sample at the time of the baseline study visit. The study team will also notify patients who do not meet criteria for SIADH that they will not be eligible to participate in the study and will offer them follow up in the UPMC Kidney Clinic for routine clinical care of their hyponatremia.

5.2 INFORMED CONSENT PROCEDURES

After IRB is approved then we will identify patients that meet inclusion criteria (see first stage above) from the University of Pittsburgh Health Records Research Request patient-generated list, the Pitt+Me University of Pittsburgh research registry, direct patient referrals from other physicians, andreview of medical records of Nephrology outpatients at the UPMC Kidney Clinic and Nephrology inpatients at UPMC Presbyterian hospital, UPMC Shadyside hospital, UPMC Magee-Womens hospital, and UPMC Mercy hospital.The electronic medical records of these patients will be reviewed to confirm inclusion criteria and select patients who do not meet exclusion criteria (see second screening stage above). For all patients meeting these inclusion and exclusion criteria, we will contact their primary care provider (PCP) to discuss the study and their patients' potential eligibility as well as ask for permission to mail a letter to their patient on the PCP's behalf that introduces the study and describes how the patient can contact our study team if they are interested in participating. The letter will also state that if patients do not contact

our team within 2 weeks, we will call them to inquire about their interest in participating in the study. This process of identifying and contacting potential study subjects has been deemed acceptable by the UPMC Institutional Review Board. For Nephrology outpatients and inpatients, if the patient is not known to a study team clinician, the study team will obtain permission from a clinical caregiver known to the subject to approach about study participation.
For patients who are interested in potentially participating, we will ask them to come for an in-person visit and the PI will obtain written informed consent from the patients to perform screening tests, including a physical examination, as well as laboratory testing (see third screening stage above). For Nephrology inpatients who meet eligibility for the study and have not started urea in the hospital, we will obtain informed consent during their hospitalization and schedule them for a screening visit within approximately 5-7 days after discharge. For Nephrology inpatients who meet eligibility for the study including a nadir serum sodium of no less than 125 mmol/L and have already started urea in the hospital, we will obtain informed consent during hospitalization, discuss with the nephrologist and/or hospitalist, we will ask them if the patient can come off urea and if so, to discontinue urea. We will then schedule these patient for a screening visit within 5-7 days after discharge. This will ensure that if urea is discontinued, that the process is approved and implemented by the treating physician and that the patient will have a follow up plasma sodium checked within approximately 5-7 days after urea discontinuation. We are specifically limiting the inclusion of Nephrology inpatients, including those who are on urea in the hospital, to those with plasma sodium levels no less than 125 mmol/L to avoid enrolling patients with plasma sodium levels that are more severely reduced.

This informed consent will also cover patient participation in the study. The information communicated as part of the patient participation in the study will include:

- Name of the study
- Name of the Principal Investigators
- Explanation that the study involves research
- Explanation of the purpose of the study
- Explanation of the treatment procedures
- Description of randomization
- Description of the risks and benefits of participation in the study
- Description of alternatives to participation in the study
- Explanation that all records will be kept confidential, but that records may be examined by representatives of the NIH and/or the FDA
- Whom to contact for questions about the research and about subjects' rights
- Whom to contact in the event of a research-related injury
- A statement that participation in the study is voluntary and that a decision not to participate or to withdraw from the study after initially agreeing involves no penalty, loss of benefits, or reduction in access to medical care
- A statement that the treatments provided as part of this study are free
- Payments offered for their participation

There will be no waiting period between informing the prospective participant and obtaining consent. If participants are unable to consent for themselves then they won't be included in the study as this constitutes an exclusion criteria. Patients deemed eligible to participate based on this testing will be notified by the study team within 2 business days. Patients who fail this screening testing will have their consent to participate withdrawn and the study team will notify them and offer them follow up in the UPMC Kidney Clinic for routine clinical care of their

hyponatremia. Subjects will not be informed of the outcome of the research unless they request so in writing.

6. POTENTIAL RISKS AND BENEFITS

6.1 POTENTIAL RISKS

6.1.1 Risk of Experimental Drug Intervention

Overall, this is a low risk study.

Physical risks

Urea is a dietary supplement considered by the US Food and Drug administration under the GRAS (Generally Regarded As Safe) category. Urea is now available for the treatment of hyponatremia in the United States and evidence from case series in Europe and from a recent study by our group suggests that urea is safe and well-tolerated. Nevertheless, some patients might experience distaste, nausea, vomiting, diarrhea, and/or headaches associated with the use of urea. Osmotic demyelination syndrome is a complication of overly rapid correction of PNa (increase in PNa >8 mmol/L/24 hours) in patients with severe hyponatremia (PNa < 120 mmol/L). However, this is not a concern in our study population as patients with a PNa ≥125 mmol/L who do not have serious liver disease (an exclusion criterion for our study) have a negligible risk of osmotic demyelination syndrome if overcorrection of hyponatremia occurs.

Psychological risks
There are no anticipated psychological risks.

No social, cultural, financial or legal risks for the study participants are anticipated. Pregnant women are excluded from this study. However, no birth defect have been observed and currently there is inadequate information to conclude whether urea is mutagenic.

7.1.2 Risk of Study Procedures

- Blood draws: It may cause pain and bruise. Some people may become light-headed (dizzy) or faint after blood drawing. There is also a rare risk of infection at the site of the blood draw
- Fluid restriction: it may cause thirst.
- Neurocognition assessment: no known risks are associated with this study procedure.
- Posture stability assessment: There is a risk of falling while undertaking the balance testing, and this is heighted with increased age. All participants will use a safety harness during posture stability assessment.
- Collection and storing of PHI and Bio specimens: low risk of breach of confidentiality
- Discontinuing clinically prescribed urea in the hospital: This might cause the blood sodium level to decrease. To minimize this risk, we will first obtain approval from the participant's treating physician in the hospital to make sure is safe to do so and schedule participants for the screening visit within 5-7 days of hospital discharge to ensure a blood sodium level will be checked after urea discontinuation. In addition, we are only enrolling participants with a blood sodium level no lower of 125 mmol/L where the risk of experiencing any symptoms related to low blood sodium is very low.

