University of Minnesota
Clinical Research Study Protocol

Interval Bolus Delivery of Subcutaneous Hydrocortisone via Infusion Pump in Children With Congenital Adrenal Hyperplasia (FDA IND#125640)

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Version Date: September 20, 2021

Confidential

Revision History

Version Date	Summary of Changes	Consent Change? Y/N	Consent Version Date
2/4/15	Original to FDA	**N/A**	
3/2/15	• 7.1.3 Dosage adjustment section was added • 9.3 Methods to minimize risk section was expanded • 10.4 Stopping rules section expanded detail on adrenal crisis	**N/A**	
12/29/15	• 6.2 Treatment Period section was changed to reflect that subjects will be discharged after second admission and continue on their usual HC oral dosing schedule for 1 week prior to initiation of the SQHC pump.	**N/A**	
6/30/16	• 9.3 Methods to minimize risk changed to reflect weekly visits and blood draws while on pump.	**N/A**	
3/1/17	• In section 8 Study Device, we added that the Medtronic quick-set will be the ONLY infusion set used in this study. • 7.1.3 Dosage adjustment was changed to reflect that in the event of pump failure, patients will be instructed to resume their usual oral HC dose and notify the investigators. • 5.2.2. Exclusion criteria section was changed to reflect that patients with body surface areas under $1m^2$ or over $2m^2$ will be excluded from the study. • 7.1.3 Dosage adjustment section was changed to reflect procedure if a pump malfunction results in delivery of the entire reservoir (worst case scenario 100 mg of hydrocortisone) and for missed doses due to occlusions.	**N/A**	
9/15/17	• 3.1 Moved Admission 3, that was part of Primary Objective 2, and combined it with Primary Objective 1. There is no change to the specifications of Admission 3. • 3.2 Moved the nonlinear mixed-effects population analysis from Primary Objective 2 to the Secondary Objectives section. There is no change to the approach of the analysis. With the moving of both Admission 3 and the analysis, Primary Objective 2 was no longer needed as the approaches have been moved to other sections. • In Section 4 Study Design information was added to explain in more detail the criteria that will be used for dosing changes during the 6-week SQHC trial. Also, the number of home visits by the nurse have been increased from 7 to 8 home visits. • 7.1.2 Dosage and Administration section was expanded to provide more detail the criteria that will be used for dosing changes during the 6-week SQHC trial. • In Section 6, the Studies Procedure table was changed to reflect the additional home nursing visit and additional safety measure of daily blood pressure measurements while on 6-week SQHC trial. • Section 9.1 has been reorganized so that the potential risks of HC more specific to this study are listed before the listing of all of the potential risks of HC that can happen in any age group and population. • Section 9.3 has been changed to reflect that parents will be provided with an automatic upper arm blood pressure monitor with	N/A	

	cuff as an additional safety measure for monitoring pump failure that does not trigger an alarm. •11.1 – 11.2 The statistical analysis of what was formerly Primary Objective 2 has been moved from Section 11.1 (Primary Outcome Measures) to Section 11.2 (Secondary Outcome Measures).		
6/14/18	• Per request from the FDA and from University of Minnesota IRB, the title of protocol has been changed to remove reference to "pulsatile." The protocol has been renamed: "Interval Bolus Delivery of Subcutaneous Hydrocortisone via Infusion Pump in Children With Congenital Adrenal Hyperplasia." • Per request from the University of Minnesota IRB, when referencing the pump within the text of the protocol, "pulsatile delivery" has been replaced with "interval bolus delivery." • Section 5.2: Added description/explanation of "Inclusion of Children," "Rationale for Specific Age Range," and "Expertise of the investigative team for working with the age group." • Section 5.3: Added description/explanation of "Resources available," "Provisions to Protect the Privacy Interests of Participants," "Consent Process" and "Compensation for Research-Related Injury." • Section 12.2: Added description/explanation of "Confidentiality – Data Security," "Data Banking" and "Sharing of Results with Participants."	N/A	
3/12/19	• Sec 6.0: Study procedures: addition of ACTH to the lab results during PKPD study blood samples • Sec 6.0: Study procedures: addition of 24 hour urine collection during admission 1, 2 and 3 • Sec 6.0: Study procedures: addition of salvia samples during admission 1, 2 and 3 • Section 10.5 medical monitoring by physician outside of trial • Section 10.2 will not use UMCC SAE form but will used MedWatch3500 • Section 3.2 and 6.0 addition of sleep and mood surveys to be administered		
3/29/19	• Sec 8: The CRONO P is designed to be used with any commercially available infusion sets so that the decision of which set to use could be made by the healthcare professionals following the patient. Based on the pediatric patient population we will ONLY use the following infusion sets in this protocol, both of which are cleared for use in the United States: **a) Tandem Diabetes Care AutoSoft™ XC infusion set.** **b) Tandem Diabetes Care TruSteel™ infusion set.**		
5/20/19	• Add co-I, Bomberg • Sec 3.2 NIH took box includes Flanker assessment • Sec 5.1 confirmed 3 outpatient visits at Prism • Sec 6.0 add subject diary to study procedures, used through week 0-20 • Sec 6.2.1.1 increased to 3mL blood draws for total 75mL over visit period • Sec 6.2.1.1 added that Quest samples will be saved for Quest future research, if approved by participant, through consent • Sec 6.2.1.3 Week 6 removed surveys and NIH testing, added RN assessment		

	• Sec 6.2.1.4 instruction on use of blood pressure cuff, started on week 7 through 13 • Sec 6.2.1.4 Dilution will occur with 8 mL of 0.9% normal saline and NOT sterile water • Sec 6.2.1.4 added provision and instruction on blood pressure cuff • 6.2.1.6 Week 8, phone call from RN for assessment • 6.2.2.1 Week 9 outpatient visit includes physical exam and RN assessment. Addressed that after outpatient visit, RN will be making phone calls regarding pump, events, questions. Home visits Week 9-13 Add ACTH to home visits, RN assessments, actigraph provided • 6.2.2.2 Week 13 removed • Sec 6.2.2.2 Week 14 increased to Week 14 + 5 days in order to ensure adequate time period of intervention. Physical exam performed. Surveys & NIH toolbox repeated. • Sec 6.2.3.1 to ensure adequate time period of intervention, adjusted 15 weeks to 15 week + 1 day • Sec 6.2.3.2 week 19 is a home visit. Removed surveys, NIH toolbox tests • Sec 6.2.3.3 Week 20 separated out to include outpatient visit procedures (physical, surveys/NIH toolbox, assessments) • Removed study table, added new schedule of events table clarifying study procedure adjustments (on page 19) • Sec 7.1.2 updated sterile 0.9% saline to 0.9% normal saline for consistency in terminology • Sec 10.3.2 utilizing MedWatch 3500A for reporting		
7/3/2019	Clarification that only infusion set to use is now branded under the Unomedical/Animas® Inset™. This is brand equivalent to the Tandem Diabetes Care AutoSoft™ set and TruSteel™ set.The CRONO P is designed to be used with any commercially available infusion sets so that the decision of which set to use could be made by the healthcare professionals following the patient.		
8/1/2019	Clarification that the infusion set to be used in this study is the Comfort and Inset Subcutaneous Infusion Sets that are made by Unomedical (510K #: K051264)		
8/12/2019	Update schedule of events to note that study visits will have a time window of +/- 1 day	Na	na
9/10/2019	Page 19: updated schedule of events to include an unscheduled visit column Page 22: updated to include clarification that if pump treatment is delayed/stopped the pump will be resumed to meet the required 6-week of pump treatment	No	
11/14/2019	Increase to twelve participants consented to ensure analysis for eight completed participants (pgs 11, 12, 14) Specify that placement of pump is based upon evaluation of body composition to ensure best placement for subcutaneous infusion. (pgs 13, 19) Updated 6.2.2.1 regarding use of VEROFY for saliva sampling will be based upon availability from supplying company	YES	11/14/19

2/6/2020	Update to exclusion criteria in section 5.2.2 to expand body surface area to < 0.95m^2 or > 2.04 m^2	No	Na
10/13/2020	Remove the exclusion criteria regarding body surface area in section 5.2.2 to reflect the following explanation: *This amendment to the exclusion criteria is based on the rationale that body surface area should not preclude potential patients from participating in this study, as the appropriate dosing of the hydrocortisone through the pump is not based on the child's body surface area. Use of the pump is based on the need of the volume of hydrocortisone that can be delivered through the infusion pump. To ensure the pump volume is being met, the investigator(s) will review the standard total daily dose and calculate the needed dosing for each patient's pump volume. If the appropriate volume for dosage cannot be reached with the pump, the child will be excluded from participation until that minimum volume can be met.* Addition of exclusion criteria regarding the minimum total daily dose for the patient's HC regimen that is needed in order to provide the minimum volume needed for the pump dosing	No	Na
2/17/2021	Sec 5.2.1 updated to include children aged 3 years old (decreased from the prior minimum of 4 years old). This change is reflects the patient population that would meet all remainder of inclusion/exclusion criteria.	Yes (parent & adult)	2/17/2021
5/7/2021	In order to align the approved protocol (2/17/2021) this date's tracked changes are to update missed sections regarding the approved change from 4 years to 3 years old. See pages 12 and 14 in "all markup." Protocol remains under date of 2/17/2021.	No	NA
9/20/2021	Update location to Nucleus Network (formerly Prism Clinical Research) as Nucleus Network acquired Prism though conducting same procedures and at same physical location	No	

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1 Introduction

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is a rare form of adrenal insufficiency with a prevalence of 1:16,000,[1] characterized by impaired cortisol synthesis leading to excessive adrenal androgen production. The currently recommended oral hydrocortisone (HC) therapy for children with CAH[2] is unsatisfactory because it results in alternating hypo- and hypercortisolemia[3,4], thereby incurring increased risk for the deleterious effects of both these states. Hypocortisolemia triggers increased production of 17-hydroxyprogesterone (17OHP) and the adrenal androgen androstenedione (D4A), which can lead to premature fusion of the growth plates, genital virilization, precocious puberty, adrenal rests, polycystic ovarian syndrome and infertility.[5-9] Hypercortisolemia also has untoward long-term effects such as osteoporosis, short stature, and increased risk for cardiovascular disease in adulthood.[10-16] Current oral therapy is suboptimal due to resulting non-physiologic levels of cortisol and the inability to replicate circadian and ultradian cortisol secretion rhythms associated with normal adrenal function.[17-26] Therefore, an improved and personalized drug delivery system is needed[27] which more closely replicates physiological pulsatile cortisol secretion and limits periods of hyper- and hypocortisolemia in children with a resulting tighter control of adrenal androgens.

