Physiologic Approach to Sodium Supplementation in Premature Infants (Salt to Grow)

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SECTION 1. ABSTRACT

Study Hypothesis/Question

Aim: To determine the effects on somatic growth (weight, length, head circumference) of adherence to a clinical practice algorithm utilizing urinary sodium concentration to guide sodium supplementation as compared to current treatment practices in preterm infants.

Study Design Type

Prospective, pragmatic, randomized trial comparing a clinical practice algorithm to current treatment practices.

Sample Size

N = 90 patients (Infant and mother dyads)

Eligibility Criteria

Inclusion:

1. Infants with gestational age 25 0/7 – 29 6/7 at birth
2. Birth weight ≥ 500 grams
3. Admitted within the 1st week of life
4. < 17 days of age at time of enrollment

Exclusion:

1. Infants admitted after the 1st week of life
2. Major congenital anomalies
3. Structural genitourinary abnormality
4. Renal dysfunction (serum creatinine > 1.0 mg/dl or an increase of ≥ 0.3 mg/dl between the 2 most recent consecutive measurements) immediately prior to the initiation of study procedures.
5. Diuretic use less than 48 hours prior to initiation of study procedures
6. Infant with an ostomy (infants receiving an ostomy after study entry will be withdrawn)
7. Infant with a diagnosis or suspicion of diabetes insipidus

This study will also include the mother of the infant to collect information from the mother's chart.

Study Intervention/Methods

Beginning on the 14-16th postnatal day and continuing until 36 weeks postmenstrual age, infants randomized to the algorithm will have a spot urine sodium concentration determined every two weeks with additional sodium supplementation provided according to the algorithm.
Assessment of total body water and energy expenditure will be made at 32 weeks corrected age by the doubly-labeled water method on all enrolled patients.

Primary Outcome

The change in somatic growth (weight gain, head circumference, and length) evaluated by the change in Z-score between 2 weeks of age and 36 weeks conceptual age or transfer from NICU.

Secondary Outcomes

1. The change in somatic growth (weight gain, head circumference and length) evaluated by the change in Z-score between 2 weeks of age and discharge or transfer from the NICU or 44 weeks corrected age (whichever occurs first)
2. Total body water and energy expenditure at 32 weeks corrected age.
3. Incidence and severity of bronchopulmonary dysplasia
4. Duration of mechanical ventilation
5. Need for supplemental oxygen
6. Use of diuretic therapy
7. Retinopathy of prematurity

SECTION 2. STATEMENT OF PROBLEM

2.1 PRIMARY HYPOTHESES OR QUESTIONS

Aim 1. To determine the effects on somatic growth of adherence to a clinical practice algorithm guiding sodium supplementation as compared to current treatment practices in preterm infants.

Aim 2. To determine the effects on total body water and energy expenditure of adherence to a clinical practice algorithm guiding sodium supplementation as compared to current treatment practices in preterm infants.

Aim 3. To assess whether adherence to the algorithm impacts the incidence of secondary outcomes including, duration of mechanical ventilation, rates of bronchopulmonary dysplasia, duration of supplemental oxygen, severe retinopathy of prematurity (stage ≥3 or requiring treatment), length of hospitalization, and neurodevelopmental impairment.

Hypothesis 1. Use of this innovative algorithm to direct sodium supplementation will result in improved in-hospital somatic growth (weight, length and head circumference) compared to standard treatment.

Hypothesis 2. Use of the clinical practice algorithm to guide sodium supplementation in premature infants will result in no differences in total body water but decreased energy expenditure compared to the control group.

Hypothesis 3. Use of the algorithm will not negatively impact the incidence of common neonatal morbidities, including duration of mechanical ventilation, rates of bronchopulmonary dysplasia, duration of supplemental oxygen, severe retinopathy of prematurity, length of hospitalization, and neurodevelopmental impairment.

2.2 BACKGROUND AND RATIONAL

Postnatal growth failure is a significant morbidity in very low birth weight (VLBW, <1500 grams at birth) infants. Recent efforts to promote growth and optimize nutritional support in this population have included earlier initiation of parenteral nutrition and increased caloric and protein administration. While these advances in nutritional practices have resulted in improved growth velocity, up to 50% of VLBW infants continue to experience postnatal growth failure (defined as discharge weight <10th percentile by Fenton growth charts[1]) and over 25% experience severe postnatal growth failure (<3rd percentile)[2, 3]. Furthermore, approximately 40% of VLBW infants have a fall in weight Z-score of >1 between birth and discharge. Strong associations have been identified between in-hospital growth failure and impaired short term (18–22 months) and long-term (up to 25 years) neurodevelopment[4-7]. Thus, it is imperative that evidenced-based initiatives be designed to address this major issue impacting the care of preterm infants.

Sodium and Growth

While recent efforts have focused on increasing caloric and protein intake in VLBW infants, little attention has been given to identifying sodium requirements for optimal growth[8, 9]. The need for adequate sodium intake to optimize growth is apparent from studies in animals and humans[10-16]. In young, growing rats, feeding a sodium deficient diet impairs weight and length gain, diminishes nitrogen retention and decreases

muscle protein synthesis and RNA concentrations[16]. Salt supplementation to sodium depleted animals restores normal rates of weight and length growth and protein synthesis[17]. Improved growth resulted from increased efficiency of weight gain per gram of food intake; there was no difference in caloric intake. In a separate study, weanling rats fed low sodium diets for 5 weeks displayed impaired bone growth, fat-free dry weight and total body fat and nitrogen accretion compared to sodium replete animals[11]. Notably, animals provided a broad range of sodium intake (0.1 to 3 times the minimal daily requirement of sodium for normal growth) displayed similar body water content (% body weight) and serum sodium values. Several groups identified that infants with short bowl syndrome at risk for sodium depletion due to intestinal losses displayed improved growth with sodium supplementation and sodium repletion[10, 14, 18].

The mechanisms linking sodium depletion and impaired growth and tissue repair have not been fully defined. It is hypothesized that reduced availability of sodium in the extracellular fluid space and a decreased transmembrane gradient for sodium results in decreased Na^+-H^+ antiporter activity across the cell membrane, increased intracellular H^+ and decreased intracellular pH[13, 19]. In tissue culture, DNA synthesis and the action of growth factors on cell growth is inhibited when the sodium concentration in the culture medium is reduced; and restored when sodium is replaced[20, 21]. Specifically, reduced sodium concentration of culture medium inhibits Na^+-H^+ exchange, decreasing intracellular pH (pHi) and impairing cell growth[20-22]. Ray et al. demonstrated in weanling rats that a sodium-deficient diet results in growth restriction and a decrease in muscle pHi [19]. Thus, there appears to be a strong relationship between sodium availability and its effects on Na^+-H^+ exchange activity, pHi, and cell growth, both *in vitro* and *in vivo*[21, 23-25]. It is therefore not surprising to find that sodium depletion impairs growth during the postnatal period.

Provision of Sodium

Current nutritional recommendations for preterm infants from the American Academy of Pediatrics include enteral or parenteral intake of sodium of 3-5 mEq/kg/d and are based on a factorial approach calculated from fetal accretion of body components[26-28]. This recommendation has not been updated since 1985[29] and fails to take into account the degree of renal immaturity present in extremely preterm infants who are surviving after birth far earlier in gestation now than 30 years ago. Furthermore, many infants fail to receive the currently recommended quantity of sodium. In a review of 70 infants with birth weights less than 1000 grams admitted to a level IV NICU January 2009 through June 2011, only 11% of infants received an average sodium intake of over 3 mEq/kg/d from weeks 2 through 12 of life[30]. Growth velocity in this cohort averaged only 10.7 g/kg/d, well below the goal outlined by the AAP[29]. Delgado, *et al* demonstrated that infants born at 23-31 weeks gestation and receiving the AAP recommended sodium intake do not consistently achieve a state of positive sodium balance (intake > urine sodium losses) until after 32 weeks postmenstrual age[31]. Considering these calculations did not account for non-renal sodium losses or sodium accretion associated with growth, (approximately 1.4 mEq/kg/d at these gestational ages), it is unlikely sufficient sodium was provided at any time point. The University of Iowa Children's Hospital found average daily sodium intake for 26–29 6/7-week gestation infants born during 2014–2015 to be 3.6, 3.8, and 2.9 mEq/kg/d at 4, 6 and 8 weeks of postnatal age, respectively. Thus, even current recommendations, which likely underestimate the needs of many infants, are often not met. Reasons for this likely include that formulas designed specifically for premature infants (Enfamil Premature, 2.5 mEq/100 calories; Similac Special Care, 1.87 mEq/100 calories) fail to meet the higher end of these recommended requirements as does human milk supplemented with commercial fortifiers (2.1 mEq/100 calories, fortification to 24 kcal/oz)[26].