6.2 ALTERNATIVE TREATMENTS

Alternative treatments include fluid restriction alone, loop diuretics with salt tablets, and tolvaptan.

6.3 POTENTIAL BENEFITS

There is no direct benefit to study participants. The results will provide proof of concept data and inform the design of a future randomized controlled trial which may profoundly impact the treatment of patients with chronic hyponatremia. However, the study interventions convey a very low risk in relationship to the therapeutic benefits for future patients with chronic hyponatremia. All patients will be offered the opportunity to be seen in the UPMC kidney clinic after completion of the study for consideration of continuation of urea therapy as part of their routine clinical care.

7. RISKS MANAGEMENT PROCEDURES

7.1 PROTECTION AGAINST RISKS

General Risks of Study Protocol and Procedures
All research interventions/activities will be conducted in private patient care areas. The collection of sensitive information about subjects is limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is being collected. All demographic and clinical information about the subject will be stored in an electronic password-guarded study database under the supervision of the Investigator for this protocol.

The data will be stripped of individual identifiers and stored anonymously with a subject number. Information linking subject identifiers with the coded subject number will be stored under password protection on computers in locked areas, with access only to the database manager. All staff will sign confidentiality statements. Access to the database will be limited to the data manager and staff under the supervision of the Investigator.

All staff involved in this study are properly credentialed and instructed in the areas of testing, confidentiality, and safety.

The investigators will retain the data for the entire period of this study and will retain the specified records and reports for up to two years after the marketing application is approved for the investigational drug; or, if a marketing application is not submitted or approved for the investigational drug, until two years after investigations under the IND have been discontinued and the FDA so notified. The investigators may continue to use and disclose subjects' de-identified information for the purpose of this study for a minimum of seven years after final reporting or publication of the study. If the subject and/or legal representative decide to withdraw or be withdrawn from study participation, they may request that the study data be destroyed. Subject names or other directly identifiable information will not appear on any reports, publications, or other disclosures of clinical study outcomes.

Clinical study data that will be recorded directly on the electronic database include patient demographics, medications, comorbidities, and laboratory data whereupon the electronic data is to be considered Source Data. Source Data are the clinical findings and observations, laboratory and test data, and other information contained in Source Documents. Source documents may include, but are not limited to, hospital records, clinical and office charts,

laboratory notes, memoranda, subjects' diaries or evaluation, checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm, or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at the medico-technical departments involved in the clinical trial. When applicable, information recorded on the CRF shall match the Source Data recorded on the Source Documents.

We will account for any missed, unused, and/or spurious data as follows: the extent and reasons that data are missing will be described. A comparison of baseline characteristics and intermediate outcomes between those who complete the study and those who do not will be conducted. We will investigate the randomness of missing data using available information on patient characteristics to help discern patterns in the missing data and to identify the possible covert missing data mechanisms. The primary efficacy analytic approach (mixed models) can handle data that are ignorable missing (i.e., either missing completely at random or missing at random). However, strategies to handle missing data (single/multiple imputation, selection models, pattern-mixture models, etc.) and sensitivity analyses to encompass different scenarios of assumptions will be considered as appropriate.

7.2 PROTECTION AGAINST POTENTIAL RISKS OF EXPERIMENTAL INTERVENTION

- Involvement of trained staff / investigators with experience in the administration of the study drug
- Continuous monitoring by the Data and Safety Monitoring Board
- Required Education in the Protection of Human Research Participants (CITI Good Clinical Practice Module)

8.0 ADVERSE EVENTS

The proposed study will use the FDA definition of adverse events (AE) and serious adverse events (SAE). Any SAE, which is unexpected and related to study intervention, will be reported immediately to the IRB and FDA and will include all known details regarding the nature of the SAE. In the event that a participant either withdraws from the study or the investigators decide to discontinue a participant due to a SAE, the participant will be monitored by the PI and treating investigator until (a) a resolution is reached (e.g., the problem has resolved or stabilized with no further change expected), (b) the SAE is determined to be clearly unrelated to the study intervention, or (c) the SAE results in death. Outcomes of SAEs not previously reported will be reported to the sponsor, IRB and FDA via a follow-up report. A summary of the SAEs that occurred during the previous year will be included in the FDA annual progress report as well as in the annual IRB renewal.

The severity of adverse changes in physical signs or symptoms will be classified as follows:

- Grade 1 (Mild): asymptomatic or mild symptoms; clinical or diagnostic observation only; intervention not indicated.
- Grade 2 (Moderate): minimal, local or noninvasive intervention indicated; limiting age-appropriate ADL (Activities of Daily Living).

- Grade 3 (Severe): medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care/ADL.

- Grade 4 (Life-threatening): consequences; urgent intervention indicated.

- Grade 5 (Death): event is a direct cause of death.

8.1 REPORTABLE ADVERSE EVENTS

For this study, a serious adverse event is any untoward clinical event that is thought by either the investigator or the sponsor to be unexpected and at least possibly related to the study and results in any of the following:

1. Death
2. A life-threatening adverse event
3. Inpatient hospitalization or prolongation of an existing hospitalization
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. A congenital anomaly or birth defect
6. Important medical events that may not result in death, be life threatening, or require
7. Hospitalization may be considered serious 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 serious outcomes listed above.