Various strategies to “optimize” glucocorticoid (GC) replacement therapy include modified or dual-release preparations,[28,29] *continuous* subcutaneous (SQ) infusions[30-33] and personalized treatment based on oral cortisol pharmacokinetic (PK) and 17OHP and D4A pharmacodynamic (PD) response studies. However, none of these strategies addresses that endogenous GCs are released in a *pulsatile* manner and that this ultradian rhythm is important in maintaining homeostatic control through GC receptor-dependent transcriptional regulation, which rapidly responds to changes in circulating cortisol levels.[17-20,25,34] The diametrically opposite effects of SQ pulsatile vs continuous hormone delivery are well described in other hormone systems. Pulsatile delivery of gonadotropin releasing hormone results in ovulation induction[35] whereas continuous delivery causes anovulation and suppresses the hypothalamic-pituitary-gonadal axis.[36,37] We propose to more closely replicate normal cortisol physiology by conducting the first clinical trial in children with CAH of a SQ hydrocortisone (SQHC) pump that will mimic pulsatility through interval bolus delivery system. An open-label, non-randomized crossover design will be used for this proof-of-concept study to inform the design of a larger clinical trial. Our **long term goal** is to improve clinical outcomes in children with CAH through optimizing the dosing and scheduling of replacement therapy and avoid the hyperandrogenemia that is specific to CAH. This **study's objective** is to demonstrate that a SQHC pump with interval bolus delivery more closely replicates circadian and ultradian rhythms of cortisol and improves control of adrenal androgens. Our **study's rationale** is that cortisol profiles more consistent with physiologic rhythms of cortisol secretion will produce better health outcomes.

2 Background and Significance

Congenital adrenal hyperplasia (CAH) and the inadequacy of current therapy.

Classic CAH due to 21-hydroxylase deficiency (21OHD) is a rare form of adrenal insufficiency (prevalence 1:16,000)[1] characterized by both impaired cortisol synthesis and excessive androgen production starting *in utero*. Normally, in the hypothalamic-pituitary-adrenal (HPA) axis a negative feedback loop exists wherein cortisol and adrenocorticotropic hormone (ACTH) are in a state of dynamic equilibrium. Hypocortisolemia in CAH leads to ACTH-driven adrenal gland stimulation where cortisol precursors, such as 17-hydroxyprogesterone (17OHP), are shunted to the androgen pathway as androgen synthesis does not require the 21-hydroxylase enzyme. In turn, this leads to excess production of adrenal androgens such as androstenedione (D4A) and testosterone (T) (**Fig. 1**).[38] Elevated D4A and T in utero and postnatally can cause masculinization of the external genitalia in both sexes. Untreated or poorly controlled females will continue to masculinize which in turn can lead to extensive and multiple corrective genital surgeries (**Fig. 2**).

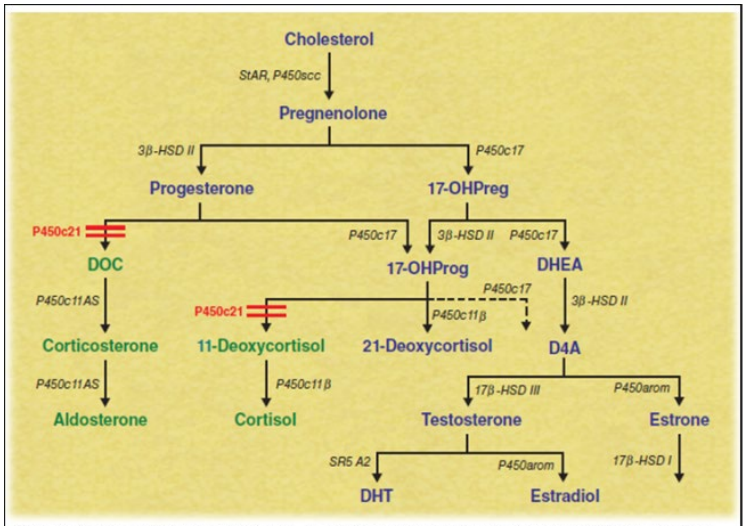

Fig. 1: Adrenal steroidogenesis in CAH due to 21-hydroxylase deficiency. Red bars represent the enzyme block. Increased metabolites are in blue. Decreased metabolites are in green (Sarafoglou, et al, 2017, In Press).

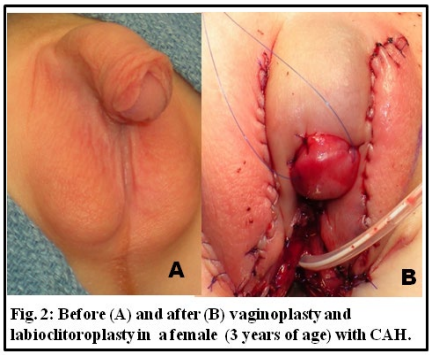

Fig. 2: Before (A) and after (B) vaginoplasty and labioclitoroplasty in a female (3 years of age) with CAH.

Patients with CAH require life-long glucocorticoid replacement. *The main treatment challenge for CAH* is to find the right balance of delivering sufficient glucocorticoid replacement to suppress overproduction of adrenal androgens (hyperandrogenemia), while avoiding the effects of glucocorticoid excess (hypercortisolemia). The current CAH consensus guidelines recommend treating children with oral hydrocortisone (HC; cortisol) at a dose of 10-15 mg/m^2/day given 3 times per day.[2] Response to HC therapy and disease control is evaluated by single measurements of 17OHP and/or D4A at clinic visits only every 3 to 6 months along with clinical impressions including growth velocity, weight gain, blood pressure, pubertal development and bone age maturation.[2] This practice provides limited information for several reasons: 1) Clinical findings reflect current drug therapy and adverse findings are a consequence of chronic over- and under- exposure to cortisol and androgen. While these findings may indicate the current HC regimen is inadequate, it does not indicate which specific aspects of the regimen (dose, timing, frequency) need to be changed. 2) Obtaining 17OHP and D4A concentrations three to four times a year does not provide a complete picture of disease control. These biomarkers are in a state of dynamic equilibrium in response to the HC dosing regimen and its negative feedback loop of the HPA axis. Blood samples obtained at various times after a dose can lead to highly variable concentrations, thereby confounding interpretation of 17OHP and D4A. 3) Single biomarker determinations only provide assessment of control at a single point in time and do not imply control over 24 hours.

Another challenge with HC therapy is its short median elimination half-life in CAH children of 58 min (range: 41-105 min) allowing most of the HC dose to be eliminated from the body within 4-5 hrs.[3,39] This is of particular concern as the cortisol concentration from the evening HC dose washes out over night resulting in unopposed ACTH-stimulated adrenal androgen production and significant hyperandrogenemia each morning when ACTH is at its peak (~0600). Because of HC's short half life, the recommended 3 times a day dosing schedule is inadequate and children are inevitably exposed to alternating periods of hypo- and hypercortisolemia throughout each day (**Fig. 3**). Hypocortisolemia leading to hyperandrogenemia, besides causing genital virilization, can also lead to premature fusion of the growth plates, precocious puberty, adrenal rests, polycystic ovarian syndrome, infertility and endothelial dysfunction.[5-9,40] Working memory performance and executive function are lower in children with CAH than in unaffected relatives[41,42] and quality of life is reported to be reduced.[43] *Hypercortisolemia* or overtreatment also have untoward long-term effects, such as osteoporosis, short stature, insulin resistance and increased risk for cardiovascular disease.[10-12,14-16,44,45] Increasing the challenge of treatment is the interindividual variability of pharmacokinetic (PK) and pharmacodynamic (PD) response to HC due to variations in glucocorticoid receptor and tissue sensitivity.[3,46,47] For example, about 7% of the general population harbor polymorphisms in the human glucocorticoid receptor that enhance responsiveness to GCs.[48]

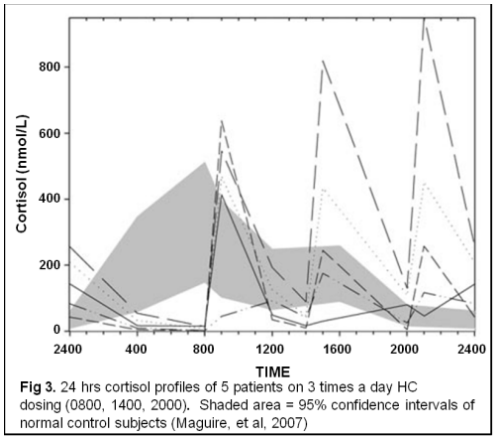

Fig 3. 24 hrs cortisol profiles of 5 patients on 3 times a day HC dosing (0800, 1400, 2000). Shaded area = 95% confidence intervals of normal control subjects (Maguire, et al, 2007)

Furthermore, cortisol PK in children with CAH differ from those individuals with other forms of adrenal insufficiency as their intermittently increased adrenal sex steroid production throughout the day could alter the enzyme activity of 11β-hydroxysteroid dehydrogenase (11β-HSD) isoenzymes and other enzymes that have a role in cortisol metabolism.[49-51] The interindividual cortisol PKPD variability and the shortcomings of the current recommended therapy and associated adverse clinical outcomes[10,11,52] underscore the importance of examining new ways of cortisol delivery that account for the inherently dynamic patterns of cortisol secretion and individual cortisol PK and 17OHP and D4A PD responses.