Studies in preterm infants suggest sodium supplementation above that provided in the diet may optimize weight gain. Vanpee *et al* provided 4 mEq/kg/d of supplemental sodium chloride to infants with gestational age 29-34 weeks from 4-14 days of life[32]. At 2 weeks of age, supplemented infants weighed about 6%

above birthweight while non-supplemented infants were approximately 2% below birthweight (<0.01); fluid intake and urine output were similar between groups. Recently, Isemann *et al* reported that sodium supplementation to infants <32 weeks gestation at a dose of 4 mEq/kg/d from days 7–35 of life enhanced weight gain (26.9 $\pm$ 3.1 vs. 22.9 $\pm$ 4.7 g/kg/d)[33]. Importantly, 79% of patients in the supplemented group maintained their Fenton growth curve birth percentile at 6 weeks of age compared to 13% in the non-supplemented group. This study was limited by its small sample size as well as the lack of a patient-focused assessment of the need for supplementation.

Clinical relevance of the study

A primary issue in preterm infants failing to receive adequate sodium intake is the current lack of a clinical measure of total body sodium status. Most neonatologists base the decision to provide supplemental sodium on serum sodium levels (personal communication), however the physiologic basis for this practice is limited given the poor relationship between serum sodium concentration and body sodium content[34-37]. Animal and human studies support that i) sodium deficiency impairs growth; ii) many preterm infants fail to receive sufficient sodium in their diet; iii) there is currently no accepted approach to identifying infants with sodium deficiency; and iv) impaired postnatal growth is associated with impaired neurodevelopmental outcome. Sodium supplementation improves growth of preterm infants, however previous studies were limited in sample size, provided supplementation for only relatively short period of time and failed to identify which infants required supplementation and those in whom supplementation was inadequate. As such, there is a vital need for early identification of those infants requiring sodium supplementation and to develop a rational, physiology-based approach guiding the amount of supplementation. The proposed trial addresses these limitations and gaps in knowledge. Specifically, this pragmatic trial utilizing a sodium supplementation algorithm is powered to address an important, yet underappreciated clinical aspect of nutritional management of preterm infants, and will produce substantial evidence on which to base recommendations to guide sodium supplementation in extremely preterm infants. Results from this trial will have the potential to change clinical practice and result in a paradigm shift in how physicians use dietary approaches to optimize growth and potentially neurodevelopmental outcomes.

Supportive preliminary data

An underappreciated though convenient approach to the assessment of sodium homeostasis is the use of urine sodium concentration measured from a spot urine sample[38]. While evidence is limited, there is general agreement that in patients with normal and mature renal function, urine sodium >30 mEq/L is reflective of positive sodium balance, measurements <30 mEq/L represent total body sodium depletion and measurements <10 mEq/L indicate severe deficit. Limitations of this approach have recently been highlighted as urine sodium excretion is dependent upon diet, fluid consumption and circadian cycle[39]. However, in contrast to older children and adults, there is typically a consistent pattern of provision of diet and fluid to preterm infants, ameliorating some of this concern.

Several studies have now correlated low urine sodium concentration with poor growth during infancy and demonstrated that sodium supplementation to achieve urine sodium >30 mEq/L is associated with improved weight gain[10, 14, 18]. Butterworth *et al* described 39 infants (mean gestational age at birth 32 weeks and birth weight 1.43 kg) with intestinal stomas in place, 92% had total sodium deficiency (defined as urine Na <30 mEq/L) and 64% had severe deficiency (<10 mEq/L)[10]. Sodium repletion and maintenance therapy were governed by a clinical guideline primarily using urine sodium values. On multiple regression analysis, urine sodium but not serum sodium, caloric or fluid intake was significantly associated with weight gain. A separate study of 40 infants <1 year of age with ileostomies reported similar

findings, optimum growth was achieved when urine sodium exceeded 30 mEq/L[14]. These findings highlight the correlation between sodium deficit and impaired growth in infants.

Development of algorithm using urine sodium concentrations. Our novel approach takes advantage of the fact that urine sodium concentration is reflective of total body sodium status. However, in the preterm population such an approach is confounded by renal functional immaturity. Specifically, preterm neonates have a lower glomerular filtration rate (GFR) and higher fractional excretion of sodium (FeNa) compared to term infants[40, 41]. Furthermore, there is ongoing maturation of renal function with postnatal age; GFR and FeNa being inversely correlated with postnatal age[40, 41]. To account for these maturational changes in developing a physiologically based algorithm for guiding sodium supplementation in the preterm population, we used recently published measures of glomerular and tubular function to calculate expected urine sodium concentrations with various and advancing gestational and postnatal ages. Values for weight and length were based on standardized growth charts as well as data from our NICU. Body surface area was calculated according to a standardized formula[42]. Glomerular filtration rate was obtained from the literature[41]. GFR in ml/min was calculated based on estimated GFR in ml/min/1.73 m^2 and estimated body surface area for each gestational and postnatal age. Total filtered volume was calculated from GFR (ml/min) x 1440 min/day. Filtered sodium was calculated using an estimated serum sodium concentration of 135 mEq/L. We purposefully chose a value at the lower limit of normal to avoid overestimating urine sodium losses. Fractional excretion of sodium was based on interpolation and extrapolation of recently published longitudinal data on FeNa in preterm infants less than 28 weeks and 29–31 weeks gestation[40]. Total urine sodium was calculated based on the total amount of filtered sodium and FeNa (filtered sodium x FeNa). The value chosen for urine volume was based on values reported in the literature and verified in our own patient population. Finally, urine sodium concentrations for each gestational and postnatal age were calculated based on total urine sodium and estimated urine volume (UNa (mEq/d)/urine volume (ml/d) x 1000 ml/L).

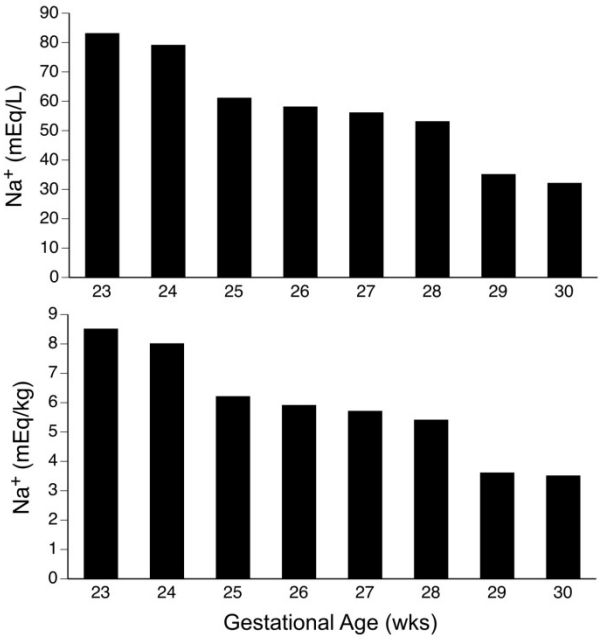

Figure 1. Calculated urine sodium concentration (top) and daily urine sodium losses (bottom) at 2 weeks of age for various gestational ages. Values calculated as described in text.

As expected there is large variation in urine sodium concentration over the ranges of gestational and postnatal age. Figure 1 summarizes the calculated urine Na concentrations (top) and daily urine sodium losses (bottom) at 2 weeks of postnatal age for a range of gestational ages. Not unexpectedly, the urine values consistently equal or exceed the 30 mEq/L value used in children with more mature renal function to signify sufficient body sodium. Furthermore, estimated urine sodium losses often exceeded the 3–5 mEq/kg/d of Na currently recommended by AAP for preterm infants.