If clinically important and unexpected adverse experiences or clinically important study-related adverse experiences occur, they will be recorded on the adverse event case report form.

8.2 ADVERSE EVENTS REPORTING TIMELINES

Life-threatening or fatal unexpected adverse events associated with the use of the study drug or procedures must be reported to the IRB within 24 hours of discovery of the incident with subsequent follow-up submission of a detailed written report.

The FDA will be notified by telephone or facsimile transmission of a human adverse event that is fatal or life-threatening no later than 7 calendar days after receiving the respective human adverse event information, followed by the subsequent submission of a written IND Safety Report.

Serious and unexpected adverse events associated with the use of the study drug or procedures will be reported to the IRB with subsequent follow-up submission of a detailed written report in accordance with the respective policies and procedures of the IRB. Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the investigator-sponsor's receipt of the respective adverse event information.

A summary report of the findings will be prepared and submitted to the regulatory agencies as required.

9. DATA SAFETY MONITORING

9.1 DATA SAFETY MONITORING BOARD

A Data Safety Monitoring Board (DSMB) will be appointed for this study. The DSMB will be composed of 3 members to be named.

9.2 DATA SAFETY MONITORING PLAN

Monitoring of safety and data quality in the proposed study will be the responsibility of all personnel on the project, with primary responsibility and supervision by the Investigator. The Institutional Review Board will approve the Statement of Informed Consent for the study and provide institutional oversight of data and safety issues. The study protocol will be approved prior to recruiting or obtaining consent from any participants. Moreover, the study will be reviewed at a minimum of annual basis (or more frequently as deemed necessary) by the IRB committee. Each participant will sign the Informed Consent Form described above prior to participating in the study. To ensure participant safety, once participants are enrolled in the study, study staff will immediately report all adverse and serious adverse events to one of the Investigators. The Investigator will, per institutional requirements, report them to the IRB for their review. These events should also be communicated to the sponsor of the IND. With regard to monitoring of data quality and protected health information, all required personnel proposed for this project will have the required human subjects and confidentiality training, which includes information about maintaining data integrity and security. Confidentiality will be guarded using established procedures such as storing data in locked cabinets within locked offices or locked data rooms, coding by study identification numbers rather than any personally identifying information to avoid revealing the identity of subjects, and aggregating data across participants. The key linking names and study identification numbers will be kept separately from the data sets with limited access by study personnel. Only study personnel will have access to the data sets on protected servers. In order to maintain the highest standard of data entry quality, all data will be double-entered, with discrepancies highlighted so that they can be reviewed by the project coordinator. Oversight of all aspects of data management will occur with the Investigator.

9.3 PARAMETERS TO BE MONITORED

The following progress will be monitored throughout the course of the research to ensure the safety of subjects as well as the integrity and confidentiality of their data.

- An evaluation of the progress of the research study, including subject recruitment and retention, and an assessment of the timeliness and quality of the data.

- A review of collected data (including adverse events, unanticipated problems requiring reporting and those captured on the non-compliance log, and subject withdrawals) to determine whether there is a change to the anticipated benefit-to-risk assessment of study participation and whether the study should continue as originally designed, should be changed, or should be terminated.

- An assessment of external factors or relevant information (e.g. pertinent scientific literature reports or therapeutic development, results of related studies) that may have an impact on the safety and study participants or the ethics of the research study.

- A review of study procedures designed to protect the privacy of the research subjects and the confidentiality of their research data.

9.4 FREQUENCY OF MONITORING

The Investigator will review subject safety data as it is generated. The Investigator, sub-investigators, and the research staff will meet on a 4-week interval to re-evaluate study goals, subject recruitment, data coding and retention, documentation and identification of adverse events, complaints and confidentiality of subjects. There will be an evaluation of the progress of the research study, including assessments of data quality, timelines, participant recruitment, accrual, and retention. The Investigator will also review the outcome and adverse event data to determine whether there is any change to the anticipated benefit-to-risk ratio of study participation and whether the study should continue as originally designed or should it be re-evaluated and changed.

10. WITHDRAWAL OF SUBJECTS AND STOPPING CRITERIA:

Adverse Events: Oral urea use is occasionally associated with the onset of nausea, vomiting, diarrhea and headaches. If these symptoms appear then they will be initially managed by asking patients to monitor their symptoms and report to the research coordinator if symptoms persist after 24h. If symptoms persist, urea dose will be reduced by 50%. If symptoms persist 72h after reducing the dose of urea, urea will be discontinued. Participant will be asked to return remaining urea doses, and after consultation with all the members of the research team, participant will be formally withdrawn from the study. Data no longer will be collected from the withdrawn subject and subject will be referred to the UPMC kidney clinic for follow up. Subjects withdrawn from the study will be replaced using the regular procedures described for recruitment. We will continue recruitment until the target number of patients are enrolled.

Other Criteria: None

Discontinuation of the Clinical Trial: This does not apply to this study as this is a pilot study that is not designed to assess either efficacy or futility.

11. COSTS AND PAYMENTS

11.1 COSTS

The total budget for the study is $234,750.00.

11.2 PAYMENTS

Patient's time compensation will be $20 per patient per visit. In addition, participants will receive free validated parking during study visits.