The importance of GC pulsatility for normal function of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is characterized by discrete pulses of ACTH and cortisol secretion. Cortisol secretion has a classically circadian pattern: cortisol levels reach nadir at midnight, begin to rise around 0200 hrs, peak early in the morning (cortisol awakening response) and gradually decrease throughout the day.[53,54] The circadian cortisol secretion pattern is actually derived from a dynamic ultradian rhythm of discrete cortisol pulses that follow ACTH pulses[55-57], with larger and more frequent pulses occurring early in the morning.[53,57] In humans, secretory pulses of cortisol occur every 80–110 min.[17,19,55,57,58] The mean number of pulses in 18 healthy subjects was 12±0.7, and the circadian and ultradian pattern of cortisol concentrations (lower panel) and derived cortisol secretory rate (upper panel) is shown for a representative volunteer in **Fig. 4**.[57] Ultradian cortisol rhythms can be generated through a mechanism independent of input from the hypothalamic circadian pacemaker (suprachiasmatic nucleus)[26] and are evolutionarily conserved across mammals, supporting the importance of pulsatility in normal GC signaling.[59-61]

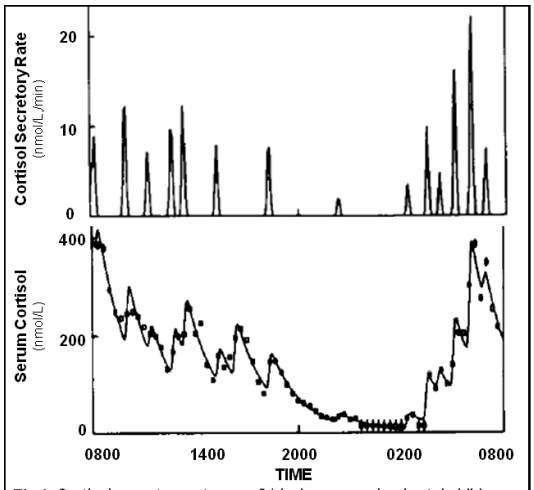

Fig 4. Cortisol secretory rate over 24 hr in a normal pubertal child (top); measured cortisol concentration in the same child (bottom). Both circadian and ultradian aspects of cortisol secretion can be seen. Kerrigan, et al, 1993

Pulsatile access of glucocorticoids to their receptors has been previously shown *in vitro* and *in vivo* in animal models to be of critical importance for gene regulation, non-genomic glucocorticoid signaling, HPA axis regulation, and endocrine and behavioral responses.[23,25,62-64,22] Pulsatile changes in plasma GC levels result in gene pulsing mediated by transient GC receptor activation, which rapidly responds to circulating hormone levels. Conversely, constant, non-oscillatory hormone levels result in continuous transcription, aberrant mRNA accumulation, abnormal protein levels, and GC resistance.[25,59] Rodent experiments show a differential c-fos response in the amygdala, hippocampus, and prefrontal cortex depending on a constant versus pulsatile pattern of GC presentation, underscoring the significance of gene pulsing.[23] The different and often completely opposite effects of pulsatile vs continuous hormone presentation is well described in other hormone systems. For example, pulsatile delivery of gonadotropin releasing hormone (GnRH) induces ovulation[35] whereas continuous delivery results in anovulation and suppression of the hypothalamic-pituitary-gonadal axis.[36,37] Deregulation of the ultradian GC rhythm influences gene transcription[63] and neuroendocrine responsiveness to GC challenge,[23,24] and alters electro-physiological properties of the hippocampus.[65] These effects can even be brain-region specific.[18] For example, the *Per 1* gene is rapidly and robustly induced after a single GC dose in the hippocampus in rodents, but requires two or three pulses before transcription can proceed in the prefrontal cortex.[18] Such tissue specificity likely allows differential physiological induction of appropriate genes under different conditions. Although yet to be determined, if such differential effects are present in cardio-metabolic tissues, this may have important implications for therapeutic development of ultradian delivery of GC replacement.[34,66] While recent advances in GC replacement have attempted to mimic the circadian pattern of cortisol secretion, we will be the first to use subcutaneous (SQ) hydrocortisone (HC) pump with interval bolus delivery to provide both circadian rhythm and ultradian pulsatility in children with CAH.

Alternative GC delivery modalities tested so far do not address cortisol pulsatility. Recent efforts to improve treatment of patients with adrenal insufficiency have focused on *continuous* SQHC infusions and modified release oral GC preparations, with mixed results. Studies, nearly all in adults,

have shown that SQHC via a pump is safe and can closely mimic the circadian rhythm.[30-33,67] Oksnes *et al*., found no differences in height, weight, blood pressure, and sleep patterns between oral and SQHC therapy, although Health Related Quality of Life was improved with SQHC.[31] Gagliardi *et al*., found no improvement in subjective health status.[32] A recent study in 8 adults by Nella, et al, found that that continuous SQHC was a well-tolerated and safe modality of HC replacement over 6 months resulting in improved adrenal steroid control and positive effects on quality of life and fatigue.[67] The study concluded earlier (childhood) intervention strategies are needed. Although all three studies tried to replicate circadian rhythm, they did not address *pulsatile*, ultradian rhythm of cortisol secretion. Johannson *et al*. showed modest improvement in blood pressure and weight reduction with a dual-release HC tablet therapy compared to immediate-release HC at the same daily dose (despite lower bioavailability) but patients were hypocortisolemic in the evening and early morning hrs.[28] Another twice daily modified-release HC preparation reduces the magnitude of hypocortisolemia at night, but again does not provide ultradian pulsatility.[29,68]

Current treatment guidelines for children with CAH[2] do not consider individual cortisol PK or adrenal steroid PD parameters, or the inherent circadian and ultradian patterns of cortisol secretion. The methodology used in this proposed research is conceptually and *in praxis* novel for 2 reasons. First, in contrast to studies that used *continuous* SQHC to parallel circadian rhythm,[30-33] we will be the first to use SQHC interval bolus delivery to capture physiologic cortisol circadian rhythm and ultradian pulsatility in children with CAH. Second, our study incorporates a single integrated PKPD model able to simultaneously describe the time courses of cortisol, 17OHP and D4A concentrations over 24 hours in CAH patients. Our approaches could result in an important therapeutic paradigm shift in treating children with CAH and significantly improve health outcomes, and establish a new standard of care for dosing of patients with any form of adrenal insufficiency.

3 Objectives

3.1 Primary Objectives

1. **To implement an interval bolus SQHC dosing regimen that more closely mimics cortisol, 17OHP and D4A circadian and ultradian rhythms than conventional oral HC dosing.** Up to twelve children (eight analyzable) with CAH, ages 4-18 yrs, will have 24-hr PKPD profiles of cortisol, 17OHP and D4A serum concentrations measured while on oral HC therapy (admission 1), during an initial trial of the SQHC pump (admission 2), and after 6 weeks of SQHC pump treatment (admission 3). An integrated PKPD model will be used to determine cortisol, 17OHP and D4A parameters to compare the duration of time subjects have these concentrations outside their acceptable ranges.

Hypothesis 1: Duration of hypocortisolemia and hypercortisolemia will be significantly shorter on SQHC pump therapy (admissions 2 and 3) than with conventional oral HC dosing (admission 1).

Hypothesis 2: Duration of time 17OHP and D4A serum concentrations are outside an acceptable range of suppression will be significantly shorter while on the SQHC pump (admissions 2 and 3) than with oral dosing (admission 1).

3.2 Secondary/Exploratory Objectives

1. Physical activity levels: Daily activity and sleep patterns will be assessed by wearing an Actigraph Link accelerometer (Pensacola, FL) for 7 full days (5 weekdays and 2 weekend days) during each study period.

2. Quality of Life and cognitive measures: Quality of life (QoL) measures including NIH toolbox will be measured four times during the study (baseline, and once during each study period). QoL will be measured through the validated PedsQL™ Generic Core Scale and the PedsQL™ Fatigue Scale. *NIH Toolbox Cognitive Tasks:* We will use NIH Toolbox Dimensional Change Card Sort Test (DCCS) from the computer-administered NIH Toolbox to measure children's attention and executive functioning and cognitive flexibility, along with the Flanker Inhibitory Control & Attention Test A validated sleep and mood survey will also be administered.

3. This proposal will include a utility assessment of a real-time (20 minutes) noninvasive point-of-care salivary testing device [VerOFy® cortisol (VCORT)] in a home setting. While we are not using VCORT to make dosing decisions or diagnoses and formal monitoring will be accomplished using serum cortisol sampling within the protocol, we believe it is important to determine the utility of such a device.