Using these data, we developed a novel algorithm to guide sodium supplementation in infants 25–29 6/7 weeks gestation. The algorithm has undergone slight modification since its original development, although changes have been minimal. The algorithm is as follows:

Postnatal age (weeks)	2	3	4	5	6	7	8	9	10	11	12	13	14
GA (birth)													
25 wk	<50		<40		<40		<40		<40		<30		<30
26 - 29 6/7 wk	<40		<40		<40		<40		<30		<30		

i. Values represent urine [Na], expressed as mEq/L, obtained every other week
ii. If urine [Na] is below threshold value, initiate supplementation at 4 mEq/kg/d above current Na intake. Adjust supplementation weekly to account for weight gain
iii. Increase by 2 mEq/kg at subsequent time points if <urine [Na] goal. (Values should not be obtained within 48 hr of use of diuretic agent)
iv. Consider supplementation or determination of urine [Na] if serum [Na] is ≤132 mEq/L prior to first scheduled urine [Na] determination
v. Provide supplementation if serum [Na] ≤132 mEq/L, regardless of urine [Na] unless there is evidence of acute fluid overload (significant increase in weight). Hyponatremia in presence of high urine [Na] likely represents a condition of severe urinary sodium loss leading to hyponatremia
vi. Continue supplementation unless serum [Na] >144 mEq/L
vii. Consider discontinuation of supplementation at 38 weeks postmenstrual age

Initial supplementation of 4 mEq/kg/d was chosen based on experience in the literature[33, 43]. These studies suggest this degree of supplementation overcomes sodium depletion and improves growth without development of hypernatremia.

This algorithm was initiated as a standardized protocol to guide sodium supplementation at the University of Iowa Children's Hospital NICU in 2016 for infants 26 – 29 6/7 weeks gestation. The algorithm was applied to all infants excluding those with underlying renal abnormalities, or other conditions that may affect fluid and electrolyte balance. As expected, there was little relationship between serum and urine sodium concentrations at 2 weeks of postnatal age (Figure 2). Notable, a significant number of infants with "normal" serum sodium concentrations had urine sodium concentrations that we interpreted as being consistent with sodium depletion, based on the algorithm.

To evaluate the impact of adapting the algorithm, data were compared for 50 infants, 26 – 29 6/7 weeks gestation cared for in the 2014-2015, prior to implementation of the algorithm and 40 infants cared for in 2016, after implementation of the algorithm. Results from this study have recently been published[44].

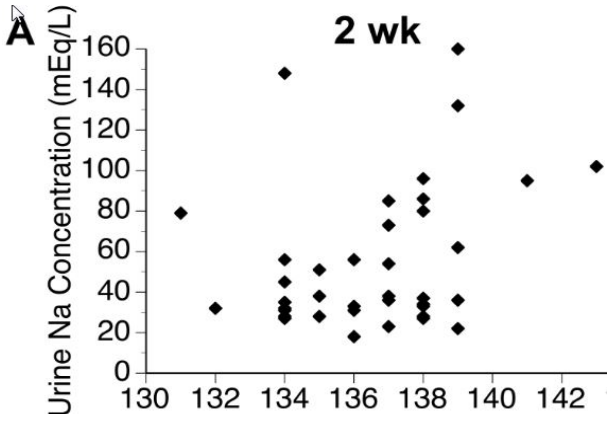

Figure 2. Serum sodium vs. urine sodium concentrations measured at 2 weeks postnatal

Twenty-three (58%) of infants had initial (2-week measurement) urine Na concentration warranting supplementation; while another 7 of infants had low urine sodium concentration] at 4 wk, warranting supplementation. Thus, 75% of infants cared for using this algorithm received sodium supplementation above that in their normal diet. Daily Na intake was similar at 2 wk in both cohorts, but significantly greater ($p < 0.05$) at 4, 6 and 8 wk in the 2016 vs. 2014–2015 cohort (Table). **Weight gain, determined as change in weight Z-score between 2 and 8 wk of age, was significantly greater in 2016 vs. 2014–2015 cohort (0.32 ± 0.06 vs. -0.01 ± 0.08, $p<0.01$)**. Caloric, protein and total fluid intakes as well as daily urine output were similar between cohorts at the measured time points of 2, 4, 6 and 8 weeks of age (Table). Use of the algorithm did not negatively impact respiratory morbidity at either 28 days of postnatal age (or at 36 weeks postmenstrual age (2014-2015 cohort: 60% receiving supplemental oxygen; 2016 cohort: 48% receiving supplemental oxygen). Since initiating use of this algorithm, no infant has required sodium supplementation to be discontinued due to hypernatremia (serum [Na] >144 mEq/L). These data suggest the physiologically based algorithm to individualize identification of need for sodium supplementation in the preterm infant resulted in increased sodium intake and growth in this population. Given the potential benefits of this approach on growth and ultimately neurodevelopmental outcome, a randomized controlled trial to evaluate the efficacy of this approach is needed.

Table. Sodium, caloric and protein intakes at increasing postmenstrual ages

	Postmenstrual Age			
	2 wk	4 wk	6 wk	8 wk
Sodium Intake (mEq/kg/d)				
2014-2015 cohort	3.5 ± 0.2	3.8 ± 0.2	3.8 ± 0.2	3.2 ±0.1
2016 cohort	3.7 ± 0.3	5.3 ± 0.2*	5.7 ± 0.3*	4.4 ±0.2*
Caloric Intake (Kcal/kg/d)				
2014-2015 cohort	112 ± 3	119 ± 3	119 ± 3	125 ± 4
2016 cohort	115 ± 3	116 ± 2	121 ± 2	120 ± 2
Protein Intake (g/kg/d)				
2014-2015 cohort	4.1 ± 0.1	3.9 ± 0.1	4.2 ± 0.1	4.0 ± 0.1
2016 cohort	4.2 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	3.9 ± 0.1
Fluid Intake (ml/kg/d)				
2014-2015 cohort	140 ± 2	147 ± 1	146 ± 1	148 ± 3
2016 cohort	139 ± 2	146 ± 2	147 ± 1	149 ± 2
Urine Output (ml/kg/d)				
2014-2015 cohort	73 ± 3	83 ± 2	86 ± 3	93 ± 2
2016 cohort	75 ± 2	85 ± 2	85 ± 3	95 ± 3

* $p<0.05$ compared to other cohort

Assumptions regarding the approach have been validated. Infants at the University of Iowa Children's Hospital NICU, have validated several of the assumptions implicit in the derived algorithm. Fractional excretion of sodium (FE_{Na}) at 2 weeks of age is similar to that used in the calculations for development of the algorithm. As previously stated, we found little relationship between serum [Na] and urine [Na] at 2 weeks of age. Importantly, many infants with urine [Na] <40 mEq/L still had serum [Na] >135 mEq/L. This represents a group of infants who likely have total body sodium depletion yet would likely not be receiving sodium supplementation due to a "normal" serum [Na]. As expected, Na intake correlated positively with urine [Na] at and beyond the 2-week time point. Recently Dr. Segar collected urine samples 4 times over the course of 24 hrs from 10 preterm infants (25-28 weeks gestation) at 2 weeks of postnatal age and measured urine sodium concentration and found the coefficient of variation (Standard deviation/mean) to be about 0.15. Thus we are confident that a spot urine sodium concentration is reflective of daily sodium losses (*i.e.* those obtained by a 24 hr urine collection).

SECTION 3. METHODS

3.1 STUDY POPULATION

Premature infants < 17 days of age and their mothers

3.1.1 Inclusion Criteria

1. Infants with gestational age 25 0/7 – 29 6/7 at birth
2. Birth weight ≥ 500 grams
3. Admitted within the 1st week of life
4. < 17 days of age at time of enrollment

3.1.2 Exclusion Criteria

1. Infants admitted after the 1st week of life
2. Major congenital anomalies
3. Structural genitourinary abnormality
4. Renal dysfunction (serum creatinine > 1.0 mg/dl or an increase of ≥ 0.3 mg/dl between the 2 most recent consecutive measurements) immediately prior to the initiation of study procedures.
5. Diuretic use less than 48 hours prior to initiation of study procedures
6. Infant with an ostomy (infants receiving an ostomy after study entry will be withdrawn)
7. Infant with a diagnosis or suspicion of diabetes insipidus

This study will also include the mother of the infant to collect information from the mother's chart.

3.2 DETAILED STUDY PROCEDURES

- All study events described below have a ±2 day range for execution.
- Doubly labeled water procedure will occur from corrected week 32 to corrected week 32 + 6 days
- Date of birth is considered day 0
- See Appendix for table of events

3.2.1 Screening

All patients admitted to Riley Hospital at IU Health's NICUs (Simon Family Tower, Methodist, IU North), and Eskenazi Health NICU, will be evaluated for eligibility. Outborn infants will be included if admitted within the first week of life.

3.2.2 Consent Procedure

Eligible infant's guardians will be approached for consent by study personnel after the infant's first week of life.

3.2.3 Randomization Procedure

An infant must meet all inclusion criteria and no exclusion criteria at the time of randomization. After meeting eligibility and obtaining consent, randomization will be carried out in REDCap to management arms 1:1, using a permutated block scheme stratified by gestational age at birth: 25 0/7 – 27 6/7 and 28 0/7 – 29 6/7 weeks.