12. QUALIFICATIONS AND SOURCE OF SUPPORT

12.1 QUALIFICATIONS OF THE INVESTIGATORS

Helbert Rondon Berrios, MD, MS, FACP, FASN, FNKF (Principal Investigator)

Dr. Rondon Berrios is an Associate Professor of Medicine at the University of Pittsburgh School of Medicine and Program Director of the Nephrology Fellowship Training Program at UPMC Medical Education. Dr. Rondon Berrios is a physician trained in Internal Medicine and Nephrology with over 10 years of clinical experience in both private practice and academia. Within the field of nephrology, Dr. Rondon Berrios has a special interest in fluid, electrolytes, and acid-base disorders with an emphasis on the diagnosis, management, and outcomes of chronic non-severe hyponatremia. Dr. Rondon Berrios has authored several peer-reviewed publications and book chapters on the topic of hyponatremia and has given invited talks on this topic at regional and national meetings. Dr. Rondon Berrios recently served as the primary investigator and author on a retrospective observational study describing the use of urea for the treatment of hyponatremia among hospitalized patients. This was the first study describing the use of this therapy in the United States. In association with Dr. Steven Weisbord who is co-Principal Investigator on this proposal and who has extensive experience conducting clinical trials in the field of nephrology, Dr. Rondon Berrios is uniquely positioned to successfully carry out the proposed research project. In close collaboration with Dr. Weisbord, Dr. Rondon Berrios will oversee all aspects of this study including, obtaining IRB approval, hiring and training the study coordinator, enrolling patient participants, ensuring compliance with the study protocol, overseeing data collection and analysis, and dissemination of study results.

Steven D. Weisbord, MD, MSc, FASN (Co-Principal Investigator)

Dr. Weisbord is a Professor of Medicine (with Tenure) and Clinical and Translational Sciences at the University of Pittsburgh School of Medicine and Staff Nephrologist and clinical investigator at the VA Pittsburgh Healthcare System. Dr. Weisbord has conducted two multicenter clinical trials. Most recently, Dr. Weisbord was the Study Chairman and Principal Investigator of the Prevention of Serious Adverse Events Following Angiography (PRESERVE) study, a multicenter, randomized, VA clinical trial that compared interventions for the prevention of serious adverse outcomes among high-risk Veterans undergoing angiography. Dr. Weisbord was also Principal Investigator of the NIH-supported Biomarker Collection and Analysis in the PRESERVE Trial Cohort study, which involved the collection and banking of blood and urine specimens from PRESERVE trial participants for analyses of specific kidney biomarkers. With Dr. Weisbord's experience as a Principal Investigator on prior clinical trials, he is uniquely positioned to assist Dr. Rondon Berrios conduct the proposed pilot study. Specifically, Dr. Weisbord will assist Dr. Rondon Berrios on all aspects of the proposed research, including patient screening and recruitment, data collection, data analysis, presentation of study results, and dissemination of study findings.

Paul M. Palevsky, MD, FASN (Co-Investigator)

Dr. Palevsky is a Professor of Medicine (with Tenure) and Clinical and Translational Sciences at the University of Pittsburgh School of Medicine and chief of the Renal Section and clinical investigator at the VA Pittsburgh Healthcare System. Dr. Palevsky's primary research interests are in the areas of acute kidney injury, critical care nephrology, chronic kidney disease and acute and chronic dialysis. Dr. Palevsky has helped design and conduct multiple large, multicenter, randomized controlled trials including the VA/NIH Acute renal failure Trial Network (ATN) study (VA CSP #530; NCT000769219) for which he was the study chair; the VA Nephropathy in Diabetes (NEPHRON-D) study (VA CSP #565; NCT00555217) for which he was on the planning and executive committees; the EUPHRATES trial, evaluating the safety and efficacy of polymyxin B hemoperfusion in patients with endotoxemia and septic shock (NCT01046669) for which he was a member of the study executive committee; the Prevention of Serious Adverse Events Following Angiography (PRESERVE) trial (VA CSP #578;

NCT01467466) for which he was the co-principal investigator and study co-chair; and the ongoing Stop GOUT (Comparative Effectiveness in Gout: Allopurinol vs. Febuxostat; VA CSP #594; NCT02579096) for which he was a member of the planning committee and is a member of the study executive committee. Dr. Palevsky has also been a co-investigator for the Protocolized Goaldirected Resuscitation of Septic Shock to prevent AKI (ProGReSS-AKI) study (1R01DK083961), an ancillary study to the recently completed Protocolized Care for Early Septic Shock (ProCESS) trial (NCT00510835), which evaluated the effect of protocolized goal-directed therapy in sepsis on the development and long-term outcomes of AKI and he is one of three principal investigator for Phenotyping Renal Cases In Sepsis and surgery for Early Acute Kidney Injury (PReCISE AKI), the Pittsburgh AKI recruitment site for the NIDDK Kidney Precision Medicine Project (1UG3 DK114861-01). Among other research roles, he is a member of the OSMB for the Chronic Renal Insufficiency Cohort (CRIC) study, served as the chair of the DSMB for the NIDDK's Hemodialysis Novel Therapies (HDNT) Consortium, is a member of the DSMB for the Optimal Management of HIV Positive Adults at Risk for Kidney Disease in Nigeria study and is a member of the EPP for the United States Renal Data System. With his experience as a Principal Investigator on prior clinical trials, Dr. Palevsky is uniquely positioned to assist Dr. Rondon Berrios and Dr. Weisbord conduct the proposed project.