4. An analysis to determine whether PKPD system parameters change with the switch in the route of HC administration from oral to SQHC pump, and after six weeks of SQHC treatment. A preferred pharmacometric analysis for this outcome is to use nonlinear mixed-effects (NLME) regression modeling to estimate PKPD parameters from all data at weeks 1, 7 and 14 simultaneously.

4 Study Design

This is an open-label, non-randomized crossover feasibility trial which will compare oral HC administration with SQHC pump administration in pediatric patients with CAH. Up to twelve children (eight analyzable) with CAH, ages 3-18 years, will have serial cortisol, 17OHP, ACTH and D4A serum concentrations determined over 24 hours while taking conventional oral therapy (Admission 1).

Admission 2 will serve three purposes: a 33-hr SQHC pump evaluation period, patient and parent training with the pump, and a 24-hr PKPD study. During admission 2, we will use each patient's total daily dose (TDD) of HC to determine the doses to be delivered during the 33-hr period. The 24-hr schedule and percentage of the TDD of HC will be as follows: approximately 60% of the TDD of HC will be delivered in 3 equal pulses at 0300, 0600 and 0900. Another 35% will be delivered in 3 equal pulses at 1200, 1500 and 1800 and the remaining 5% at 2100 and 2400. Following delivery of the 9:00 pm, 12:00 am, and 3:00 am pulses, a 24-hr PKPD study will be initiated at the 6:00 am pulse using the same sampling schedule of cortisol, 17OHP, ACTH and D4A as the first admission. After the 24-hr serum sample at 0600 the next morning, the pump will be disconnected and the subject will be sent home on their usual oral HC dosing regimen.

After the SQHC pump therapy PKPD study during admission 2 at week 7, an analysis of the pattern of concentrations will be performed as a safety check. If the cortisol or adrenal steroid concentrations

are generally within acceptable ranges (defined in Aim 1), the same pump settings will be used when initiating the ambulatory pump phase at week 9. If concentrations are outside acceptable ranges, the initial SQHC pump settings will be adjusted from the Admission 2 dosing as needed to attain acceptable concentration ranges. Individual pulse doses may be increased or decreased but the frequency of pulses will be fixed to every 3 hours. After determining appropriate SQHC pump settings, the subjects will return to clinic at week 9 to initiate the 6-week SQHC ambulatory pump therapy phase.

6-WEEK PUMP TREATMENT

All subcutaneous pump settings of hydrocortisone (timing and dose) can only be set by the researchers. The SQ infusion site will be changed every 3 days. As there may be variability in drug absorption associated with different sites of SQ infusion therapy,[69,70].Each subject will be evaluated for appropriate site for SQ infusion (i.e. gluteal area, abdominal area) to ensure proper placement.. A clinician will establish contact every day for the first week via telephone. All patients will be seen at home twice each week during the first and second week at the time of injection site changes and once a week thereafter while on the pump to evaluate proficiency in changing the injection site; to collect blood for cortisol, 17OHP, ACTH, and D4A concentrations; and to inspect the injection site integrity.

Dose Adjustments During Pump Therapy. During the 6-week pump treatment, multiple cortisol, D4A, ACTH and 17OHP concentrations will become available and they may suggest, along with clinical impressions, that the pump dosing setting needs to be revised. It is critical to note that subjects will not be required to stay on the initial dosing schedule for 6 weeks. Rather, standard clinical practice will be used to guide SQHC pump dosing adjustments. Specifically, D4A and 17OHP concentrations will be evaluated along with clinical information provided by the patient that are indicative of hypocortisolemia such as fatigue, nausea, diaphoresis, low blood pressure, dizziness or headache. This is currently the standard of care for pediatric CAH patients. Should incongruous information be observed, clinical impressions take precedence along with D4A concentrations, and then 17OHP concentration. Cortisol concentrations are not generally part of routine patient care and will play a lesser role in dosing changes. The changes in dose settings may involve increasing or decreasing the HC TDD, or perhaps changing the percentage of TDD administered at a given time, but the 3-hr dosing intervals will remain fixed.

A 24-hr PKPD study will be performed during admission 3 (week 14) using the same sampling schedule as admission 2.

5 Subjects

5.1 Study Setting

There will be three admissions and three outpatient visits held at Nucleus Network (formerly Prism Clinical Research), a 52-bed early phase research site that specializes in complex, phase I and II clinical trials in healthy and numerous patient volunteer populations including pediatrics. It is located within 1 mile from the University of Minnesota campus. Aside from close proximity to the University of Minnesota, Nucleus Network (Prism Research) is located in the epicenter of the Minneapolis/St. Paul Metropolitan area with easy access for all residents to the facility as well as ample free parking for clinical research subjects.

Nucleus Network (formerly Prism Clinical Research) has performed studies in pediatric populations and collaborative trials with University of Minnesota Faculty. Nucleus Network (Prism Clinical Research) staff are trained in pediatric phlebotomy. Volunteers and their guardians will be provided a private bedroom with satellite television and wireless internet access that is convenient for both children and their guardians. Nucleus Network (formerly Prism Research) also provides games and movies suitable for all ages.

5.2 Eligibility Criteria

A total of 12 children (6 boys and 6 girls), aged 3-18 years, may be enrolled on this study, to ensure available analysis of 8 completed participants. This study entry is open to children regardless of gender or ethnic background.

5.2.1 Inclusion Criteria

- Children 3 - 18 years of age.
- Classic CAH as confirmed by hormonal and molecular testing.
- Patients who have been on the same HC dosing regimen for 1 month

5.2.2 Exclusion Criteria

- Patients with non-classic CAH.
- Patients on:
  - Dexamethasone
  - Prednisone, or
  - inhaled steroids.
- Patients with total daily dose of HC dosing regimen (min 10 mg) that cannot meet the required smallest volume to be delivered by the pump (0.2 mg)
- Non-English speaking patients

Inclusion of Children

The proposed study will enroll children 3-18 years of age. Justification for the inclusion of children in this study includes: 1) relevance of the disease to the pediatric population; 2) subcutaneous infusion pumps are currently used in the pediatric population to treat diabetes; 3) hydrocortisone succinate, the injectable form of oral hydrocortisone that will be used for hydrocortisone delivery in the subcutaneous infusion pumps, is currently used intramuscularly at home for children with CAH for emergency treatment or when children are sick and unable to take their medicine orally; 4) subcutaneous continuous delivery of hydrocortisone succinate has been used in adults with adrenal insufficiency and in an adolescent with CAH and was well tolerated and safe (Lovas and Husebye, Eur J Endocrinol, 2007; Gagliardi, et al., J Clin Endocrinol Metab, 2014; Oksnes, et al, J Clin Endocrinol Metab, 2014; Bryan, et al., J Clin Endocrinol Metab, 2009); 5) pulsatile delivery of hydrocortisone succinate was performed in normal adults without adverse effects in a study conducted by our collaborator Professor Stafford Lightman (Russell, et al., Clin Endocrinol, 2014); 6) The Crono P ambulatory subcutaneous infusion pump (CANÈ S.p.A. Medical Technology, Italy) used in Professor Lightman's study is the only pump specifically designed for preprogrammed (bolus) delivery of glucocorticoids. Inclusion of children in this trial is in accordance with 45 CFR part 46.406—research involving greater than minimal risk and no prospect of direct benefit to individual subjects, but likely to yield generalizable knowledge about the subject's disorder or condition.

Rationale for specific age range

We propose to include children 3-18 years of age because the sequelae of the disease and treatment with steroids such as early epiphyseal closure and short stature, precocious puberty, virilization, adrenal rests, polycystic ovarian disease, and increased risk for osteoporosis and developing metabolic syndrome-related atherosclerotic cardiovascular disease in adult life impact children at an early age and some of them may not be reversible. It is therefore important in this disease to improve treatment early in life to avoid these adverse outcomes.

Expertise of the investigative team for working with the age group

Dr. Kyriakie Sarafoglou, a pediatric endocrinologist at the University of Minnesota, and has worked extensively with children with CAH for over 20 years and established a dedicated multidisciplinary CAH center in 2005. Dr. Richard Brundage has modeled PK/PD systems in many pediatric studies. Dr. Megan Gunnar is Director of the Institute of Child Development at the University of Minnesota. Her work focuses on stress responses of the hypothalamic pituitary-adrenocortical axis and its dysregulation in children and adolescents under various adverse conditions She has extensively studied correlations between cortisol levels and neurobehavioral outcomes in children. Dr. Zan Gao's Kinesiology Laboratory focuses on field-based physical activity intervention to fight childhood obesity and application of modern technology in interventions to promote physical activity and health.

5.3 Recruitment

Subjects will be recruited from the current CAH patient population at the University of Minnesota Masonic Children's Hospital. The study will be offered to all subjects with CAH at their routine clinical visits.

Resources available

The multidisciplinary pediatric CAH center at the University of Minnesota, which is the primary referral center for the State and neighboring states, follows over 120 children. Over 50 children have already participated in 6 hour cortisol pharmacokinetic studies (Sarafoglou, et al., J Invest Med, 2015; Sarafoglou, et al., J of Clin Pharma, 2014). 98 children and their families with CAH have participated in an ongoing Dr. Sarafoglou's March of Dimes (#6-FY14-440) funded study "Can Molecular Testing Improve Newborn Screening Performance and Outcomes for Congenital Adrenal Hyperplasia?" Overall our experience is that this is a population eager to participate in clinical research because of limitations in current treatments. The PI will delegate much of the screening and recruitment responsibilities to a research nurse and research coordinator. The PI has dedicated research time in her faculty appointment, allowing ample time to provide appropriate oversight and review eligibility of potential participants with study staff.

Provisions to Protect the Privacy Interests of Participants

Protecting Privacy: The study consent form will describe in detail any intrusive, uncomfortable, or unfamiliar questions, procedures, or interactions with researchers or study personnel that the participant will be asked to complete. Furthermore, the study consent form will communicate that it is the participant's right to opt-out of any study procedures or the study as a whole or withdraw from the study at any time and this information will be reiterated and revisited periodically throughout the study in advance of intrusive, uncomfortable, or unfamiliar questions procedures or interactions. Participants will not be compelled or pressured to provide information or specimens or study data that they do not wish to provide.

Access to Participants: Participants have been fully informed of the ways in which their data will/may be used during the informed consent process. The research team has been trained in conducting these conversations and the participants are also assessed for their understanding of consent prior to signing the consent form or initiating any study procedures

Consent Process

Consent forms describing in detail the study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study product. Consent will take place at CAH clinic at the University of Minnesota Children's Hospital in a private and confidential room. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants will be given the opportunity to take the consent home to discuss with family and caregivers and return on a subsequent day to sign the consent and enroll in the study. Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with others or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Consent Process for Participants Who Are Not Yet Adults (infants, children, teenagers under 18 years of age): Prior to screening for the study each participant and parent(s)/legal guardian will be informed in detail about the study with its risks and discomforts to be expected. A written parental permission will be obtained from each patient to be involved in the clinical trial by using the IRB-approved Informed Consent Form (ICF) prior to the conduct of any study-related activity. Each participant will be given a copy of the written ICF. The participants will also be instructed that they are free to withdraw their consent and discontinue their participation in the study at any time. The assent from the child or adolescent will be obtained when this is appropriate and when the potential participant is capable of providing assent. The determination of appropriateness and capacity of children in the study to provide assent is made by the PI. For clinical investigations involving greater than minimal risk, permission is generally required from both parents unless only one parent has legal custody, or one parent is deceased, unknown, incompetent, or not reasonably available.

Compensation for Research-Related Injury

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to the participant or his/her insurance company.

6 Study Procedures

6.1 Screening/Baseline

Once consent has been obtained, the following tests and procedures will be done to determine if the subject is eligible:

- Physical Exam
- Medical History

A subject diary will be provided after consent. This diary will be used by the subject throughout the study to log items such as signs & symptoms, adverse events, medications and dosing.

6.2 Treatment period (weeks 1-20)

6.2.1 Weeks 1-8 (Oral HC)

6.2.1.1 Week 1

Once eligibility has been determined, subjects will be admitted into the research unit. Subjects will be admitted for approximately 36 hours. Subjects will have a physical exam and quality of life questionnaire, sleep and mood surveys and NIH toolbox cognitive domain measures administered for baseline values. During Admission 1, the subject's usual doses will be administered at 3 standardized dosing times for the PKPD study: 0600, 1200 and 1800. Changes from usual dosing times are expected to be small and inconsequential for the patient. Immediately prior to the morning dose at 6am, a 3-mL blood sample will be obtained for quantification of cortisol, 17OHP, ACTH and D4A. Samples will then be obtained at 0.5, 1, 1.5, 2, 3, 4, and 6 (noon) hrs after the first dose. The same sampling intervals will be used following the 1200 and 1800 doses, with additional samples collected during the night at 0200, 0400 and 0600. The total blood volume over 36 hrs will be 75 ml, which is well within the acceptable blood volume for this patient population and age range. If a patient is <35 kg, the blood draw will be adjusted based on weight. Also during admission, a 24-hour urine collection will be performed and an aliquot of 20mLwill be extracted from two timed 12 hour timed urine collections over the course of 24 hours. Urine samples will be stored under PI supervision for necessary analysis in the future. The IRB will be notified of these analyses when determined. In addition, a total of six saliva samples will be collected at: 0600 (prior to first dose), 0700, 1200 (prior to second dose), 1300, 1800 (prior to third dose) and 1900. Salivary samples will be sent to Quest Diagnostics for adrenal steroid measurements through LC-MS/MS assay. After processing, any remaining saliva samples (during week 1 and the remainder of the study) will be retained by Quest for future research, per subject/family consent. The nurse coordinator will review adverse events, medications and the subject's dairy

6.2.1.2 Weeks 1-6

Upon discharge, subjects will continue their standard of care oral administration of HC for 6 weeks.

6.2.1.3 Weeks 6

At the home visit, an Actigraph Link accelerometer will be provided and used to monitor daily activity and sleep patterns for one week. These will be monitored by study personnel. The nurse coordinator will complete their assessment and will review adverse events, medications and the subject's dairy.

6.2.1.4 Week 7 (SQHC Pump 33 hours)

At week 7, subjects will be re-admitted for Crono P subcutaneous HC (SQHC) infusion pump training in which the device will be used for 33 hrs and include a 24-hr PKPD study while on the SQHC pump. Training will emphasize proper aseptic catheter placement, care of the insertion site, reconstituting the solution and filling the infusion syringe. HC sodium succinate (SOLU-CORTEF) sterile powder will be used for the SQHC pump as it is FDA approved for intramuscular and intravenous routes for CAH and Addison's disease. HC sodium succinate comes in a two-compartment vial (Act-O-Vial) containing a 100 mg dose in the lower compartment and 2 ml sterile water in the upper compartment (100 mg/2 ml). Parents will be instructed to further dilute the solution with another 8 ml of 0.9% normal salineinto the infusion syringe, for a final concentration of 100 mg/10 ml (10 mg/ml). Parents will be trained to visually inspect the HC sodium succinate solution for particulate matter and discoloration. The parents of the children seen at the CAH Center are already familiar with the 100 mg Act-O-Vial as the IM HC sodium succinate injection is used at home in cases of acute stress or illness when the children are not able to take oral medication.

Subjects will have a physical exam. Pump therapy during this admission will begin at 9 pm and continue for 33 hours. We will use each patient's total daily dose (TDD) of HC to determine the doses to be delivered during the 33-hr period. The 24-hr schedule and percentage of the TDD of HC will be as follows: approximately 60% of the TDD of HC will be delivered in 3 equal pulses at 0300, 0600 and 0900. Another 35% will be delivered in 3 equal pulses at 1200, 1500 and 1800 and the remaining 5% at 2100 and 2400. Following delivery of the 9:00 pm, 12:00 am, and 3:00 am pulses, a 24-hr PKPD study will be initiated at the 6:00 am pulse using the same sampling schedule of cortisol, 17OHP, ACTH and D4A as the first admission. Urine collection will proceed as the first admission beginning at 0600. Saliva samples will be collected at the same time points as in admission 1 (0600, 0700, 1200, 1300, 1800, and 1900) irrespective of dosing. Salivary samples will be sent to Quest Diagnostics for adrenal steroid measurements through LC-MS/MS assay.

Subjects and families will be given and instructed on how to use an automatic, upper arm blood pressure monitor with a cuff appropriate for the child's size. Subjects will begin monitoring of daily blood pressure upon discharge (see section 9.3 for instructions), to week 13. The staff will review adverse events, medications and the subject's dairy. The Actigraph will be returned at this visit.

6.2.1.5 Weeks 7-8

After completing the 24-hr PKPD study, subjects will be discharged from the research facility and continue on their usual HC oral dosing schedule for 1 week prior to initiation of the SQHC pump. During this time, cortisol, 17OHP, and D4A concentrations will be analyzed to determine that the pump has delivered HC as expected and cortisol, 17OHP, and D4A concentrations are in an acceptable range. SQHC will be recalculated as needed before initiating the pump if cortisol, 17OHP, or D4A concentrations are not generally in a desired range.

6.2.1.6 Week 8

The nurse coordinator will call the subject/family to review daily blood pressure readings, adverse events, medications and the subject's dairy.

6.2.2 Weeks 9-14 (SQHC Pump)

6.2.2.1 Weeks 9-13

Week 9 (outpatient visit) The patient will meet Dr. Sarafoglou and the study nurse coordinator at Nucleus (Prism) for initiating the 6-wk SQHC pump phase. The patient will have a physical exam and the nurse coordinator will review the patient's recorded daily blood pressure, subject diary, adverse events and any medications taken. It is our standard policy to make sure that families have parenteral hydrocortisone to use during emergencies. We will instruct the patients to call Dr. Sarafoglou before administering any parenteral dose during this phase. After the first four supervised home visits by the nurse coordinator, the family, as instructed will replace the HC infusion syringe and solution, and the site of SQ infusion every 72 hours. As with insulin, we recognize that there may be variability in drug absorption associated with different sites of SQ injection pump infusion therapy. Each subject will be evaluated for appropriate site for SQ infusion (i.e. gluteal area, abdominal area).. Parents will be instructed to visually inspect the parenteral HC solution for particulate matter and discoloration prior to administration into the pump.

After the outpatient visit, during week 9, the subject/family will be called on the days that the RN coordinator is not completing home visits. This call is the review pump use, adverse events and other questions or concerns.

Weeks 9-13: The nurse coordinator will visit subjects at home seven times for safety monitoring, which includes a nursing assessment, and measurement of cortisol, 17OHP, ACTH and D4A concentrations during the SQHC pump period. During the home visits, along with the blood draws, saliva will be collected and measured through VerOFy® cortisol (VCORT) with the remaining salivary sample sent to Quest Diagnostics for free cortisol measurements through LC-MS/MS assay. VCORT is IDE exempt per the requirements outlined in 21CFR812.2(c) and in 21CFR809.10(c). The nurse coordinator will complete their assessment and review the patient's recorded daily blood pressure, subject diary, adverse events and any medications taken. At Visit 13, an Actigraph Link accelerometer will be provided for monitoring daily activity and sleep patterns for one week.
To note, the use of the VerOFy® may be restricted based upon the company's availability to provide the device to our site site. As noted under the secondary/exploratory objectives, the VerOFy® will not be used to "make dosing decisions or diagnoses and formal monitoring will be accomplished using serum cortisol sampling." We therefore will collect and record results only at time of availability for use of the device. The informed consent will notify participants/families that this sampling and measurement will be based upon when the device is available at the time of study visit..

6.2.2.2 Week 14 + 5 days

At week 14 + 5days, subjects will be admitted for a final SQHC pump 24-hr PKPD study. A physical exam will be performed. This study will occur at the 6:00 am pulse using the same sampling schedule of cortisol, 17OHP, ACTH and D4A as the previous two admissions. Urine collection will proceed as the first admission. Saliva samples will be collected at the same time points as in admission 1 and 2. Salivary samples will be sent to Quest Diagnostics for adrenal steroid measurements through LC-MS/MS assay. QoL, sleep and mood surveys and NIH toolbox cognitive domain measures will be repeated. Once the PKPD study is complete, subject will discontinue use of the pump and resume their usual oral HC regimen. The subject's diary, adverse events and medications taken will be reviewed. The Actigraph will be returned.

6.2.3 Weeks 15 (+ 1 day)-20 (Oral HC follow-up)

6.2.3.1 Week 15 (+ 1 day)-18

We will continue to follow subjects with weekly phone calls while they are on their usual oral HC dose regimen. The coordinator will discuss any adverse events and medications taken, along with reviewing the subject's diary.

6.2.3.2 Week 19

The nurse coordinator will visit the subject at home. The will include a nurse coordinator assessment that includes reviewing the patient's diary, adverse events and medications taken. An Actigraph Link accelerometer will be provided and used to monitor daily activity and sleep patterns for one week. These will be monitored by study personnel.

6.2.3.3 Week 20

Outpatient visit. Subjects will have a physical exam. Quality of life questionnaire, sleep and mood surveys and NIH toolbox cognitive domain measures administered. Patient's diary will be reviewed and collected by the study staff. Adverse events and medications will be reviewed.

Schedule of Events

Week	0	1	6	7	8	9	9 #1	9 #2	10 #1	10 #2	11	12	13	14 + 5 Days	15+1 Day - 18	19	20	Unscheduled Visit[f]
Med	Oral HC			SQHC (1day)	Oral HC	Home SQHC Pump									Oral HC			
Visit Type	Outpatient (UMMC)	Overnight	Home	Overnight	Phone	Outpatient (PRISM)	Home	Home	Home	Home	Home	Home	Home	Overnight	Phone	Home	Outpatient (PRISM)	Home/ Outpatient
Consent	X																	
Eligibility	X																	
Medical History	X																	
MD Exam	X	X		X		X								X			X	X
Tanner Staging	X																X	
RN Assessment			X				X	X	X	X	X	X	X	X		X		X
Pump/Syringe Education				X		X	X	X	X	X								X
QoL, Mood, Sleep Surveys		X												X			X	
Cognitive Tests		X												X			X	
Actigraphy[a]			X										X			X		
PKPD sampling[b]		X		X										X				
24hr urine collection[c]		X		X										X				
Cortisol, 17OHP, D4A, ACTH							X	X	X	X	X	X	X					X
VerOFy cortisol (saliva)							X	X	X	X	X	X	X					X
Saliva (to Quest)		X		X			X	X	X	X	X	X	X	X				X
Daily BP				X	X	X	X	X	X	X	X	X	X					X
Subject Diary	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Con Meds	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Phone Call					X	X[d]									X[e]			

NOTES:

[a] Actigraph to be provided at visit for use for 7 days, returning at next visit

[b] PKPD: samples to Quest for cortisol (free & total), ACTH plasma, 17-OHP, D4A, & ACTH at all timepoints

[c] Urine collection of 20 mL q12h, store frozen at -80C

[d] Phone call will occur daily during week 9 (except for outpatient & home visit days)

[e] Phone call will occur once a week for weeks 15 through 18

[f] any of the study activites noted below may be performed per investigator discretion

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* Schedule of events will follow as described above with a time window of +/- 1 day for completion of study visits

6.2.4 Subject Withdrawal

Subjects make be taken off the study by the Principal Investigator for the following reasons:

- Safety reasons
- Failure to adhere to the protocol requirements
- Subject withdraws consent
- The study terminates early.

Subjects will be instructed not to stop taking the cortisol if they withdraw from the study. Subjects receiving SQHC pump therapy will be asked to return to the clinic for pump removal and to resume taking oral steroids. If subjects are willing, we will ask them to complete the follow up visit at the time they withdraw from study to capture any final data.

7 Study Drug

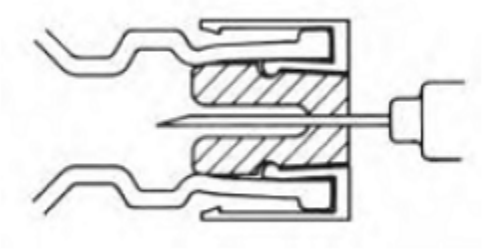
7.1 Solu-Cortef® (hydrocortisone sodium succinate for injection)

7.1.1 Description

SOLU-CORTEF Sterile Powder is an anti-inflammatory glucocorticoid, which contains hydrocortisone sodium succinate as the active ingredient. SOLU-CORTEF Sterile Powder is available in several packages for intravenous or intramuscular administration. Our patients use the single-dose 100 mg ACT-O-VIAL that reconstitutes the hydrocortisone powder to provide a final concentration of 100mg/2 mL .

DIRECTIONS FOR USING THE ACT-O-VIAL SYSTEM

1. Press down on plastic activator to force diluent into the lower compartment.
2. Gently agitate to effect solution.
3. Remove plastic tab covering center of stopper.
4. Sterilize top of stopper with a suitable germicide.
5. Insert needle squarely through center of stopper until tip is just visible. Invert vial and withdraw dose.



Further dilution is not necessary for intravenous or intramuscular injection. For intravenous infusion, first prepare solution as just described. **The 100 mg** solution may then be added to 100 to 1000 mL of 5% dextrose in water (or isotonic saline solution or 5% dextrose in isotonic saline solution if patient is not on sodium restriction). The **250 mg** solution may be added to 250 to 1000 mL, the **500 mg** solution may be added to 500 to 1000 mL and the **1000 mg** solution to 1000 mL of the same diluents. In cases where administration of a small volume of fluid is desirable, 100 mg to

3000 mg of SOLU-CORTEF may be added to 50 mL of the above diluents. The resulting solutions are stable for at least 4 hours and may be administered either directly or by IV piggyback.

When reconstituted as directed, pH's of the solutions range from 7 to 8 and the tonicities are: 100 mg ACT-O-VIAL, .36 osmolar; 250 mg ACT-O-VIAL, 500 mg ACT-O-VIAL, and the 1000 mg ACT-O-VIAL, .57 osmolar. (Isotonic saline=.28 osmolar.)

7.1.2 Dosage and Administration

The parents of our children are already familiar with as the 100 mg ACT-O-VIAL as IM hydrocortisone injection is used at home in cases of acute stress or illness when the children are not able to take oral medication. Parents will be instructed to further dilute the solution with another 8 mL of 0.9% normal saline into the infusion syringe and visually inspect the hydrocortisone solution for particulate matter and discoloration.

The patients will receive their usual oral hydrocortisone dosing throughout and the first treatment period until the SQHC pump is initiated at admission 2. During admission 2, we will use each patient's total daily dose (TDD) of HC to determine the doses to be delivered during the 33-hr period. The infusion pump will be set to rapidly deliver a hydrocortisone pulse dose every 3 hr to attain an ultradian rhythm. The 24-hr schedule and percentage of the TDD of HC will be as follows: approximately 60% of the TDD of HC will be delivered in 3 equal pulses at 0300, 0600 and 0900. Another 35% will be delivered in 3 equal pulses at 1200, 1500 and 1800 and the remaining 5% at 2100 and 2400.After the 24-hr serum sample at 0600 the next morning, the pump will be disconnected and the subject will be sent home on their usual oral HC dosing regimen. If concentrations are outside acceptable ranges, the initial SQHC pump settings will be adjusted from the Admission 2 dosing as needed to attain acceptable concentration ranges. Individual pulse doses may be increased or decreased but the frequency of pulses will be fixed to every 3 hours. After determining appropriate SQHC pump settings, the subjects will return to clinic at week 9 to initiate the 6-week SQHC ambulatory pump therapy phase.

During the 6-week pump treatment, multiple cortisol, D4A and 17OHP concentrations will become available and they may suggest, along with clinical impressions, that the pump dosing setting needs to be revised. It is critical to note that subjects will not be required to stay on the initial bolus dosing schedule for 6 weeks. Rather, standard clinical practice will be used to guide SQHC pump dosing adjustments. Specifically, D4A and 17OHP concentrations will be evaluated along with clinical information provided by the patient that are indicative of hypocortisolemia such as fatigue, nausea, diaphoresis, low blood pressure, dizziness or headache. This is currently the standard of care for pediatric CAH patients. Should incongruous information be observed, clinical impressions take precedence along with D4A concentrations, and then 17OHP concentration. Cortisol concentrations are not generally part of routine patient care and will play a lesser role in dosing changes. The changes in dose settings may involve increasing or decreasing the HC TDD, or perhaps changing the percentage of TDD administered at a given time, but the 3-hr dosing intervals will remain fixed.

Every 72 hours, the family will be instructed to replace the hydrocortisone infusion syringe and solution, and the site of subcutaneous infusion will be changed.

Parents will be instructed to visually inspect the parenteral hydrocortisone solution for particulate matter and discoloration prior to administration into the pump cassette. Parents are already familiar with this process as IM hydrocortisone injection is used at home in cases of acute stress or illness when the children are not able to take oral medication.

7.1.3 Dosage adjustments

The following dosage adjustments may be made during the course of the study in the event of intercurrent illness, pump failure or symptoms of adrenal insufficiency occur.

- For fever > 101 degrees F: add oral hydrocortisone to the patient's regimen, which will be double the amount of the daily dose that the patient receives through the SQ pump. The additional oral stress hydrocortisone dose will be divided in 4 equal doses every 6 hours. The subject's family will be instructed to administer an antipyretic (Tylenol, ibuprofen, etc).
- For a serious physical injury or broken bone(s), administer a stress dose of hydrocortisone as described above.
- In the event of severe illness, trauma, or inability to tolerate oral hydrocortisone, unconsciousness, or repeated vomiting: 75 mg/m2/day of Solucortef intramuscular injection will be administered immediately. The family will be instructed to seek Emergency Department care to initiate IV hydrocortisone and fluid replacement as they typically do as part of the standard therapy to prevent adrenal crisis.
- In the event of pump failure, patients will be instructed to resume their usual oral hydrocortisone dose until a replacement pump is given to the family.
- In the event of an occlusion that results in missed hydrocortisone dosing, patients will be instructed to change the infusion set and notify the investigators. If occlusion persists, patients will be instructed to resume their usual oral hydrocortisone dose until occlusion is resolved.
- A pump malfunction that results in delivery of the entire reservoir (worst case scenario 100 mg of hydrocortisone) is not a life threatening event as children receive similar or greater amounts of hydrocortisone during illness. If the entire reservoir is delivered, patients will be instructed to notify the investigators and return to their usual oral hydrocortisone dosing until a replacement pump is given to the family.

At all times the PI will be available to the families. If pump therapy is stopped at any point the investigator will evaluate the participant and re-initiate the pump treatment when deemed clinically appropriate. Once the pump is resumed, pump treatment will be continued in order to meet the total 6 weeks of pump treatment.

7.2 Cortef® hydrocortisone tablets

7.2.1 Description

Cortef Tablets contain hydrocortisone which is a glucocorticoid. Glucocorticoids are adrenocortical steroids, both naturally occurring and synthetic, which are readily absorbed from the gastrointestinal tract. Hydrocortisone USP is white to practically white, odorless, crystalline powder with a melting point of about 215° C. It is very slightly soluble in water and in ether; sparingly soluble in acetone and in alcohol; slightly soluble in chloroform.

7.2.2 Dosage and Administration

Patients will be receiving their usual dosing regimen of hydrocortisone tablets during the first treatment period.

7.3 Receiving, Storage, and Dispensing

7.3.1 Receipt of Drug Supplies

Upon receipt of the of the study treatment supplies, an inventory will be performed and a drug receipt log filled out and signed by the person accepting the shipment. The designated study staff will count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files.

7.3.2 Storage

Store unreconstituted product at controlled room temperature 20° to 25(C(68° to 77(F). Store solution at controlled room temperature 20° to 25(C (68° to 77(F) and protect from light. Use solution only if it is clear. Unused solution should be discarded after 3 days.

7.3.3 Dispensing of Study Drug

Drug will be prepared and dispensed by the Investigational Drug Services Pharmacy at the Nucleus Network (Prism Clinical Research) center.

8 Study Device- *CRONO* P

CRONO P is an ambulatory infusion pump with reservoir*,* intended for bolus subcutaneous administration for long-term treatment with glucocorticoids.

A mechanism pushes against the reservoir's rubber plunger, enabling the pump to combine high delivery pressure with excellent precision while administering the drug.

An innovative infusion control system allows the pump to automatically restart and finish an infusion after an occlusion has been removed.

CRONO P has a liquid crystal display (LCD) which provides the doctor and patient with useful information regarding the settings, operations and diagnostics of the pump.

The CRONO P is designed to be used with any commercially available infusion sets with luer lock connector so that the decision of which set to use could be made by the healthcare professionals following the patient. For this protocol we are going to use the following set:

Comfort and Inset Subcutaneous Infusion Sets made by Unomedical (510K #: K051264)
The Unomedical infusion set uses the standard luer lock which is compatible with all pumps that use a luer lock, like the Cane` Crono P that we are using in our study. The Unomedical set is commercially available and cleared for use in the United States. This infusion Set is an all-in-one straight-set infusion system. The 90-degree soft cannula is pre-loaded in an automatic spring inserter, to allow for one-handed insertions and it also has a built-in needle cover.

9 Risks and Benefits

9.1 Potential Risks

Risks of Hydrocortisone

While there are risks associated with chronic corticosteroid use, this CAH patient population does not produce adequate levels of cortisol and therefore require this treatment for survival. Patients with CAH, whether in the study or not, take corticosteroids every day of their life. The potential risks specific to this study are mainly dermatologic: Allergic dermatitis, cutaneous and subcutaneous atrophy, erythema, hyperpigmentation, hypopigmentation, rash, sterile abscess, striae, urticaria.

Potential risks of hydrocortisone in children and adults not specific to our study population and study design include:

- Gasping syndrome due to the presence of benzyl alcohol in the injectable cortisol.
- Allergic reactions: allergic or hypersensitivity reactions, anaphylactoid reaction, anaphylaxis, angioedema.
- Cardiovascular: Bradycardia, cardiac arrest, cardiac arrhythmias, cardiac enlargement, circulatory collapse, congestive heart failure, fat embolism, hypertension, hypertrophic cardiomyopathy in premature infants, myocardial rupture following recent myocardial infarction (see WARNINGS), pulmonary edema, syncope, tachycardia, thromboembolism, thrombophlebitis, vasculitis.
- Dermatologic: Acne, allergic dermatitis, burning or tingling (especially in the perineal area, after intravenous injection), cutaneous and subcutaneous atrophy, dry scaly skin, ecchymoses and petechiae, edema, erythema, hyperpigmentation, hypopigmentation, impaired wound healing, increased sweating, rash, sterile abscess, striae, suppressed reactions to skin tests, thin fragile skin, thinning scalp hair, urticaria.
- Endocrine: Decreased carbohydrate and glucose tolerance, development of cushingoid state, glycosuria, hirsutism, hypertrichosis, increased requirements for insulin or oral hypoglycemic agents in diabetes, manifestations of latent diabetes mellitus, menstrual irregularities, secondary adrenocortical and pituitary unresponsiveness (particularly in times of stress, as in trauma, surgery, or illness).
- Fluid and electrolyte disturbances: Congestive heart failure in susceptible patients, fluid retention, hypokalemic alkalosis, potassium loss, sodium retention.
- Gastrointestinal: Abdominal distention, bowel/bladder dysfunction (after intrathecal administration), elevation in serum liver enzyme levels (usually reversible upon discontinuation), hepatomegaly, increased appetite, nausea, pancreatitis, peptic ulcer with possible perforation and hemorrhage, perforation of the small and large intestine (particularly in patients with inflammatory bowel disease), ulcerative esophagitis.
- Metabolic: Negative nitrogen balance due to protein catabolism.
- Musculoskeletal: Aseptic necrosis of femoral and humeral heads, Charcot-like arthropathy, loss of muscle mass, muscle weakness, osteoporosis, pathologic fracture of long bones, postinjection flare (following intra-articular use), steroid myopathy, tendon rupture, vertebral compression fractures.
- Neurologic/Psychiatric: Convulsions, depression, emotional instability, euphoria, headache, increased intracranial pressure with papilledema (pseudotumor cerebri) usually following discontinuation of treatment, insomnia, mood swings, neuritis, neuropathy, paresthesia, personality changes, psychic disorders, vertigo. Arachnoiditis, meningitis,

paraparesis/paraplegia, and sensory disturbances have occurred after intrathecal administration (see WARNINGS, Neurologic).
- Ophthalmic: Exophthalmoses, glaucoma, increased intraocular pressure, posterior subcapsular cataracts, rare instances of blindness associated with periocular injections.
- Other: Abnormal fat deposits, decreased resistance to infection, hiccups, increased or decreased motility and number of spermatozoa, injection site infections following non-sterile administration (see WARNINGS), malaise, moon face, weight gain.

9.2 Potential Risks of the CRONO P Device

The major risk of this device that could cause serious harm to the patient is the use of incorrect settings and/or incomplete understanding of the operational functions and of the alarms.

9.3 Methods to minimize risk

Subjects will be monitored as per standard of care during treatment with oral cortisol. During the treatment with the SQHC pump, subjects will be monitored inpatient through physical exams for the first 36 hours. Following discharge, for the first four changes in SQ infusion site, a nurse coordinator will travel to the patient's home to evaluate proficiency in changing the injection site; to collect blood for cortisol, 17OHP, ACTH and D4A concentrations; and to inspect the injection site integrity. All patients will be seen at home twice during the first week and second week and once a week thereafter while on the pump. As an additional safety measure for monitoring pump failure that does not trigger an alarm, families will be given and instructed on how to use an automatic, upper arm blood pressure monitor with a cuff appropriate for the child's size. One week prior to the SQHC pump treatment, patients while on oral HC will measure and log child's blood pressure daily in the morning, after 5 minutes rest. When the child is on the pump, families will be instructed to measure and log the child's blood pressure daily. If the mean arterial pressure [MAP = [(2 x diastolic)+systolic] / 3] is less than 20 percent of the lowest MAP observed during oral treatment, the blood pressure will be measured 2 more times at 5-minute intervals. Instructions will be provided to the families to notify the PI if 2 of the 3 MAP measurements are below 20 percent of the lowest MAP obtained during oral HC treatment. Parents will be asked to inspect the injection site, tubing, and syringe volume. If no issues are identified and if the blood pressure remains outside the appropriate range after three measures, the parents will be instructed to bring the child to the clinic for clinical evaluation and assessment of pump function. Subjects and their parents will be instructed to contact the Principal Investigator immediately if they develop any signs of symptoms of adrenal insufficiency (e.g. fatigue, nausea, dizziness). The PI can be reached 24 hours a day by her pager number.