3.2.4 Study Intervention

Infants randomized to the study algorithm will have their urine [Na] measured at 2 weeks of age and every 2 weeks thereafter until 36 weeks corrected age. They will receive additional sodium supplementation after the second week of life if their urine [Na] is below the level in the diagram on the following page:

Postnatal age (weeks)	2	3	4	5	6	7	8	9	10	11	12	13	14
GA (birth)													
25 wk	<50		<40		<40		<40		<40		<30		<30
26 - 29 6/7 wk	<40		<40		<40		<40		<30		<30		

i. Values in table represent urine [Na], expressed as mEq/L, obtained every other week
ii. If urine [Na] is below threshold value, initiate supplementation at 4 mEq/kg/d above current Na intake. Adjust supplementation weekly to account for weight gain
iii. Increase supplementation by 2 mEq/kg/d at subsequent biweekly points if <urine [Na] goal. Urine [Na] should not be obtained within 48 hr of use of diuretic agent
iv. Provide supplementation if serum [Na] ≤132 mEq/L regardless of urine [Na] unless there is evidence of acute fluid overload (significant increase in weight). Hyponatremia in presence of high urine [Na] likely represents a condition of severe urinary sodium loss leading to hyponatremia
v. Continue supplementation unless serum [Na] >144 mEq/L
   If serum [Na] 145 – 146 mEq/L; reduce sodium supplement by ½ and consider measuring serum [Na] on subsequent day
   If serum [Na] ≥ 147 mEq/L; discontinue sodium supplementation
vi. Continuation of supplementation after 38 weeks postmenstrual age will be at the discretion of the clinical care team

Routes of sodium administration:

We will allow flexibility in how supplemental sodium is delivered since this is not standardized in usual care practices.

For infants receiving ≤80 ml/kg/d of enteral feedings: The designated amount of sodium supplementation will be added to parenteral fluids to be infused over 24 hours. Sodium may be provided as sodium chloride acetate/citrate/bicarbonate or any mixture thereof.

For infants receiving >80 ml/kg/d of enteral feedings: The designated amount of sodium will be added to the feedings (sole route of supplementation) or provided by a combination of enteral and parenteral route. When provided enterally, sodium will be administered in equally divided quantities with every other feeding, (*i.e*., 4 feedings each day for infants fed every 3 hours, 3 feedings each day for infants fed every 4 hours). Enteral supplemental sodium may be administered in the form (sodium chloride, sodium acetate, sodium citrate, sodium bicarbonate, or any mixture) chosen by the care team.

3.2.5 Data collection:

Growth and nutrition data, including fluid, caloric, protein, carbohydrate and lipid intakes and weight, will be abstracted from the infants' medical records and entered on to study data collection instruments. Standardized data collection forms will be used for collection all clinical outcome data and entered into a REDCap data base as described below. Other variables will be recorded on data forms created for this trial. The potential confounding variables include but are not limited to:

- Maternal demographic variables: maternal age, maternal education level, maternal race/ethnicity, maternal health issues, presence of chorioamnionitis, preeclampsia, reason for preterm delivery

- Labor and delivery: rupture of membranes, steroid exposure, antibiotics, mode of delivery

- Infant health demographic variables: gestational age at birth, birthweight, length, sex, small for gestational age status, parity, Apgar scores, delivery room resuscitation, initial respiratory support, blood pressure, body temperature, pH, PaO2, FiO2.

- Infant neurologic and medical morbidity: death, presence and severity of intracranial hemorrhage, presence of periventricular leukomalacia, seizures, patent ductus arteriosus, early and late onset sepsis, bronchopulmonary dysplasia, retinopathy of prematurity (stage ≥3 or requiring treatment), age at final tracheal extubation, length of hospital stay, duration of need for supplemental oxygen and diagnosis of hypertension as defined as need for scheduled antihypertensive agents.

- **Nutritional intake data:** Parenteral intake of fluid, protein, lipid, calories and sodium will be recorded each Monday, Wednesday and Friday from day of life until 38 weeks postmenstrual age or hospital discharge. Enteral intake of milk/formula volume, sodium and calories will similarly be recorded each Monday, Wednesday and Friday. Sodium content of breast milk will be estimated at 0.8 mEq/100 ml. Milk fortified to 24 and 27 kcal/oz with human milk fortifier (HMF) will be estimated to have sodium content of 1.4 and 1.8 mEq/100 ml, respectively. Sodium content of formula will be as described by the manufacturer.

- **Anthropomorphic measurements:** Measurements will be obtained weekly until discharge/transfer or until 44 weeks corrected age (whichever occurs first). Weights will be

extracted from the medical record. Research staff will obtain the length (using a length board) and OFC.

3.2.6 Measurement of Total Body Water and Energy Expenditure

Total body water (TBW) and energy expenditure (EE) will be determined at 32 weeks postmenstrual age. This approach will provide TBW and EE measurements over a range of postnatal ages following initial supplementation (*i.e*. 3 weeks for infants born at 29 weeks gestation, 7 weeks for infants born at 25 weeks gestation) allowing an understanding of changes in TBW and EE over time after initiating supplemental Na. EE and TBW will be measured using the doubly labeled water (DLW, $^{2}H_{2}{}^{18}O$) method which has been validated in preterm and term infants[53-56]. The method uses non-radioactive isotopes, is non-invasive and does not require blood sampling. The method is based on the principle that labeled hydrogen (^{2}H) is eliminated as water, disappearance of the oxygen isotope (^{18}O) is eliminated as water and as expired carbon dioxide. By measuring the difference in the elimination rates, carbon dioxide production can be calculated according to the following equations:

$$rCO_2 = 0.445N \times [(1.01 \times ko) – (1.04 \times kh]$$

where rCO_2 is carbon dioxide production rate, N is the mean of the initial and final TBW volume calculated as described below, ko and kh are the fractional elimination rates for ^{18}O and $^{2}H_2$, respectively.

Metabolic rate is estimated from rCO_2 using Weir's equation based on the food quotient (FQ) of dietary intake:

$$EE(kcal/d) = 1.44 \times [[3.941 \times rCO_2(ml/min)/FQ] + 1.11 \times rCO_2(ml/min)]$$

Food quotient (FQ), used as an estimate of respiratory quotient is calculated as:

$$FQ = (P \times 0.81) + (F \times 0.71) + (C \times 1.00)$$

Where P, F and C represent the energy contributed by protein (P), fat (F) and carbohydrate (C) expressed as a fraction of the total metabolizable energy of the diet[57]. Published values for human milk and infant formula will be used as applicable and modified as necessary for any dietary supplementation (*i.e.* human milk fortifier) provided.

TBW (g) is estimated from the dilution of ^{2}H using the following equation as previously described by us and others[58-60].

$$TBW = [Dose \times W \times (\delta_d - \delta_w)]/(D \times I_0 \times 1.041)$$

where Dose is the weight (g) of the DLW administered, W is the weight (g) of water used to prepare the dose dilution, δ_d is the δ^2H value (°/oo) of the dose dilution, δ_w is the δ^2H value (°/oo) of the water used in making the dose dilution, D is the weight (g) of the DLW used to prepare the dose dilution, I_0 is the zero-time intercept (°/oo) of the ^{2}H enrichment over time, and 1.041 is the correction for deuterium distributed into non-aqueous tissues. Urine $^{2}H_2O$ enrichment at time 0 is extrapolated by linear regression analysis after plotting the logarithm of $^{2}H_2O$ enrichment in urine versus time.

A typical study protocol using the DLW method starts with a baseline urine collection before the dose of DLW to determine baseline values for ^{2}H and ^{18}O. An accurately weighed dose of DLW (1.5g/kg body weight, $^{2}H_2O$ (99.9 atom %) and $H_2{}^{18}O$ (10.0 atom %) (both from Children's Nutrition Research Center at Baylor College of Medicine) is administered through a nasogastric feeding tube. DLW sterility is ensured using microfiltration into single use sterilized serum vials that are then covered with parafilm. The dose (approximately 1.5 ml/kg) is flushed through the tube with 2 ml of sterile water. The syringes containing the isotopes are weighed to the nearest 0.1 mg before and after the dose is given. Urine samples are then collected between 6-24 h after dosing and, and again 2 and 5 days after dosing. Timing of urine collection is carefully recorded. These urine samples will be analyzed for ^{2}H and ^{18}O enrichment by isotope ratio mass spectrometry, which will allow for calculation of TBW and EE as described above[61,62].