Christopher Connaboy, PhD (Co-Investigator)

Dr. Connaboy is an Assistant Professor in the Department of Sport Medicine and Nutrition, working within the Neuromuscular Research Laboratory and Warrior Human Performance Research Center. Dr. Connaboy completed his PhD in Biomechanics and Motor Control and his MSc in Biomechanics at the University of Edinburgh. Prior to coming to the University of Pittsburgh, Dr. Connaboy worked at the University of Houston, TX and Edinburgh Napier University, Scotland. During Dr. Connaboy's time at Edinburgh Napier University, he led the successful development of a collaborative, interdisciplinary research group: The Military and Veterans Health Research Consortium (MVHRC). The MVHRC is a network of academic partners from around Scotland, the UK, and USA. During his tenure as Director of MHVRC, Dr. Connaboy oversaw the inclusion of the MVHRC as a preferred supplier on the Ministry of Defense/Defense Science and Technology Laboratories Human Capabilities Research Program, from which they were successful in receiving over £1,000,000+ of research funding. Prior to undertaking his academic career Dr. Connaboy was an enlisted soldier in the Black Watch, Royal Highland Regiment in the UK Armed Forces. As a researcher, he has expertise in human performance optimization with a specific focus on movement, coordination and the perceptuo-motor processes involved in performing skilled actions in occupational settings, and for both elite warfighters and athletes. Dr. Connaboy has received funding from the U.S. Department of Defense (ARMY, NAVY, AIR FORCE and MARINES), NASA, and the UK Ministry of Defense. Therefore, Dr. Connaboy is well positioned to provide Dr. Rondon Berrios and Dr. Weisbord the expertise in the measurement of neurocognition and postural stability for the proposed project.

Shawn Flanagan, PhD (Co-investigator)

Dr. Flanagan is an Assistant Professor of Sports Medicine and Nutrition, and his overall research agenda emphasizes neural contributions and adaptations related to human performance, resilience, and injury. He has a broad background in neuroscience and physiology with specific training in brain stimulation, imaging, endocrinology, and physical exercise. I use multimodal and complementary neuroimaging, perturbational, biochemical, and electrophysiological techniques combined with ethologically-relevant behavioral assays to better understand and improve human brain-body interactions in health and disease. His research is

interdisciplinary in nature and has involved collaborations with physiologists, biomechanists, neuroscientists, psychologists, biochemists, mathematicians, psychiatrists, nutritionists, and neurologists. As a PI or co-Investigator on several federally-funded grants, his current work includes efforts to better understand psychological and physiological resilience, novel techniques to optimize human performance, the influence of injury on the brain, and biomarkers for performance adaptations and injury. In this capacity, he has successfully administered the projects, cultivated strong collaborations, produced several peer-reviewed publications in leading journals, and developed communication and management practices that facilitate realistic research plans, timelines, and budgets. In summary, He has the expertise, resources, and leadership needed to successfully complete the proposed research, which represents a logical extension of his prior work.

Jonathan G. Yabes, PhD (Co-Investigator)

Dr. Yabes is an Assistant Professor of Medicine at the University of Pittsburgh School of Medicine and a biostatistician at the University of Pittsburgh Center for Research on Health Care Data Center (CRHC-DC), where he works with various investigators in different clinical research projects. Dr. Yabes has been collaborating with the Nephrology division on several projects. Dr. Yabes is currently a co-investigator and biostatistician in several other randomized trials studying interventions for patients on hemodialysis, with chronic kidney disease, insomnia, lower respiratory tract infection, and diabetes. Dr. Yabes also work on health services research projects on urologic oncology using large administrative datasets. Dr. Yabes was a member of the Multidisciplinary Clinical Research Scholars Program advisory committee where he collaborated with junior faculties working on career development proposals. As the study biostatistician, Dr. Yabes will work closely with the study team in overseeing and executing the statistical aspects of trial including randomization, report generation for data monitoring and dissemination, and implementation of the statistical analyses plan. Dr. Yabes has worked with Dr. Rondon Berrios and Dr. Weisbord in developing the study design and statistical aspects of this proposed work. In summary, Dr. Yabes' research experience and rigorous statistical training are well-suited for the biostatistical needs of the proposed work.