The parents of the subjects will be thoroughly trained on the use of the pump, the infusion sets which will be used, and the operational functions of the alarms.

9.4 Potential Benefits

Based upon guidance provided in 45 CFR 46, subpart D regarding research in children, the proposed study presents greater than minimal risk without direct benefit to the individual subject (45 CFR part 46.406). However, this study has potential to yield generalizable knowledge about the subject's pharmacokinetics and dynamic response (45 CFR 46.406) to cortisol. An indirect benefit is this alternative pulse dosing strategy could spur the development of novel methods of HC drug delivery that could improve long term outcomes of patients with CAH.

10 Safety and Adverse Events

10.1 Definitions

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries will be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs a hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality will be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

10.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

10.3 Reporting of Serious Adverse Events

10.3.1 IRB Notification by Investigator

Reports of all serious adverse events (including follow-up information) must be submitted to the IRB within 5 working days if it falls under the UPIRTSO guidelines. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder.

10.3.2 FDA Notification by Sponsor

The study sponsor shall notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the sponsor's original receipt of the information.

If a previous adverse event that was not initially deemed reportable is later found to fit the criteria for reporting, the study sponsor will submit the adverse event in a written report to the FDA as soon as possible, but no later than 15 calendar days from the time the determination is made.

Agency	Criteria for Reporting	Timeframe	Form to Use	Submission address/fax numbers
U of MN IRB	SAE: fatal, life-threatening or serious, unexpected, at least possibly related	5 working days	MedWatch 3500A Form	MMC 820
FDA	SAE: fatal, life-threatening, unexpected, at least possible related	7 calendar days	MedWatch 3500a Form but alternative formats are acceptable (e.g. summary letter)	Fax: 1 (800) FDA - 0178
	SAE: serious, unexpected, at least possibly related	15 calendar days		

10.4 Stopping Rules

Subjects will be removed from the study if the following occur:

- An infection at the site of SQ cannulation. On the first and second occasion of an infected site, the cannula will be changed and moved to a new SQ location, and the family will receive additional training in proper hygienic technique. If infection occurs on more than two occasions, poor hygienic technique might be a cause and the patient will be removed from the study.
- A systemic allergic reaction at the site of the SQ infusion cannula; if this happens we will discontinue using the pump and return a child to their oral HC therapy.
- If a patient experiences more than two episodes of adrenal crisis (defined as a constellation of signs and symptoms caused by low cortisol that include: lethargy, hypoglycemia, low blood pressure, vomiting/nausea, abdominal pain, weakness, dizziness and cardiovascular collapse) in the SQ treatment period, the patient will be removed from the study. Periods of increased stress or illness can result in adrenal crisis and can occur in any CAH patients at any time. The adrenal crisis would be attributed to the pump if there are no other triggers such as trauma, febrile illness, gastroenteritis. Patients will be instructed to take stress dose oral steroids during physical stress as per emergency protocol that all children with CAH receive as part of standard care, in addition to the cortisol provided by the SQ pump

10.5 Medical Monitoring

The Principal Investigator will oversee the safety of the study at the University of Minnesota site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Medical monitoring will include a regular assessment of the number and type of adverse events.

This study will have a medical monitor, a University of Minnesota physician, not involved in the trial. The medical monitor will review adverse events, Serious Adverse Events, and any other event considered to be reportable to the University of Minnesota Institutional Review Board. The Principal Investigator will submit copies of these reports to the medical monitor.. The medical monitor will summarize its findings and/or recommendations in a letter to the Principal Investigator. The Principal Investigator will submit a copy of the monitor's report to the Institutional Review Board per their policy.

11 Statistical Considerations

11.1 Primary Outcomes Measure

Primary objective 1: Time periods of hyper- and hypocortisolemia and over- and under-suppression of 17OHP and D4A will be compared between the oral and SQHC PKPD admissions using a paired t-test (with times log-transformed if needed to satisfy the normality assumption). A sample of 8 children gives 80% power to detect a population-average difference in outcome (undesired time period) between oral HC and SQHC pump of 1.2 times the standard deviation describing variation between children in the outcome for oral HC minus the outcome for SQHC pump (Cohen's d=1.2). This study design and analysis have not been performed to our knowledge in humans or animals, and data to estimate this standard deviation do not exist.

11.2 Secondary Outcomes Measures

Statistical analysis for secondary measures of actigraph, quality of life, sleep survey and NIH toolbox: Descriptive statistics for each outcome variable will be provided and used to discern changes in the outcome variables. Repeated measures ANOVAs will be used to examine differences in each outcome variable from Oral HC to SQHC Pump, and then back to Oral HC.

Statistical analysis for VCORT: Correspondence between VCORT and salivary cortisol by LC-MS/MS (56 samples total) will be described using a scatter plot identifying the child who provided each measurement; the intra-class correlation (ICC) describing association of VCORT with LC-MS/MS; a test for bias of VCORT relative to LC-MS/MS using a paired t-test; and percentiles of absolute differences between the VCORT and LC-MS/MS measurements (e.g., "90% of differences were less than X").

The Likelihood Ratio Test will be used to determine the statistical significance of the changes in PKPD parameters. The difference between objective function values (OFV; a goodness of fit measure similar to a sum of squares) is approximately chi-square distributed and if the OFV decreases by more than 3.8 (chi-square, p=0.05, df=1) the model with the added parameter is significantly better. No formal power calculations are currently available for the NLME modeling approach. If a persistent difference within subjects exists among the TVCL estimates across the 3 PKPD treatment arms, it will be detected as a significant difference among THETA1, THETA2 and THETA3.

12 Administrative Considerations

12.1 Conduct of the Trial

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP), the Declaration of Helsinki, and the appropriate regulatory requirement(s).

The University Of Minnesota IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB/EC or Campus Administrator approval has been obtained. The protocol, informed consent, written information given to the patients, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

12.2 Data Management

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Data generated by the methods described in the protocol will be recorded on paper forms and transcribed to electronic case report forms (eCRFs).

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write “N/D”. If the item is not applicable to the individual case, write “N/A”. Electronic data capture will be utilized for case report forms. Data will be entered into a secure, password-protected REDCap database set up for this study and administered at the University of Minnesota.

Confidentiality – Data Security

All data obtained from this study will be linked to a study specific code and not to personal identifying information (e.g., name, social security number, medical record number). Data will be entered into REDCap database which will be password secured, and encrypted and only accessible to the investigator and research staff of this project. The REDCap database uses a MySQL database via a secure web interface with data checks used during data entry to ensure data quality. REDCap includes a complete suite of features to support HIPAA compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL database and the web server will both be housed on secure servers operated by the University of Minnesota Academic Health Center’s Information Systems group (AHC-IS). The servers are in a physically secure location on campus and are backed up nightly, with the backups stored in accordance with the AHC-IS retention schedule of daily, weekly, and monthly tapes retained for 1 month, 3 months, and 6 months, respectively. Weekly backup tapes are stored offsite. The AHC-IS servers provide a stable, secure, well-maintained, and high-capacity data storage environment, and both REDCap and MySQL are widely-used, powerful, reliable, well-supported systems. Access to the study's data in REDCap will be restricted to the members of the study team by username and password.Only members of the study team will be granted access to the REDCap database and will receive protocol and data entry training prior to access being granted. Study data will not be shared outside the study team. The consent and study data will not be placed in medical, employment, or educational records.

Data Banking

Storage and Access: Data will be stored indefinitely in the study REDCap database. Only the study team will have access to these records for the duration of storage.

Data: This data set will include no identifying information, simply the study ID and participant name as maintained in paper form as part of the participants file (original signed ICF, enrollment CRF).

Release/Sharing: Data will not be released or shared outside the study team

Sharing of Results with Participants

Data will not be released or shared outside the study team.

12.3 Data Monitoring

Data will be monitored by the clinical monitors at the University of Minnesota Clinical and Translational Science Institute. This study will be monitored according to FDA/GCP guidelines. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

12.4 Event Reporting to the IRB

Safety concerns for this project are expected to be minimal with *(with list of any expected risk(s))* as the primary expected concern. Any events meeting an unexpected, serious adverse event defined as reportable (such as hospitalization) on the IRB's website at http://www.research.umn.edu/irb/ae/.

In addition, to be in compliance with local and federal regulations the following events/problems will be reported to the IRB within the 5 working day time frame:

- Any serious event (including on-site and off-site adverse events, injuries, side effects, deaths or other problems) which in the opinion of the local researcher was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures
- Any serious accidental or unintentional change to the IRB-approved protocol that involves risk or has the potential to recur;
- Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject;
- Any publication in the literature, safety monitoring report (including Data and Safety Monitoring Reports), interim result or other finding that indicates an unexpected change to the risk/benefit ratio of the research;
- Any breach in confidentiality that may involve risk to the subject or others;
- Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the research staff.

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