Urine will be collected, labelled appropriately, including time after DLW administration, and frozen at -20˚C until shipped for analysis. Samples will be shipped from IU to the USDA/ARS Children Nutrition Research Center at Baylor College of Medicine and immediately transferred to the Central DLW Laboratory, directed by Dr. William W. Wong, a collaborator on the project. Upon arrival, samples will be logged and again frozen at -80˚C until isotope analysis is performed by gas-isotope-ratio mass spectrometry as performed in Dr. Wong's laboratory as previously described[63].

3.2.7 Blinding/Masking

None. Though randomization will be concealed preventing allocation bias, it is impossible to blind caregivers or parents to the intervention. Interim results will be provided to the Data Safety Monitoring Committee (DSMC) with treatment assignments labeled only as group "A" and "B." We will record sodium and fluid intake as well as serum and urine sodium values in order to assess protocol compliance.

3.2.8 Primary Outcome

The change in somatic growth (weight gain, head circumference, and length) evaluated by the change in Z-score between 2 weeks of age and 36 weeks conceptual age or transfer from NICU. Weights will be extracted from the medical record, consistent study personnel will obtain the length (using a length board) and OFC.

3.2.9 Secondary Outcomes

1. The change in somatic growth (weight gain, head circumference and length) evaluated by the change in Z-score between 2 weeks of age and discharge or transfer from the NICU or 44 weeks corrected age (whichever occurs first).
2. Total body water and energy expenditure at 32 weeks corrected age
3. Incidence and severity of bronchopulmonary dysplasia
4. Duration of mechanical ventilation
5. Need for supplemental oxygen
6. Use of diuretic therapy

7. Retinopathy of prematurity

SECTION 4. ADVERSE EVENTS AND REPORTING

4.1 Definitions

An adverse event (AE) is defined as any untoward medical occurrence in a subject in the investigation. AEs must be assessed for expectedness, severity, attribution (relatedness to the study intervention), and seriousness by the investigator, or another qualified study team member as characterized below. AEs deemed related to the intervention must be reported as serious adverse events (SAEs) if they become serious (become life-threatening, result in death, or prolong hospitalization). Recording of such adverse events will start at the time of the study intervention (2 weeks of age) and will stop at 36 weeks PMA or transfer/discharge (whichever occurs later).

The following criteria will be used to define relatedness:

Definite: The AE is clearly related to the study intervention

Probable: The AE is likely related to the study intervention

Possible: The AE may be related to the study intervention

Unrelated: The AE is clearly not related to the study intervention

The severity of AEs will be described as:

Mild: asymptomatic or mild symptoms, no intervention indicated

Moderate: clinically significant requiring minimal noninvasive intervention

Severe: severe or medically significant but not immediately life-threatening, need for intensive, emergent, or invasive intervention

Life-threatening: life-threatening physiological consequences, need for intensive or emergent invasive intervention

4.2 Adverse Event Monitoring and Reporting

Extremely preterm infants commonly have multiple clinical complications and laboratory abnormalities associated with prematurity. Those listed as secondary outcomes will be collected in the REDCap database and reviewed monthly by the Study Team.

Published data involving this population and at the dose of sodium supplementation to be provided in this study have not shown any harmful effects, including hypernatremia, suggesting

this risk is minimal [33, 43]. In this patient population per routine standard of care, serum [Na] concentrations are checked daily to several times/week on infants receiving majority of intake parenterally and twice weekly to weekly for infants receiving primarily enteral intake. As this is a pragmatic, unmasked study evaluating an algorithm to assist clinicians in preventing total body sodium deficiency, AE reporting will specifically focus on states of sodium inadequacy or excess, and any additional adverse event monitoring the DSMC requests.

Adverse event monitoring will include:

Hyponatremia: mild [Na] 134-130; moderate [Na] 129-125; severe [Na] <125
Hypernatremia: mild [Na] 146-150; moderate [Na] 151-155; severe [Na] >155
(To be considered an AE, 2 consecutive measurements of hyper/hyponatremia will be required to rule out laboratory measurement variation.)

Common complications of prematurity that are not considered AE's will be collected and included in a Note to File for each participant.

Other events classified as serious and unexpected
Other events resulting in death or classified as a Serious or Related (to study procedures).
All SAEs and AEs reported during this study will be followed until resolution or until the investigator confirms that the AE/SAE has stabilized and no more follow-up is required.

All deaths and serious adverse events SAEs that are at least possibly related and all unexpected SAEs will be reported to the Chair of the DSMC within 72 hours of awareness. An initial SAE form must be as complete as possible, including details of the current serious adverse event, including an Investigator assessment of the causal relationship between the event and study procedures. Information not available at the time of the initial report must be documented on a follow-up SAE form.

All AEs/SAEs that meet IU HRPP Policy on Reportable Events will be reported to the IRB within 5 working days.

SECTION 5. SAFETY, DATA MANAGEMENT, AND ANALYTICAL PLAN

5.1 Protection against risks

Potential Risks
The risks from this study are minimal, primarily consisting of mild hypernatremia. However, published data involving this population and at dose of sodium supplementation to be provided in this study have not shown any harmful effects, including hypernatremia, suggesting this risk is minimal [33, 43]. Additional risks relate to the collection of biological samples, and collection of clinical data with the potential for loss of confidentiality.

Adequacy of the Protection against Risk

Recruitment and Informed Consent

Neonatal research nurses/coordinators are a key element in the success of clinical research programs within neonatal intensive care units. The team of research coordinators will be in constant communication with the NICU care team to assist in identification of eligible patients. They will screen patients and present to the parents the details of the study and obtain informed consent. The experience of the research coordinators results in effective communication about protocols and success in recruitment. Based upon completion of a large number of studies in the NICUs, we anticipate approximately a 60% enrollment. The drop-out rate is likely to be minimal given the limited interventions and lack of anticipated side effects from the intervention.

Protecting Confidentiality

Security measures to protect subject identities include the use of coded files to unlink research records from names and other identifiers, locked storage areas, and password-protected computer files. Subjects' names are linked to their IDs as noted above. Access to computer systems housing sensitive information is strictly regulated. Credentials permitting access to these systems are granted only to essential investigators and research personnel. All systems are secured from external attack through a combination of hardware and software firewalls and are updated with necessary security patches as they become available. Related hardware and back-up storage media are maintained in a secure environment to which only essential personnel have physical access. All consent and research procedures will be compliant with Subpart B of the Code of Federal Regulations Title 45, Part 46 - Protection of Human Subjects with Additional Protections for Pregnant Women, Human Fetuses and Neonates Involved in Research (66 FR 56778, Nov. 13, 2001).

5.2.1 Data management and interpretation

Data will be collected using prepared forms, then entered and managed using REDCap electronic capture tools hosted and managed by IUPUI Biostatistics. The REDCap platform is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 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 importing data from external sources. Only IRB approved research team members will have access to the REDCap data platform. Each team's members will be granted access to the REDCap data system through a secure login. In the REDCap data platform, primary data and data backups are secured at separate Indiana University Data centers. Operating system security includes: secure logins, data encryption at rest, remote system logging and configuration and change management. Data backups are encrypted both in flight and at rest. Copies of data are replicated to the remote data center every 15 minutes. There are 100+ point-in-time copies of data available at any time. Disaster recovery has been tested and confirmed.

Performance Monitoring: Standard reports will be generated from the study database by the

Data Center. These will include monthly reports that provide the number of infants screened and enrolled, missing data, adherence to study protocols, and a variety of performance measures, including adverse events.

5.2.2 Statistical analysis

All analyses will be performed on an intent-to-treat basis. The primary analysis (and all analyses examining outcomes by treatment) will be adjusted for the stratification variable of gestational age. Since the primary outcome is continuous, linear regression will be used to estimate the adjusted mean difference in growth between the two treatment groups. Similarly, for secondary outcomes, linear regression will be used for continuous outcomes and robust Poisson regression for binary or categorical outcomes to obtain adjusted relative risk estimates for the treatment effect.