12.2 SOURCES OF SUPPORT

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14. APPENDICES

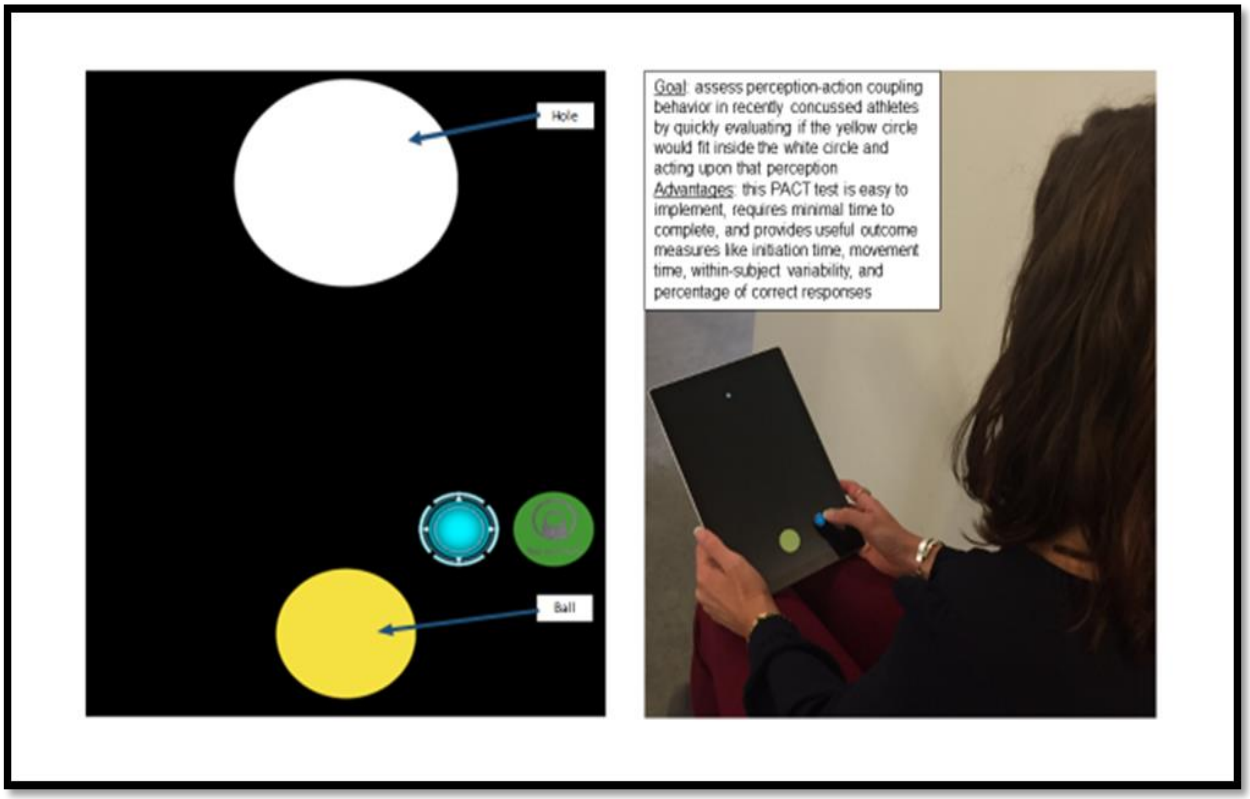
NEUROCOGNITIVE AND POSTURAL STABILITY ASSESSMENT

Neurocognition assessment

Perception-Action Coupling Task (PACT)

The PACT assesses perception-action coupling behavior; specifically, the accuracy of action-boundary perception. The PACT uses matched pairs of 'virtual' balls and 'virtual' holes to assess the subject's ability to accurately and quickly determine if a ball will fit inside a hole, when presented on an iPad screen. Subjects are presented with a series of virtual balls of different sizes and a corresponding virtual hole, also varying in size. The objective of the task is to accurately and quickly judge if the ball will fit into the hole, or not. Subject are seated, with an iPad held in both hands and forearms resting on their thighs and the thumb of their preferred hand resting on the green (home) button on the iPad screen (figure 3). Subjects will be presented with randomly

occurring series of virtual ball: virtual hole parings (see figure 1) over a 15-minute period. Time between the presentation of the virtual ball: virtual hole parings is also random. On presentation of a virtual ball: virtual hole paring, subjects react by moving their thumb from the home button to the blue joystick button. If they perceive the hole aperture (white circle) to be large enough to accommodate the ball (yellow circle) the subjects move the joystick forward towards the hole. If the subject perceives the hole to be too small compared to the size of the ball they move the joystick down, away from the ball. The ball will move in correspondence with the movement of the joystick. After completing the move, subject return their thumb to the home button and await the appearance of the next pairing. This is repeated for a total of 15 minutes. Subject are required to perform 1 cycle trial of the PACT protocol to establish familiarization with the task.
The figure below shows a zoomed in version of the test on a tablet (left), while the right-side photo shows a subject performing the test. The PACT produces multiple scores including: (1) initiation time (IT), (2) within-participant IT variability, (3) movement time, (4) within-participant MT variability, (5) percentage correct responses, (6) aperture ratio, (7) mean IT for correct and incorrect responses, (8) percentage perceived as afforded action, (9) percentage perceived as un-afforded action. Visual representation of the PACT as seen on the tablet screen (left) and a subject performing the test (right)



Senaptec™ Sensory test battery

A total of seven measures from the Senaptec Sensory Station™ test battery will be used to assess sensorimotor abilities. Measures to be assessed include: multiple object tracking (perception span), reaction time (Hand response time), perception span, go/no go, depth perception and dynamic visual acuity (near far quickness).

Multiple object tracking task

It is used to measure the ability to attentively select and successfully track several moving objects. For the first 5 trials, 6 black dots are presented on a white background. Three dots are targets and each target is paired with a corresponding distractor dot. The three target-distractor pairs are located around a cross in the center of a 13.3" tablet display. The user is instructed to track the 3 target dots, which were cued in red for 1 second, then the pairs of the target-distractor pair rotate around each other. The pairs rotate for 5 seconds with 2 reversals at random points in that time span. After the rotation stops, the user selects one dot from each target-distractor pair by using the tablet touchscreen interface. After each of the first 4 trials, the speed is adjusted based on the accuracy of the identification of the targets. The starting rotational speed for the first trial is 500 deg/sec and for the following trials can reach from 120 to 900 deg/sec after gradual adjustments. The threshold speed is defined as the final speed including the adjustment after the 5th trial. The first trial of the last 5 trials starts with 4 pairs with the rotational speed set at 80% of the threshold speed determined from the first 5 trials. Identifying all targets correctly for each trial results in adding another pair of dots for the following trial. If the user does not get all targets correct, a pair is removed for the next trial. The maximum number of pairs for the last 5 trials is 8 pairs and the minimum is 2 pairs.

Reaction time test (Hand response Test)

Subjects will keep an arm's length from the 13'3" tablet computer. Two annular patterns appear on the screen with centers 14.5 cm apart; each annulus consists of 2 concentric circles, 6.4 cm and 3.1 cm in diameter. Automated instructions direct the subject to place the fingertips of both hands on the inner circles of the annulus. If the hand was aligned correctly, the outer circles of both annuli would change color to blue-green. After a randomized delay of 2, 3, or 4 seconds, one of the annuli turns blue-green, and the subject removes the hand on that annulus as quickly as possible. Animation examples are shown, followed by 2 practice trials. Ten trials are conducted per subject to calculate average reaction and response times – 5 dominant, and 5 non-dominant trials presented in random order. Reaction Time is measured as the elapsed time between onset of the test annulus and release of the control annulus. After 10 trials, the computer calculates the averages and standard deviations for the reaction and response times. If any single measure differed from the mean by more than 2 standard deviations in either direction, another trial was conducted to replace the outlying measure for that trial.

Perception Span test

Subject seated with the 13'3" tablet computer located at arm's length. The subject is instructed to focus on a shrinking white dot in the center of a grid pattern of 30 circles arranged into a grid pattern. The disappearance of the white dot triggers the appearance of a pattern of yellow dots which simultaneously flash for 100 milliseconds. The subject is then required to recreate and identify the pattern of yellow dots by pressing on their locations on the touch screen tablet screen. If a pass rate of greater than 75% is achieved the grid pattern is increased, with additional number of potential locations – Levels 1-2: 6 circles in the grid with 2-3 dots, Levels 3-8: 18 circles with 3 to 7 dots, Levels 9-12: 30 circles with 7 to 10 dots presented. The dot pattern at each level is pseudorandomized, to maintain spatial organization/distribution and prevent clustering of dots into recognizable patterns. Examples are shown, followed by two practices trails. The score representing this measures is based on the cumulative number of correct responses, with missed responses and extra (superfluous identifications) subtracted from the cumulative score. Subjects were allowed two attempts to pass each level, if two attempts were taken on the values from the passing score were added to the cumulative score. If the subject fails the second attempt the test is terminated and total calculated.

Go/No Go test

Subjects are presented with a grid of 8 (rows) x 6 (columns) equally spaced circles on the tablet computer. A series of yellow-green or red dots are presented in a pseudorandomized order to maintain spatial distribution and prevent clustering and recognizable patterns of dot presentation. The dots, either yellow-green or red, are presented at random locations on the grid for only 450 milliseconds, and with no time gap between presentations. If a yellow-green dot is presented the subject should touch the screen, if a red dot is presented the subject is instructed not to touch it. If a yellow-green dot is touched a point is awarded, if a yellow-green dot is not touched a point is subtracted, and if a red dot is touched a point is subtracted. Subjects are instructed to touch as many yellow-green dots as possible in the time allotted. Examples are presented prior to taking the assessment, but no practice trials are given. A total of 96 are resented (64 yellow-green, 32 red). The overall score is calculated as the total number of points scored.

Depth perception

Subjects wear a pair of red/blue glasses, creating simulated depth in 1 of 4 black rings presented on an off-white background on the tablet computer. This gives the illusion of one of the rings appearing to float above the screen. The 4 rings are presented simultaneously at 1) 12 o'clock, 2) 3 o'clock, 3) 6 o'clock and 4) 9 o'clock, with one of the rings appear to float. Subjects are instructed to swipe across the screen in the direction of the 'floating ring' as quickly as possible. Examples are shown to the subjects and then 3 practice trials are allowed. Average response time for the testing is used to represent performance.

Dynamic visual acuity (Target Capture): Subjects are instructed to fixate a central white dot on the tablet computer screen until the appearance of a yellow 'Landolt' ring (a circular ring with a missing section) appears briefly in 1 of the 4 corners of the screen. Subjects are required to indicate the location of the missing section of the Landolt ring by swiping across the tablet screen in the direction of the missing section. Examples of the test procedure are given followed by 3 practice trials. If subjects cannot judge the direction of the missing segment of the ring, guessing is encouraged. The Landolt ring is initially presented for 500 milliseconds, the presentation time reducing with successive correct responses. A staircase reversal algorithm is

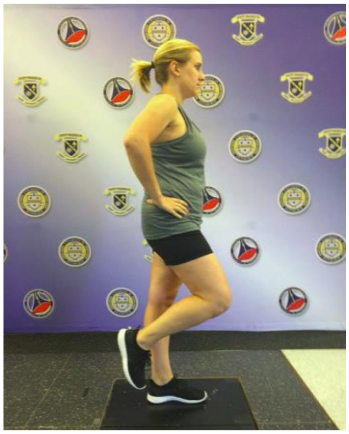
used to discern the threshold exposure duration; and is recorded as the output measure for the dynamic visual acuity.

Near-Far Quickness (accommodative-vergence facility)

In alternating style, a 20/80-equivalent black Landolt ring is presented in a box on the handheld screen, and a black Landolt ring 0.1 log unit above the threshold determined with the Visual Clarity assessment is presented on the far screen. The subject is instructed to swipe the screen of the handheld screen in the perceived direction of the gap in the ring presented on each display; incorrect responses would not change the target presentation. The assessment begins with 3 practice trials. The first Landolt ring was always presented on the far screen. After the correct response is recorded, the Landolt ring appears on the handheld screen. The subject then continually switches focus between far and near for 30 seconds, trying to correctly identify as many rings as possible. The number of correct responses determines the score.

Posture stability assessment

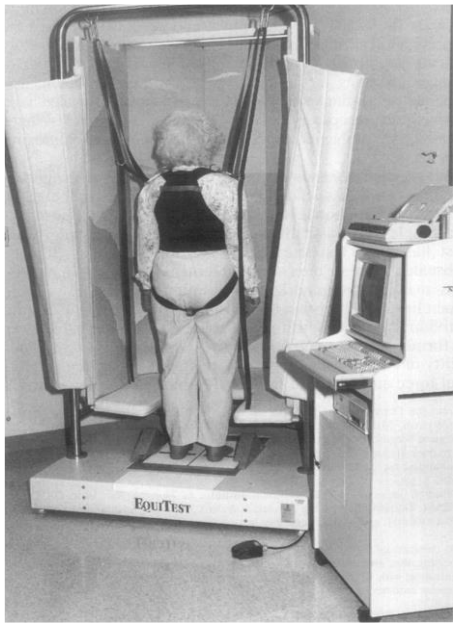
Force Plate Center of Pressure Analysis



To further assess postural stability and control, static balance in single leg and bipedal stance can be recorded for 30 seconds on a Kistler force platform (see figure below). The center of pressure (COP) data is recorded from the force platform in both eyes open and eyes closed conditions. The data produced is analyzed for the min-max excursions in the mediolateral and anteroposterior directions, the total path length, and velocity of the COP trace. In addition the sample entropy of the COP data is examined to determine the signal complexity, providing insight into factors related to postural control.