5.2.3 Sample size and power estimates

Sample size and power calculations for this study are based upon the primary outcome of differences in weight gain between the two groups of infants. Using data from our recently completed analysis, in which infants 26 – 29 6/7 weeks gestation treated according to the algorithm were compared with historical controls, and based upon identifying a difference in weight Z-score of 0.25 at 36 weeks postmenstrual age, using a SD of 0.4 with an alpha = 0.05 and power of 0.8, we calculate we will need a total sample size of 82 patients (41 per group). With regards to the secondary outcome of EE, and based upon published data of EE estimated in preterm infants using the DLW method, a sample size of 41 patients per group will allow us to detect a 6% difference in EE, assuming an alpha = 0.05 and a power of 0.80. With regards to TBW, and based upon data from these same investigators who report a mean TBW of 80.8% body weight, with SD of 3.1%, this sample size will allow us to detect a difference in TBW of 2.0% between groups, assuming an alpha = 0.05 and a power of 0.80. To acknowledge for study drop out, we will plan to enroll up to 90 patients (plus 90 mothers for chart review).

5.3 DATA SAFETY AND MONITORING PLAN

A Data Safety and Monitoring Committee (DSMC), headed by Dr. Edward Bell, Professor of Pediatrics, University of Iowa Carver College of Medicine will monitor the progress of the study when 25%, 50% and 75% of the trial subjects have reached hospital discharge. Dr. Bell, who has extensive experience with DSMCs, will be responsible for recruiting the necessary individuals with expertise in neonatology, biostatistics, bioethics and clinical trials to establish the DSMC. The DSMC will review accumulated blinded study data to ensure that the intervention is not leading to harm. If there is a significant imbalance of secondary outcome rates between the study arms, the proper course of action will be discussed by the study PIs and the DSMC. The DSMC will not be charged with assessing efficacy before conclusion of the trial; therefore, no loss of alpha will result from the planned interim safety analyses.

Safety outcomes include incidence of mortality up through 36 weeks PMA, BPD, and ROP. The DSMC may request other outcomes at their prerogative. The DSMC will examine other safety outcomes, including all reported deaths and serious adverse events by treatment group in considering a recommendation to suspend the trial for safety reasons.

SECTION 5. REFERENCES

1. Fenton, T.R. and J.H. Kim, *A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants.* BMC Pediatr, 2013. **13**: p. 59.
2. Griffin, I.J., et al., *Postnatal growth failure in very low birthweight infants born between 2005 and 2012.* Arch Dis Child Fetal Neonatal Ed, 2016. **101**(1): p. F50-5.
3. Horbar, J.D., et al., *Weight Growth Velocity and Postnatal Growth Failure in Infants 501 to 1500 Grams: 2000-2013.* Pediatrics, 2015. **136**(1): p. e84-92.
4. Ehrenkranz, R.A., et al., *Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants.* Pediatrics, 2006. **117**(4): p. 1253-61.
5. Ehrenkranz, R.A., et al., *Longitudinal growth of hospitalized very low birth weight infants.* Pediatrics, 1999. **104**(2 Pt 1): p. 280-9.
6. Franz, A.R., et al., *Intrauterine, early neonatal, and postdischarge growth and neurodevelopmental outcome at 5.4 years in extremely preterm infants after intensive neonatal nutritional support.* Pediatrics, 2009. **123**(1): p. e101-9.
7. Sammallahti, S., et al., *Infant growth after preterm birth and neurocognitive abilities in young adulthood.* J Pediatr, 2014. **165**(6): p. 1109-1115 e3.
8. Hay, W.W., Jr., *Aggressive Nutrition of the Preterm Infant.* Curr Pediatr Rep, 2013. **1**(4).
9. Prince, A. and S. Groh-Wargo, *Nutrition management for the promotion of growth in very low birth weight premature infants.* Nutr Clin Pract, 2013. **28**(6): p. 659-68.
10. Butterworth, S.A., V. Lalari, and K. Dheensaw, *Evaluation of sodium deficit in infants undergoing intestinal surgery.* J Pediatr Surg, 2014. **49**(5): p. 736-40.
11. Fine, B.P., et al., *Sodium deprivation growth failure in the rat: alterations in tissue composition and fluid spaces.* J Nutr, 1987. **117**(9): p. 1623-8.
12. Gallaher, K.J., et al., *Effect of diet-induced sodium deficiency on normal and compensatory growth of the lung in young rats.* Pediatr Res, 1990. **28**(5): p. 455-9.
13. Haycock, G.B., *The influence of sodium on growth in infancy.* Pediatr Nephrol, 1993. **7**(6): p. 871-5.
14. Mansour, F., et al., *Effect of sodium deficiency on growth of surgical infants: a retrospective observational study.* Pediatr Surg Int, 2014. **30**(12): p. 1279-84.
15. O'Neil, M., D.H. Teitelbaum, and M.B. Harris, *Total body sodium depletion and poor weight gain in children and young adults with an ileostomy: a case series.* Nutr Clin Pract, 2014. **29**(3): p. 397-401.
16. Wassner, S.J., *Altered growth and protein turnover in rats fed sodium-deficient diets.* Pediatr Res, 1989. **26**(6): p. 608-13.
17. Wassner, S.J., *The effect of sodium repletion on growth and protein turnover in sodium-depleted rats.* Pediatr Nephrol, 1991. **5**(4): p. 501-4.
18. Bower, T.R., K.C. Pringle, and R.T. Soper, *Sodium deficit causing decreased weight gain and metabolic acidosis in infants with ileostomy.* J Pediatr Surg, 1988. **23**(6): p. 567-72.
19. Ray, P.E., et al., *Sodium or chloride deficiency lowers muscle intracellular pH in growing rats.* Pediatr Nephrol, 1996. **10**(1): p. 33-7.
20. Burns, C.P. and E. Rozengurt, *Extracellular Na+ and initiation of DNA synthesis: role of intracellular pH and K+.* J Cell Biol, 1984. **98**(3): p. 1082-9.
21. Toback, F.G., *Induction of growth in kidney epithelial cells in culture by Na+.* Proc Natl Acad Sci U S A, 1980. **77**(11): p. 6654-6.
22. Moolenaar, W.H., et al., *Na+/H+ exchange and cytoplasmic pH in the action of growth factors in human fibroblasts.* Nature, 1983. **304**(5927): p. 645-8.
23. Mendoza, S.A., *The Na+-H+ antiport is a mediator of cell proliferation.* Acta Paediatr Scand, 1987. **76**(4): p. 545-7.

24. Resnick, L.M., et al., *Intracellular ionic consequences of dietary salt loading in essential hypertension. Relation to blood pressure and effects of calcium channel blockade.* J Clin Invest, 1994. **94**(3): p. 1269-76.
25. Tanaka, Y., et al., *Characterization of signaling pathways to Na+/H+ exchanger activation with epidermal growth factor in hepatocytes.* Hepatology, 1994. **20**(4 Pt 1): p. 966-74.
26. American Academy of Pediatrics. Committee on Nutrition and R.E. Kleinman, *Pediatric nutrition : policy of the American Academy of Pediatrics*. 7th edition. ed. 2014, Elk Grove Village, IL: American Academy of Pediatrics. xxvi, 1477 pages.
27. Ziegler, E.E., *Meeting the nutritional needs of the low-birth-weight infant.* Ann Nutr Metab, 2011. **58 Suppl 1**: p. 8-18.
28. Ziegler, E.E., et al., *Body composition of the reference fetus.* Growth, 1976. **40**(4): p. 329-41.
29. *American Academy of Pediatrics Committee on Nutrition: Nutritional needs of low-birth-weight infants.* Pediatrics, 1985. **75**(5): p. 976-86.
30. Kelly, D., *NICU sodium administration to extremely low birth weight infants: relationships with recommendations and growth*, 2013, Open Access Master's Theses.
31. Delgado, M.M., et al., *Sodium and potassium clearances by the maturing kidney: clinical-molecular correlates.* Pediatr Nephrol, 2003. **18**(8): p. 759-67.
32. Vanpee, M., et al., *Sodium supplementation optimizes weight gain in preterm infants.* Acta Paediatr, 1995. **84**(11): p. 1312-4.
33. Isemann, B., et al., *Impact of Early Sodium Supplementation on Hyponatremia and Growth in Premature Infants: A Randomized Controlled Trial.* JPEN J Parenter Enteral Nutr, 2016. **40**(3): p. 342-9.
34. Edelman, I.S., et al., *Interrelations between serum sodium concentration, serum osmolarity and total exchangeable sodium, total exchangeable potassium and total body water.* J Clin Invest, 1958. **37**(9): p. 1236-56.
35. Kurtz, I. and M.K. Nguyen, *Evolving concepts in the quantitative analysis of the determinants of the plasma water sodium concentration and the pathophysiology and treatment of the dysnatremias.* Kidney Int, 2005. **68**(5): p. 1982-93.
36. Nguyen, M.K. and I. Kurtz, *Quantitative interrelationship between Gibbs-Donnan equilibrium, osmolality of body fluid compartments, and plasma water sodium concentration.* J Appl Physiol (1985), 2006. **100**(4): p. 1293-300.
37. Warner, G.F., N.J. Sweet, and E.L. Dobson, *Sodium space and body sodium content, exchangeable with sodium24, in normal individuals and patients with ascites.* Circ Res, 1953. **1**(6): p. 486-90.
38. Schrier, R.W., *Diagnostic value of urinary sodium, chloride, urea, and flow.* J Am Soc Nephrol, 2011. **22**(9): p. 1610-3.
39. Bischoff, A.R., C. Tomlinson, and J. Belik, *Sodium Intake Requirements for Preterm Neonates: Review and Recommendations.* J Pediatr Gastroenterol Nutr, 2016. **63**(6): p. e123-e129.
40. Gubhaju, L., et al., *Assessment of renal functional maturation and injury in preterm neonates during the first month of life.* Am J Physiol Renal Physiol, 2014. **307**(2): p. F149-58.
41. Vieux, R., et al., *Glomerular filtration rate reference values in very preterm infants.* Pediatrics, 2010. **125**(5): p. e1186-92.
42. Haycock, G.B., G.J. Schwartz, and D.H. Wisotsky, *Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults.* J Pediatr, 1978. **93**(1): p. 62-6.
43. Al-Dahhan, J., L. Jannoun, and G.B. Haycock, *Effect of salt supplementation of newborn premature infants on neurodevelopmental outcome at 10-13 years of age.* Arch Dis Child Fetal Neonatal Ed, 2002. **86**(2): p. F120-3.
44. Segar, D.E., et al., *Physiological approach to sodium supplementation in preterm infants.* Am J Perinatol, 2018. 35(10): p. 994-1000.
45. Efird, J., *Blocked randomization with randomly selected block sizes.* Int J Environ Res Public Health, 2011. **8**(1): p. 15-20.

46. Hartnoll, G., P. Betremieux, and N. Modi, *Randomised controlled trial of postnatal sodium supplementation on body composition in 25 to 30 week gestational age infants.* Arch Dis Child Fetal Neonatal Ed, 2000. **82**(1): p. F24-8.
47. Lorenz, J.M., et al., *Water balance in very low-birth-weight infants: relationship to water and sodium intake and effect on outcome.* J Pediatr, 1982. **101**(3): p. 423-32.
48. Van Marter, L.J., et al., *Hydration during the first days of life and the risk of bronchopulmonary dysplasia in low birth weight infants.* J Pediatr, 1990. **116**(6): p. 942-9.
49. Wadhawan, R., et al., *Association between early postnatal weight loss and death or BPD in small and appropriate for gestational age extremely low-birth-weight infants.* J Perinatol, 2007. **27**(6): p. 359-64.
50. Muratore, C. and R. Dhanireddy, *Urine collection from disposable diapers in premature infants: biochemical analysis.* Clin Pediatr (Phila), 1993. **32**(5): p. 314-5.
51. Harsha, S.S. and B.R. Archana, *SNAPPE-II (Score for Neonatal Acute Physiology with Perinatal Extension-II) in Predicting Mortality and Morbidity in NICU.* J Clin Diagn Res, 2015. **9**(10): p. SC10-2.
52. Fenton, T.R. and R.S. Sauve, *Using the LMS method to calculate z-scores for the Fenton preterm infant growth chart.* Eur J Clin Nutr, 2007. **61**(12): p. 1380-5.
53. Butte, N.F., et al., *Energy expenditure and deposition of breast-fed and formula-fed infants during early infancy.* Pediatr Res, 1990. **28**(6): p. 631-40.
54. Jensen, C.L., et al., *Determining energy expenditure in preterm infants: comparison of 2H(2)18O method and indirect calorimetry.* Am J Physiol, 1992. **263**(3 Pt 2): p. R685-92.
55. Roberts, S.B., et al., *Effect of weaning on accuracy of doubly labeled water method in infants.* Am J Physiol, 1988. **254**(4 Pt 2): p. R622-7.
56. Roberts, S.B., et al., *Comparison of the doubly labeled water (2H2(18)O) method with indirect calorimetry and a nutrient-balance study for simultaneous determination of energy expenditure, water intake, and metabolizable energy intake in preterm infants.* Am J Clin Nutr, 1986. **44**(3): p. 315-22.
57. Black, A.E., A.M. Prentice, and W.A. Coward, *Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure.* Hum Nutr Clin Nutr, 1986. **40**(5): p. 381-91.
58. Baarsma, R., et al., *Measurement of total bodywater volume with deuterated water in newborn infants.* Biol Neonate, 1992. **62**(2-3): p. 108-12.
59. Segar, J.L., S. Chemtob, and E.F. Bell, *Changes in body water compartments with diuretic therapy in infants with chronic lung disease.* Early Hum Dev, 1997. **48**(1-2): p. 99-107.
60. Wells, J.C., et al., *Prediction of total body water in infants and children.* Arch Dis Child, 2005. **90**(9): p. 965-71.
61. Wong WW, Clarke LL. A hydrogen gas-water equilibration method produces accurate and precise stable hydrogen isotope ratio measurements in nutrition studies. J Nutr 2012, 142:2057-2062.
62. Wong WW, Clarke LL. Accuracy of d18O isotope ratio measurements on the same sample by continuous-flow isotope-ratio mass spectrometry. Rapid Commun Mass Spectrom 2015, 29:2252-2256.
63. Wong, W.W., et al., *The doubly labeled water method produces highly reproducible longitudinal results in nutrition studies.* J Nutr, 2014. **144**(5): p. 777-83.
64. Patel, R.M., et al., *Causes and timing of death in extremely premature infants from 2000 through 2011.* N Engl J Med, 2015. **372**(4): p. 331-40.

Section 6. Appendix

Name of form	< 17 days of life	On Study	Weeks 1-19 on Study (estimate based on birth Gestational Age)	Discharge/Transfer or week 44 of life corrected
Eligibility Check List	X			
Informed consent	X			
Enrollment Randomization		X		
Maternal Demographics		X		
Maternal Health Data		X		
Neonatal Demographics		X		
Neonatal Health Data		X		
Sodium History		X		
SOC Serum Sodium			Weekly	X
Study Urine Sodium – ARM B ONLY			Weeks 1,3,5,7,9,11,13,15,17,19	
Nutrition – Enteral/Parenteral			Weekly; Monday, Wednesday, Fridays	
Body Measurements			Weekly; Length, OFC, Weight	X
Doubly Labeled Water			Will occur at 32 - 32+6 weeks corrected age	
Doubly Labeled Water Sample			Will collect before procedure, 6-24 hr post, 36-48 hr post and 110-130 hr post	
Week 36 Status			to be completed at 36 weeks postmenstrual age or if patient discontinues study early	
Outcome Status				X
Adverse Event		Will be followed once enrolled to discharge/transfer or week 44 of life (whichever occurs first)		

INDIANA UNIVERSITY INFORMED CONSENT STATEMENT FOR RESEARCH

Physiologic Approach to Sodium Supplementation in Premature Infants (Salt to Grow)

ABOUT THIS RESEARCH

You are being asked to let your baby take part in a research study called Salt to Grow. Clinicians do research to answer important questions which might help change or improve the way we do things in the future. This consent form will give you information about the study to help you decide whether you want to let your baby take part. Please read this form, and ask any questions you have, before agreeing to let your baby be in the study.

We are also asking to collect information from your chart as described below in the "What Will Happen During the Study?" section.

This study is being done at Riley Hospital for Children (Methodist, Riley Downtown, IU North), and Eskenazi Hospital by Dr. Gregory Sokol. It is being paid for by the Thrasher Research Fund.

TAKING PART IN THIS STUDY IS VOLUNTARY

You do not have to let your baby take part in the study. Or, if you let your baby take part, you can choose to take them out of the study at any time. Your decision will not affect your baby's care or treatment at Riley Hospital for Children, Indiana University, or Eskenazi Health.

WHY IS THIS STUDY BEING DONE?

You are being asked to let your baby take part in this study because they were premature (born very early). Many premature babies do not grow well even though they get enough nutrition. We know low sodium causes poor growth. Many premature babies get extra sodium while in the hospital. Their kidneys have problems holding on to sodium because they are still developing.

There is no exact way to measure how much sodium is in the total body. Right now your baby's doctor looks at the sodium in their blood to decide when and how much sodium to give.