NeuroCom Smart Balance Master System

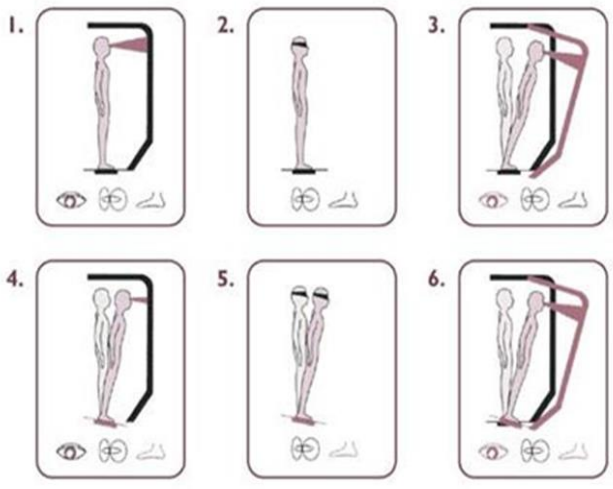
Postural control and stability will be assessed through the use of the Sensory Organization Test and Motor Control Test on the (NeuroCom International, Inc., Clackamas, OR). The NeuroCom (pictured) has been developed to provide an objective assessment of postural stability under static and dynamic test conditions. A variety of tests using the NeuroCom have been developed to objectively test sensory and motor impairments as well as functional limitations. The NeuroCom allows for the unique ability to safely assess each individual sensory system input cues (visual, vestibular, and somatosensory). The NeuroCom tests requires individuals to determine which sensory system cues are appropriate and inappropriate by manipulating the visual surround, support surface, and applying external perturbations. Traditional tests assessing postural stability using force plates are unable to determine which sensory system input cues are being used and/or suppressed. These traditional tests are only capable of identifying if impairment exists, but is unable to provide information regarding why there is impairment. While traditional measures of postural stability using force plates are frequently reported in the literature the underlying sensory systems being utilized to maintain postural stability cannot be identified, whereas, the NeuroCom has the ability to isolate the three sensory sources (somatosensory, vestibular, vision) .



<u>Sensory Organization Test (SOT)</u>

The SOT consists of 6 testing conditions designed to systematically test the visual, vestibular and somatosensory systems. The 6 test conditions consist of the following:

Condition 1: Eyes open, support surface stable;
Condition 2: Eyes closed, support surface stable;
Condition 3: Visual surround moves, support surface stable;
Condition 4: Eyes open, support surface moves;
Condition 5: Eyes closed, support surface moves;
Condition 6: Visual surround moves, support surface moves.

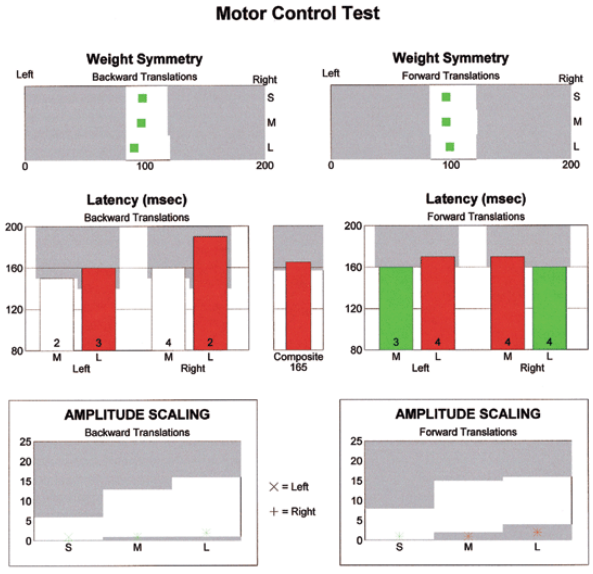


Sensory Organization Test

Subject positioning on the NeuroCom as well as subject instructions will follow the manufacturer's guidelines. During each test condition <u>subjects will wear a harness (designed to prevent fall, but not impede or assist balance)</u> and assume a two-legged stance with feet shoulder width apart on the support surface with their arms hanging down at their side. The verbal cues provided by the manufacturer for each test condition will be provided, for example: during this test (Condition 1) you are to keep your eyes open looking forward with arms hanging down by your side. After subjects verbally state that they understand the test condition and are ready to begin the test will

start. A practice trial will be provided to ensure subjects are comfortable with the test procedures. Each test condition lasts 20 seconds. A 1 minute rest period will be provided between practice trials and test trials. A 30 second rest period will be provided between trials. A total of 3 test trials will be collected for each test condition and averaged for analyses.

Motor Control Test (MCT)



The MCT assesses the ability to quickly recover from an unexpected external translation. The translations occur in the forward and backward directions. During each test condition subjects will wear a harness (designed to prevent fall, but not impede or assist balance) and assume a two-legged stance with feet shoulder width apart on the support surface with their arms hanging down at their side. For each direction (forward and backward) there are 3 translations: small (2.8 degrees/second), medium (6.0 degrees/second) and large (8.0 degrees/second). The verbal cues provided by the manufacturer for each test condition will be provided, for example: during this test condition you are to keep your eyes open looking forward, arms hanging down by your side, and the support surface will translate either forward or backward. After subjects verbally state that they understand the test condition and are ready to begin the test will start. A practice trial will be provided to ensure subjects are comfortable with the test procedures. A 1 minute rest period will be provided between practice trials and test trials. A 30 second rest period will be provided between test conditions (forward and backward). A total of 3 test trials will be collected for each speed (small, medium, large) in both directions (forward, backward) and averaged for analyses.