The purpose of this study is to see if using a baby's urine sodium and blood sodium levels to decide about giving extra sodium will keep their body's sodium normal and help them grow better.

HOW MANY BABIES WILL TAKE PART?

If you decide to let your baby be in this study, they will be one of up to 90 babies taking part. This study is only being done at Riley Hospital for Children (Methodist, Riley Downtown, IU North), and Eskenazi Health.

WHAT WILL HAPPEN DURING THE STUDY?

Information We Collect

At the beginning of the study, we will write down birth information like weight, length, head size, how long you were pregnant, your health issues, issues that you developed during your pregnancy and your race and ethnic background.

During the study we will check your baby's chart for their medical history, physical exams, labs, tests, feedings (or other nutrition) and medicines given. We will also note any problems or changes in your baby's health while in the hospital.

Study Groups

Your baby will be put into one of two study groups. One group will use urine sodium to decide when and how much sodium to give. The other group will get standard (usual) care. Both groups will have their blood sodium measured. The standard of care is to use the sodium blood test to decide when and how much sodium to give. The standard care group is needed for comparison to help us decide if this method of replacing sodium helps babies grow. Which group your baby is in will be decided by chance, like tossing a coin. Your baby will have a 50% chance (1 out of 2 chances) of being in either group.

Measuring Urine Sodium

Babies in the urine sodium group will have a urine sample collected to see how much sodium is in it. We will do this about every 2 weeks until they reach the corrected gestational age of 36 weeks. For example if your baby was born at 28 weeks and your baby is now 4 weeks old, their corrected gestational age is 32 weeks old.

If their urine sodium falls below a certain amount, extra sodium will be added every day to their IV (in the vein) fluids or feeding given by mouth or tube. This process will continue until your baby reaches a corrected gestational age of 38 weeks. Your baby's care team will decide if they will do it for a longer time.

Measuring Body Water and Energy Use

All babies in the study will have their total body water and energy use measured. This will be done when they are about 32 weeks corrected gestational age. We will use the "doubly labeled water" method. This method uses non-radioactive water isotopes. This water isotope is a kind of water found in nature. It is in things like drinking water (and other drinks), moisture in the air, rain, snow, lake and sea water, and in foods we eat every day. In fact, the oxygen isotope is found in the air we all breathe.

What we do:

1. First, we will get a urine sample from your baby.
2. Then your baby will get about ½ teaspoon of water with the isotope, followed by another ½ teaspoon of sterile water through their feeding tube.
3. Over the next 5 days, we will collect 3 more urine samples. The urine samples will be labeled with a special code number. They will not have your baby's name, initials or any other information that could identify your baby.
4. The urine samples will be studied.

Measuring Length and Head Size

A nurse will use a board and measuring tape to check your baby's length and head size every week until they are discharged from the unit, transferred or reach 44 weeks corrected age, which ever happen first. These measurements are usually done on all premature babies.

WHAT ARE THE RISKS OF TAKING PART IN THE STUDY?
Your baby was born very early. Babies born very early are at risk for many problems including bad breathing trouble (maybe needing extra oxygen and/or a breathing machine), bleeding inside the brain, infections, necrotizing enterocolitis (a serious infection of the intestine), low or high sodium in their blood, poor growth and developmental problems including cerebral palsy. These are risks whether or not your baby takes part in this study. Below are other possible risks or discomforts from participation in the study:

- Your baby may have high sodium in their blood. The care team will check it often. Other studies like this have not caused a high sodium level. We will stop giving extra sodium if the level gets above normal.
- Infection can happen when anything is added to your baby's diet. In this study, water with the isotope is added. This risk is lowered by putting the water though filters.
- Loss of confidentiality. Every effort will be made to protect your infant's confidential medical information, but this cannot be guaranteed.
- There may be other information about the study we do not yet know. If we find out any new information, including risks, while your baby is in the study, we will tell you right away.

WHAT ARE THE POTENTIAL BENEFITS OF MY BABY TAKING PART IN THE STUDY?
Your baby may or may not benefit from being in this study. Some babies may benefit if one treatment group shows benefit and your baby is in that group. But, we do not know whether there is a benefit for one group or the other. It is possible your baby will not benefit.
We hope the information we get from this study will help treat premature babies in the future.

WHAT ARE THE OTHER TREATMENT OPTIONS?
Your baby does not have to take part in this study. If you decide you do not want your baby to be in it, they will still get regular medical care at Riley Hospital for Children.

HOW WILL MY BABY'S INFORMATION BE PROTECTED?
Efforts are made to protect all research subjects from inappropriate use of their personal information. We will do everything we can to keep your baby's records confidential, but we cannot guarantee absolute confidentiality. Your baby's personal information may be shared if required by law. Your baby's identity will not be shared in reports in which this study may be published.
Personal health information (PHI) such as name, date of birth, address, email address, and phone number will be stored in an IU research clinical management and payment system and database to track your study procedures and information, schedule study visits and follow-up activities, distribute payments, link study data, and enable chart reviews.

To protect your baby's identity, your baby will be assigned a unique study ID number which will be linked (Coded) to your study information. Your information collected for this study will be kept in a password-protected database. Only research staff and collaborators can see it.

Organizations that may look at and/or copy your baby's research records for quality assurance and data analysis include groups such as the study investigator and their research associates, the Indiana University Institutional Review Board or its designees, the Thrasher Research Fund, the Indiana Clinical

Research Center,, and any state or federal agencies such as the U.S. Food and Drug Administration (FDA) who may need to access your baby's medical and/or research records (as allowed by law).

A description of this clinical trial will be available on **ClinicalTrials.gov**, as required by U.S. law. This website will not include information that can identify your baby. At most, the website will include a summary of the results. You can search this website at any time.

WILL MY BABY'S INFORMATION BE USED FOR FUTURE RESEARCH?
Information collected from your baby for this study may be used for future research studies or shared with other researchers for future research. If this happens, information which could identify your baby will be removed before any information is shared. Since identifying information will be removed, we will not ask for your additional consent.

WILL I BE PAID FOR TAKING PART?
You will not be paid for your baby's participation in this study. Your baby will receive an age-appropriate book as a thank you for participating in the study.

WILL IT COST ME ANYTHING FOR MY BABY TO BE IN THE STUDY?
Neither you nor your insurance company will have to pay for any test or visit that is done only for the purpose of this study (urine sodium, total body water and energy use measurements). The parts of your baby's care that would normally be done as standard treatment will be billed to you or your insurance company.

WHO WILL PAY FOR TREATMENT IF MY BABY IS HURT?
If your baby is hurt because of taking part in this study, they will get any needed medical treatment. This treatment will be billed as part of their medical expenses. As with any medical care your baby receives, costs not covered by your insurance company will be your responsibility. It is also your responsibility to check your health care coverage. There is no program in place for other monetary compensation for such injuries. However, you are not giving up any legal rights or benefits to which you are otherwise entitled.

WHO SHOULD I CALL WITH QUESTIONS OR PROBLEMS?
For questions about the study or a research-related injury, call Dr. Gregory Sokol at (317) 274-4768. If you need to reach a neonatologist between the hours of 5pm to 8am call the neonatologist on call at (317) 948-4371

For questions about your baby's rights as a research participant, to discuss problems, complaints, or concerns about a research study, or to get information or to offer input, please call the IU Human Subjects Office at 800-696-2949 or email irb@iu.edu.

CAN I WITHDRAW MY BABY FROM THE STUDY?
If you decide to let your baby be in this study, you can change your mind and decide to take them out of the study (withdraw) at any time. The study team will help withdraw your baby from the study safely. If you decide to withdraw, please tell a member of the study team.

The investigator can withdraw your baby from the study without your permission. If this happens, we will tell you. Possible reasons for withdrawal include: we find new information that shows the risks of the study are greater than the benefits, or if your baby develops a problem that makes us stop the study procedures.

You will be told about new information that may affect your baby's health, welfare, or willingness to stay in the study.

PARTICIPANT'S CONSENT

In consideration of all of the above, I give my consent for my baby to take part in this research study. I will get a copy of this informed consent document to keep for my records. I agree to let my baby take part in this study. I agree to the collection of information from my chart as described in this informed consent document.

Your Child's Printed Name:_____

Printed Name of Parent/Legal Guardian:_____

Signature of Parent/Legal Guardian:_____**Date**:_____

If the mother is under 18 years old please complete:

Printed Name of Parent/Legal Guardian:_____

Signature of Parent/Legal Guardian:_____**Date**:_____

Printed Name of Person Obtaining Consent:_____

Signature of Person Obtaining Consent:_____**Date**: