Vitamin E Supplementation in Hyperinsulinism/Hyperammonemia Syndrome

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ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
ATP	Adenosine triphosphate
CHOP	Children's Hospital of Philadelphia
CHPS	Center for Human Phenomic Science
CRF	Case report form
EGCG	Epigallocatechin gallate
ECG	Epicatechin gallate
GDH	Glutamate dehydrogenase
GIR	Glucose infusion rate
GTP	Guanosine triphosphate
HEK	Human embryonic kidney
HI	Hyperinsulinism
HI/HA	Hyperinsulinism/Hyperammonemia
IU	International units
OPTT	Oral protein tolerance test
SAE	Serious adverse event

ABSTRACT

Context: Congenital hyperinsulinism (HI) is a rare disorder of pancreatic beta cell insulin secretion that causes persistent and severe hypoglycemia starting at birth. Hyperinsulinism/hyperammonemia (HI/HA) syndrome is the second most common type of congenital HI and is caused by activating mutations in glutamate dehydrogenase (GDH). Patients with HI/HA exhibit fasting hyperinsulinemic hypoglycemia, protein-induced hypoglycemia, hyperammonemia, seizures, and intellectual disability independent of hypoglycemia. These effects result from abnormal GDH activity in the beta cells, liver and kidney cells, neurons, and astrocytes. The only available treatment for HI/HA syndrome is diazoxide, which acts on the beta cells to decrease insulin secretion but has no effect on GDH activity itself or on other cell types. Thus, there remains a significant unmet need for improved therapies for this disorder. Preliminary data show that Vitamin E inhibits GDH activity in cell lines and improves hypoglycemia in a GDH HI mouse model. Therefore, we hypothesize that Vitamin E will inhibit GDH activity, as measured by plasma ammonia levels and parameters of the oral protein tolerance test (OPTT), in subjects with HI/HA syndrome. This hypothesis will be tested in a future study. The intent of the current pilot study is to use Vitamin E as a supplement to obtain tolerability and feasibility data, and not to cure, prevent, treat, mitigate, or diagnose HI.

Objectives: The primary objective of this study is to determine the tolerability of short-term oral Vitamin E supplementation in subjects with HI/HA syndrome. Secondary objectives are to assess the effects of Vitamin E supplements on plasma Vitamin E levels, on plasma glucose, insulin, and ammonia concentrations during fasting oral protein tolerance test (OPTT), and on hypoglycemia episodes in subjects with HI/HA syndrome.

Study Design: This open-label pilot and feasibility clinical study will use a before-and-after design, with assessments performed prior to and after 2 weeks of daily oral Vitamin E supplementation in individuals with HI/HA syndrome.

Setting/Participants: This single-site study will recruit 14 participants from 1 to 40 years of age (inclusive) with HI/HA syndrome from CHOP's Congenital Hyperinsulinism Center. Study procedures will be performed in the outpatient Center for Human Phenomic Science.

PROTOCOL SYNOPSIS

Study Title	Vitamin E Supplementation in Hyperinsulinism/Hyperammonemia Syndrome.
Funder	University of Pennsylvania Orphan Disease Center, Pediatric Endocrine Society, Children's Hospital of Philadelphia
Clinical Phase	Pilot Study
Study Rationale	Congenital hyperinsulinism (HI) is a rare genetic disorder of dysregulated pancreatic beta cell insulin secretion that causes persistent and severe hypoglycemia starting at birth, which can result in developmental delays, brain damage, and death if inadequately controlled. The second most common type of congenital HI is caused by dominant activating mutations in glutamate dehydrogenase (GDH) that result in hyperinsulinism/hyperammonemia (HI/HA) syndrome. In addition to fasting hyperinsulinemic hypoglycemia, which is seen in all patients with HI, patients with HI/HA also exhibit protein-induced hypoglycemia, hyperammonemia, generalized seizures, and intellectual disability independent of hypoglycemia. These effects result from abnormal GDH activity in the insulin-secreting beta cells, liver and kidney cells, neurons, and astrocytes. Currently, the only treatment for HI/HA syndrome is diazoxide, which acts on beta cells to decrease insulin secretion. However, diazoxide has no effect on GDH activity itself or on other cell types. Diazoxide also has side effects of fluid retention and excessive hair growth. Thus, there remains a significant unmet need for improved therapies for patients with HI/HA syndrome. Targeting GDH activity in all cells would be a more effective approach to treat HI/HA syndrome. Our preliminary data show that alpha-tocopherol (Vitamin E) effectively inhibits GDH activity in human embryonic kidney cells with an activating GDH mutation, and it improves hypoglycemia in transgenic mice with GDH HI. Based on these preclinical *in vitro* and *in vivo* studies, we hypothesize that alpha-tocopherol will inhibit GDH activity and may impact hyperinsulinemic hypoglycemia and hyperammonemia in subjects with HI/HA syndrome. This hypothesis will be tested in a future study. In this initial pilot study, we propose the following objectives:
Study Objective(s)	**Primary**

	• To determine the tolerability of supplemental oral alpha-tocopherol in subjects with HI/HA syndrome. **Secondary** • To assess the effects of Vitamin E supplements on plasma Vitamin E levels, on plasma glucose, insulin, and ammonia concentrations during fasting oral protein tolerance test (OPTT), and on hypoglycemic episodes in subjects with HI/HA syndrome.
Test Article	Vitamin E is a fat-soluble vitamin made exclusively by plants. Vitamin E supplements are available over-the-counter in liquid and capsule formulations. Vitamin E supplements are widely used and have very low risk of intoxication or side effects.
Study Design	This is an open-label pilot and feasibility clinical study to assess tolerability of Vitamin E supplements in subjects with HI/HA syndrome. The duration of this study will be two weeks, during which subjects take a daily oral Vitamin E supplement and will undergo outpatient evaluations at baseline and at study end.
Subject Population key criteria for Inclusion and Exclusion:	**Inclusion Criteria** 1. Males or females age ≥1 year and ≤40 years. 2. Diagnosis of HI/HA syndrome. 3. Currently being treated with diazoxide. 4. Females ≥11 years of age or menstruating must have a negative urine/serum pregnancy test and must use an acceptable method of contraception, including abstinence, a barrier method (diaphragm or condom), Depo-Provera, or an oral contraceptive, for the duration of the study. 5. Informed consent for participants ≥18 years. Parental/guardian permission (informed consent) and, if appropriate, child assent for participants <18 years. **Exclusion Criteria** 1. Males or females age <1 year or >40 years. 2. Individuals who have experienced an allergic reaction to Vitamin E. 3. Individuals with a known allergy to dairy, whey, or soy. 4. On concurrent therapy with a medication known to adversely interact with Vitamin E (refer to Appendix 1). 5. Known increased risk of bleeding (bleeding disorder or on antiplatelet or anticoagulation therapy). 6. Vitamin E supplementation within 30 days prior to enrollment, including multivitamins containing Vitamin E.

	7. Severe hypoglycemia (plasma glucose <50 mg/dL on repeat checks using home glucose meter) more than once weekly within 30 days prior to enrollment. 8. Evidence of a medical condition that might alter results or compromise the interpretation of results, including active infection, kidney failure, severe liver dysfunction, severe respiratory or cardiac failure. 9. Evidence of severe hematologic abnormality including severe anemia and/or thrombocytopenia. 10. Any investigational drug use within 30 days prior to enrollment. 11. Pregnant or lactating females. 12. Parents/guardians or subjects who, in the opinion of the Investigator, may be non-compliant with study schedules or procedures. 13. Unable to provide informed consent (e.g. impaired cognition or judgment). 14. Parents/guardians or subjects with limited English proficiency.
Number Of Subjects	Fourteen total, with goal of ten evaluable
Study Duration	Each subject's participation will last approximately two weeks. The entire study is expected to last one year.
Study Phases	(1) Screening: Screen for eligibility and obtain consent (2) Visit 1: Baseline OPTT and plasma alpha-tocopherol level (3) Vitamin E oral supplementation at home for two weeks, with two study phone calls (4) Visit 2: Post-supplementation laboratory assessment, OPTT (for subjects without severe hypoglycemia or hypoglycemic symptoms during Visit 1 OPTT), plasma alpha-tocopherol level, and tolerability questionnaire
Outcome Evaluations	The primary outcome is tolerability of Vitamin E as measured by: • the change in fasting plasma alpha-tocopherol concentration before versus after alpha-tocopherol supplementation • responses to a subject/parent-reported tolerability questionnaire after Vitamin E supplementation compared to baseline (refer to Appendices 2 and 3) The secondary outcomes will assess the effects of Vitamin E supplementation on the following measurements: • delta-glucose (fasting plasma glucose - nadir plasma glucose during OPTT)

	• fasting plasma glucose • nadir plasma glucose • fasting plasma insulin • peak plasma insulin • delta-insulin (peak plasma insulin during OPTT - fasting plasma insulin) • fasting plasma ammonia • delta-ammonia (plasma ammonia at 60 minutes - plasma ammonia at 0 minutes) • hypoglycemic episodes (plasma glucose <70 mg/dL) detected on home glucose meter
Safety Evaluations	Subjects will remain under the care of their primary medical team and continue to receive their usual clinical care during this study. Adverse events will be monitored by the study PI.
Statistical And Analytic Plan	In this pilot study, paired t-tests will be conducted to evaluate differences in the primary and secondary objectives before versus after Vitamin E supplementation. Descriptive statistics will be used to characterize subject demographics.
DATA AND SAFETY MONITORING PLAN	The study investigators will be responsible for data quality management and ongoing assessment of safety.

TABLE 1: SCHEDULE OF STUDY PROCEDURES

	Screening Visit/Visit 1*	**Telephone Call 1**	**Telephone Call 2**	**Visit 2**
	Day 0	Day 4 (± 1 day)	Day 8 (± 1 day)	Day 14 (± 1 day)
Informed consent	X			
Review inclusion/exclusion criteria	X			
Demographics/medical history review	X			
Home glucose meter review	X			X
Physical exam	X			X
Fasting laboratory studies	X			X
Oral protein tolerance test	X			X†
Tolerability questionnaire		X	X	X
Prior/concomitant medication review				X
Adverse event monitoring	X			

* Vitamin E supplements will be dispensed to parent/caregiver or subject during Visit 1.

† Visit 2 oral protein tolerance test will not be performed for subjects who experience severe hypoglycemia or hypoglycemic symptoms during Visit 1 oral protein tolerance test.

Participants will be asked to partake in a 15 minute phone interview with study staff, during which the tolerability questionnaire will be administered, on Day 4 (± 1 day) and Day 8 (± 1 day), between study visits.

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Congenital hyperinsulinism (HI) is the most common cause of permanent neonatal hypoglycemia. It is a rare disease and occurs in approximately 1 in 40,000 individuals. This disease results from various mutations that affect the secretion of insulin from the beta cell of the pancreas. Gain of function mutations in the glutamic dehydrogenase (GDH) enzyme result in the second most common form of congenital HI, also known as hyperinsulinism/hyperammonemia (HI/HA) syndrome (1-3).

In addition to fasting hyperinsulinemic hypoglycemia, which is observed in all patients with congenital HI (4), patients with HI/HA syndrome also exhibit protein-induced hypoglycemia, hyperammonemia, and epilepsy (1). The severity of the hypoglycemia is greatest in younger children, with a milder phenotype observed in adults. The only current mode of treatment for hypoglycemia caused by GDH HI is diazoxide, which works at the level of the beta cell to inhibit insulin secretion. However, the other consequences of GDH activity (due to gain-of-function mutations in the kidneys, liver, and brain) including hyperammonemia, epilepsy, and intellectual disability persist and remain untreated. Furthermore, diazoxide causes side effects including fluid retention, pulmonary hypertension, heart failure, and neutropenia (5; 6) that may be avoided by use of a GDH-specific therapy.

There has been an ongoing search for a multisystem therapy for HI/HA syndrome that targets the GDH enzyme. Recently, we identified that Vitamin E is an effective GDH inhibitor (see Section 1.3.1). Furthermore, in a mouse model of GDH HI, Vitamin E (alpha-tocopherol) oral administration improved fasting hypoglycemia compared to mice who did not receive Vitamin E.

We hypothesize that Vitamin E will be a feasible, targeted, multisystem treatment for HI/HA syndrome due to its inhibition of GDH activity in all cells. In this pilot tolerability and feasibility study, we will evaluate plasma alpha-tocopherol levels and adverse events, in addition to plasma ammonia, glucose, and insulin levels during an oral protein tolerance test (OPTT) as initial, short-term, measurable effects of Vitamin E supplementation, while the subjects remain on their usual diazoxide treatment regimen. <u>These initial tolerability measures will inform future studies addressing clinical efficacy of Vitamin E alone in treating the hyperinsulinemic hypoglycemia, hyperammonemia, and neurological manifestations of HI/HA syndrome.</u>

1.2 Name and Description of Investigational Product or Intervention

Vitamin E is an essential nutrient only synthesized by plants. Over-the-counter Vitamin E supplements are commonly used for the anti-oxidant properties and have very few side effects. In this study, Vitamin E will be administered orally in either liquid (50 IU/mL, aqueous) or capsule (200 IU) formulation, depending on subject age and preference.

1.3 Findings from Non-Clinical and Clinical Studies

1.3.1 Non-Clinical Studies

Previous studies from our group identified the green tea extracts epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) (**Figure 1**) as potent allosteric inhibitors of wild-type and mutant GDH activity *in vitro* (7) (**Figure 2B**), as well as in transgenic mice (8). However, these compounds have very poor intestinal absorption, low bioavailability, and are rapidly metabolized, making them unsuitable therapeutic candidates. To identify other potential candidates, our group conducted several *in vitro* chemical screens (9) and found several small molecules that effectively inhibited wild-type and mutant GDH activity *in vitro* with low toxicity profiles (unpublished data). Some of these lead compounds have a similar structure to alpha-tocopherol, the bioavailable form of Vitamin E (**Figure 1**). Therefore, **we hypothesize that Vitamin E inhibits mutant GDH activity.**

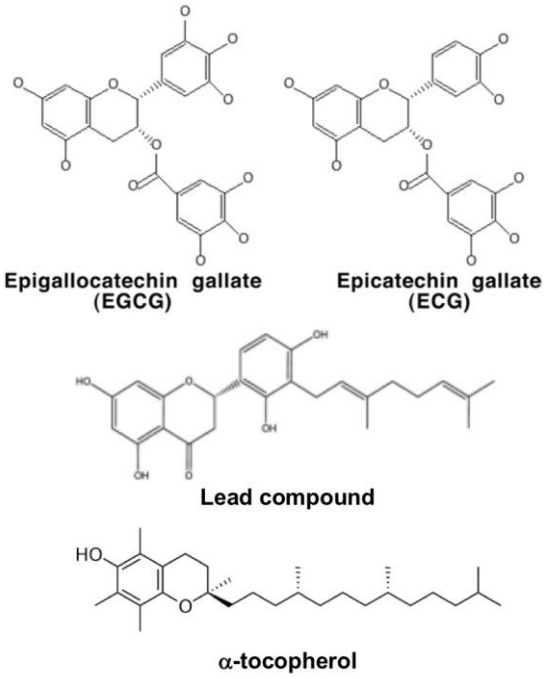

Figure 1. Chemical structures of GDH inhibitors and Vitamin E.

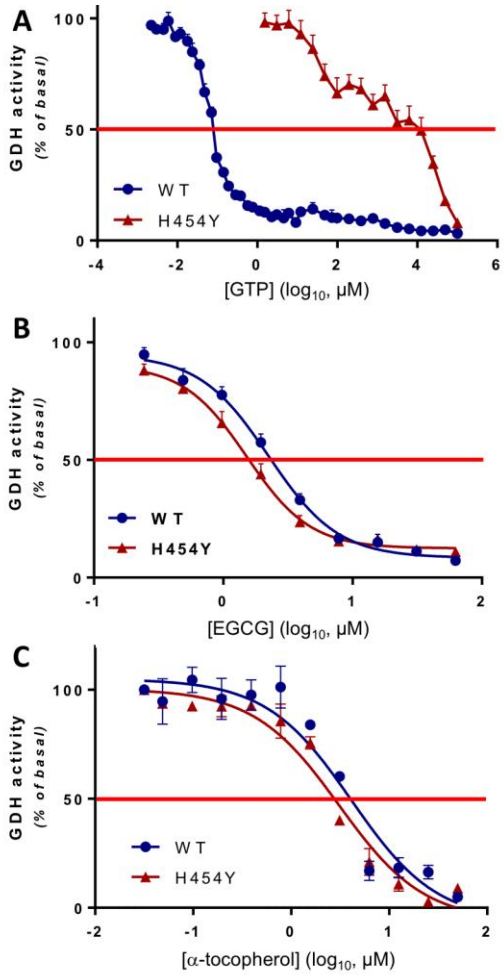

Figure 2. (A) GTP-mediated inhibition of GDH activity was reduced in HEK293T cells over-expressing the H454Y mutant (red) compared to wild-type (WT) GDH (blue). **(B)** EGCG effectively inhibited WT and H454Y mutant GDH activity. **(C)** α-tocopherol inhibited WT and H454Y mutant GDH activity with similar efficacy as EGCG.

We first evaluated alpha-tocopherol's effect on GDH activity *in vitro*. In this established model, human embryonic kidney (HEK293T) cells were transduced with lentivirus to over-express either wild-type GDH or a common mutant form of GDH, H454Y, in which the 454th amino acid is changed from histidine to tyrosine. This mutation has been described in many patients with HI/HA syndrome (3) and has been shown to prevent enzymatic inhibition by guanosine triphosphate (GTP) in cells isolated from these patients, as well as in

cell lines over-expressing this GDH mutant (**Figure 2A**). We found that alpha-tocopherol effectively inhibited activity of wild-type and H454Y mutant GDH in these human cells (**Figure 2C**), with 50% inhibitory concentration (IC_{50}) of 4.1 and 3.1 μM, respectively. Alpha-tocopherol also effectively inhibited the activity of other GDH mutants previously identified in patients with HI/HA syndrome, with IC_{50} <10 μM (**Figure 3A**), including mutations that affect the GTP binding site (**Figure 3B**) and those that do not (**Figure 3C**).

We also evaluated alpha-tocopherol's effect in transgenic mice expressing the H454Y mutant GDH in beta cells, which causes fasting hypoglycemia (8) similar to patients with HI/HA syndrome. We treated these transgenic mice with oral alpha-tocopherol for 2 days and then assessed fasting hypoglycemia, which was significantly improved in alpha-tocopherol-treated transgenic mice (nadir plasma glucose 86 ± 4 mg/dL) versus vehicle-treated transgenic mice (nadir plasma glucose 67 ± 5 mg/dL, P <0.01, **Figure 4**). These preclinical *in vitro* and *in vivo* studies suggest that alpha-tocopherol is a promising treatment for HI/HA syndrome via direct GDH inhibition.

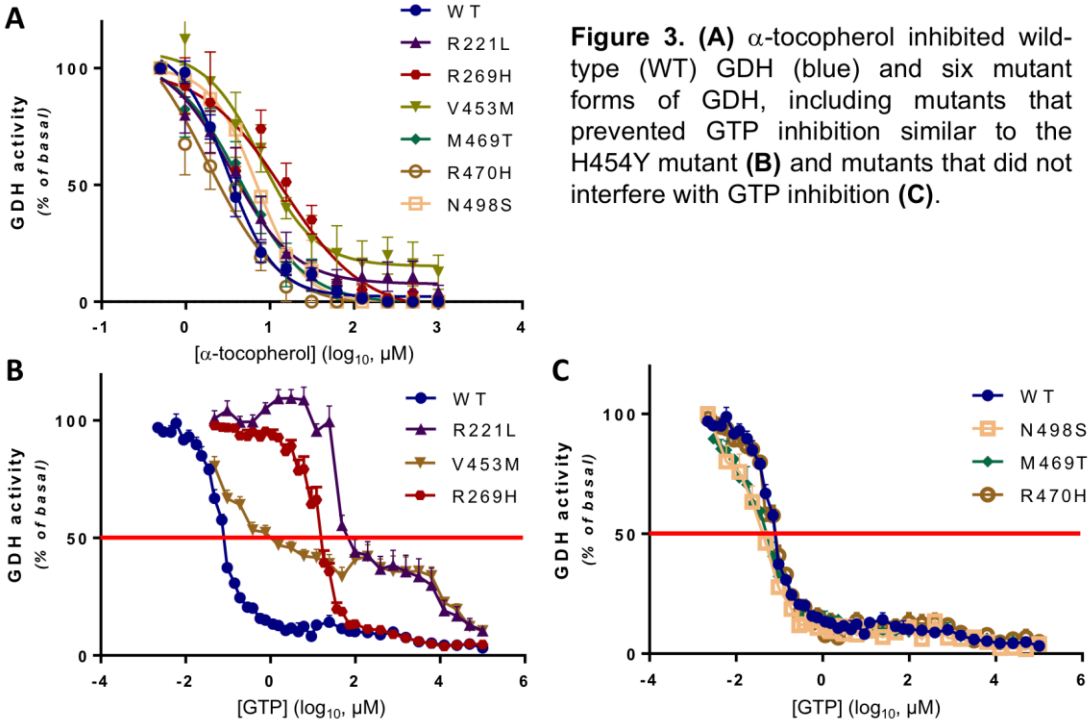

Figure 3. (A) α-tocopherol inhibited wild-type (WT) GDH (blue) and six mutant forms of GDH, including mutants that prevented GTP inhibition similar to the H454Y mutant **(B)** and mutants that did not interfere with GTP inhibition **(C)**.

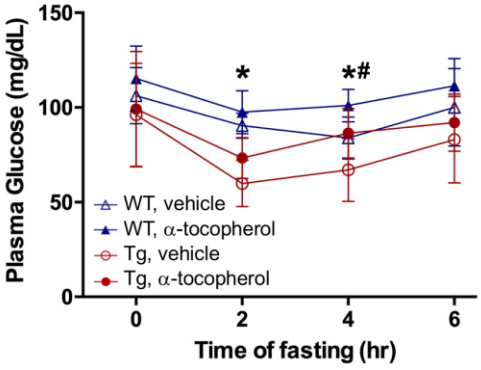

Figure 4. Wild-type (WT) and H454Y GDH transgenic (Tg) adult mice were treated with oral α-tocopherol (75 μg/g body weight per dose) or the equivalent amount of oil vehicle for 3 doses over 2 days and then fasted. Plasma glucose was lower in Tg vehicle-treated mice (red open circles) compared to WT vehicle-treated mice (blue open triangles), consistent with previous studies. Tg mice treated with α-tocopherol (red filled circles) had significantly higher plasma glucose levels after fasting for 2 and 4 hours, compared to vehicle-treated Tg mice. WT mice treated with α-tocopherol (blue filled triangles) also had significantly higher plasma glucose levels after fasting for 4 hours, compared to vehicle-treated WT mice.
n = 10 mice per group, *P < 0.01 Tg α-tocopherol versus Tg vehicle, #P < 0.01 WT α-tocopherol versus WT vehicle using t-test with Holm-Sidak correction for multiple comparisons.

1.3.2 Clinical Studies

1.3.2.1 Human Vitamin E Plasma Concentrations

Vitamin E is a fat-soluble antioxidant that protects cell membranes from oxidative damage. *In vitro* studies of Vitamin E have also demonstrated a role in immune and endothelial cell function as well as inhibition of platelet aggregation (10). Prior national surveys have found that approximately 90% of children and adults in the United States have inadequate Vitamin E dietary intake (11) and Vitamin E insufficiency based on plasma alpha-tocopherol concentration <30 μM, while only 1% are truly deficient based on plasma concentration <12 μM (12). Vitamin E deficiency typically causes neurological deficits, which can be ameliorated by Vitamin E supplementation because alpha-tocopherol crosses the blood-brain barrier (13). However, clinically apparent Vitamin E deficiency is rare and has not been reported to occur in healthy individuals. Based upon results of the 2005-2006 National Health and Nutrition Examination Survey, the mean serum Vitamin E concentration in individuals age 6 and older was 25.3 μM (14).

1.3.2.2 Pharmacokinetic Data

Pharmacokinetic studies in adults administered 800 mg all racemic alpha-tocopherol found a mean absorption time of 3 hours and mean elimination half-life of 73 hours (15). Maximum plasma levels occurred at 12-13 hours. Pharmacokinetic studies in healthy children are lacking. Serum levels achieved after varying doses of Vitamin E administration in pediatric studies are detailed in Section 1.3.2.4.

1.3.2.3 Clinical Studies in Adults

Adults supplemented with 400 or 800 IU/day for 4 weeks demonstrated increased plasma alpha-tocopherol concentrations from 26.8±2.6 to 41.8±4.3 and from 25.2±2.4 to 53.5±3.86 μM, respectively (16). A meta-analysis of randomized studies in adults showed an association between alpha-tocopherol doses ≥400 IU/day and all-cause mortality, which was not observed with lower doses (17). Furthermore, a prospective randomized placebo-controlled study of 14,641 men >50 years old given 400 IU Vitamin E every other day for 10 years showed increased risk of hemorrhagic stroke (HR, 1.74 [95% CI, 1.04-2.91]; P=.04) (18). *In vitro* and rodent studies indicate that alpha-tocopherol inhibits platelet aggregation (19). Although higher plasma alpha-tocopherol levels were associated with increased risk of bleeding in patients on anticoagulation therapy (20), healthy adults supplemented with alpha-tocopherol 800 IU/day for 2 weeks had no differences in platelet aggregation, coagulation profile, or bleeding time (21). No other specific side effects or risks have been associated with Vitamin E supplementation.

1.3.2.4 Clinical Studies in Children

Most children's multivitamins contain 18-30 IU of Vitamin E. Higher supplementation doses are commonly used for children and adults with malabsorption disorders, including cystic fibrosis. Vitamin E oral supplementation in children has been evaluated in multiple studies, including:

- a retrospective study of 232 children ages 0-17 years with cystic fibrosis chronically supplemented with age-based doses of alpha-tocopherol ranging from approximately 47.5-137.2 mg daily (equivalent to approximately 70-200 IU daily), median serum alpha -tocopherol levels ranged between 18 and 25 mmol/l (775 and 1079 mg/dL) (22)

- a randomized double-blind placebo-controlled study in which 120 children ages 5-17 years with moderate persistent asthma were supplemented with alpha-tocopherol 50 mg daily (equivalent to 75 IU daily) for 8 weeks, pre-treatment serum vitamin E levels were 4.1±1.41 μg/dL and 4.5±1.8 μg/dL in treated and untreated groups, respectively, with post-treatment levels of 8.2±2.4 μg/dL and 4.7±0.9 μg/dL (p=0.012), in the treated and untreated groups, respectively (23)
- a randomized placebo-controlled study in which 49 children ages 7-15 years with Down syndrome were supplemented with alpha-tocopherol 400 IU daily for 4 months (24)
- a randomized double-blind placebo-controlled study in which 76 female children ages 5-12 years with pyelonephritis were supplemented with alpha-tocopherol 100 IU daily for 2 weeks (25)
- an open-label study of 9 children ages 8-15 years with type 1 diabetes mellitus were supplemented with weight-based doses of alpha-tocopherol (100 IU daily if <30 kg, 200 IU daily if 30-60 kg, 300 IU daily if >60 kg) (26)
- an open-label study of 18 children and adults ages 0.6-42 years with glycogen storage disease 1b (GSD1b) supplemented with Vitamin E either 600 mg (800 IU) daily if pre-pubertal or 900 mg (1,350 IU) daily if post-pubertal for 1 year (27)

These studies reported no adverse events in subjects receiving Vitamin E supplements. It is reassuring that Vitamin E has been given to children with disorders of glucose regulation (hyperglycemia in type 1 diabetes, and hypoglycemia in GSD1b). In summary, **alpha-tocopherol supplementation is generally considered safe in children and adults without increased bleeding risk**. Based upon a literature review, no clinical studies of Vitamin E supplementation in subjects with HI/HA syndrome have yet been conducted. There are no other ongoing studies at CHOP targeting this patient population.

1.4 Selection of Supplements and Dosages

Oral Vitamin E supplements to be used in this study will be provided by the CHOP Pharmacy, the formulations of which are on the CHOP formulary for inpatient and outpatient administration. Age-based oral Vitamin E supplement dose ranges established by the Food and Nutrition Board at the Institute of Medicine (**Table 2**) were used to select the age-based doses that will be used in this study (**Table 3**). Liquid and capsule formulations were chosen to allow for age-based dosing and subject preference. Study duration of 2 weeks was determined based on the elimination half-life of Vitamin E of 73 hours; five half-lives will allow for evaluation at steady-state plasma levels of the study supplement.

Age	RDA (IU)	UL (IU)
0-6 months	6	nd
7-12 months	7.5	nd
1-3 years	9	300
4-8 years	10.4	450
9-13 years	16.4	900
14-18 years	22.4	1,200
19+ years	22.4	1,500

Table 2. Recommended Dietary Allowance (RDA) and Tolerable Upper Intake Level (UL) of d-alpha-tocopherol for different age groups.

Age	Daily Dose (mg) Active Form	Equivalent Daily Dose (IU) Natural Form	Equivalent Daily Dose (IU) Synthetic Form	Liquid Dose (50 IU/mL) Natural	Liquid Dose (50 IU/ mL) Synthetic	Capsule Dose (200 IU/capsule) Natural	Capsule Dose (200 IU/capsule) Synthetic
1-3 years	67	100	148.7 IU – ***round to 150 IU***	2 mL	3 mL	--	--
4-8 years	134	200	297 IU – ***round to 300 IU***	4 mL	6 mL	--	--
9-17 years	201	300	446.2 IU – ***round to 450 IU***	6 mL	9 mL	--	--
18+ years	268	400	594.9 IU – ***round to 600 IU***	8 mL	12 mL	2 capsules	3 capsules

Table 3. Alpha-tocopherol daily doses to be used in this study. 1 mg of alpha-tocopherol active form is equivalent to 1.49 IU of natural form (d-alpha-tocopherol) or 2.22 IU of synthetic form (dl-alpha-tocopherol).

1.5 Relevant Literature and Data

HI/HA syndrome is the second most common cause of congenital HI and the most common cause of diazoxide-responsive congenital HI (1; 2). Patients with HI/HA syndrome have several clinical characteristics:

Fasting hyperinsulinemic hypoglycemia in patients with HI/HA syndrome is relatively mild compared to other forms of congenital HI. Average fasting tolerance without hypoglycemia in patients with HI/HA syndrome is approximately 12 hours and improves with age (3). Diazoxide treatment effectively prevents significant fasting hypoglycemia in HI/HA syndrome (1).

Protein-induced hyperinsulinemic hypoglycemia is acute and profound in patients with HI/HA syndrome, with decrease of plasma glucose concentration by 9-62 mg/dL (mean 23 mg/dL) within 3 hours of an oral protein load (1.5 g/kg body weight), without carbohydrate intake (3). This decline in plasma glucose results in significant hypoglycemia in most patients with HI/HA syndrome, compared to controls who only experience a decrease in plasma glucose of 4 mg/dL. Patients with HI/HA syndrome can avoid protein-induced hypoglycemia by consuming carbohydrates with protein-containing meals. Diazoxide treatment is moderately effective in preventing significant protein-induced hypoglycemia.

Hyperammonemia with plasma ammonia levels typically 2-5 times the upper limit of normal (35 μmol/L) occurs in patients with HI/HA syndrome (3). However, there is no clear correlation between plasma ammonia and plasma glucose levels, likely because the primary source of elevated plasma ammonia is the kidney, rather than the beta cell (28). Diazoxide treatment does not affect plasma ammonia levels.

Central nervous system (CNS) effects, including seizures and developmental delays, are seen in patients with HI/HA syndrome, although the underlying mechanisms are not understood (1). While hyperinsulinemic hypoglycemia likely contributes to some degree of

neurodevelopmental deficits, as is seen in other forms of congenital HI, seizures (particularly absence seizures) are observed in patients with HI/HA syndrome even when plasma glucose is normal. In comparison to patients with other genetic causes of hyperammonemia, such as urea cycle defects, CNS effects in patients with HI/HA syndrome are relatively mild. It is likely that these outcomes are consequences of GDH hyperactivity in the CNS, rather than solely due to hyperammonemia. However, the direct effects of GDH hyperactivity in the CNS are not understood.

The only current treatment available for HI/HA syndrome is diazoxide, which opens the ATP-gated potassium (K_{ATP}) channels on beta cells to reduce insulin secretion. Diazoxide effectively prevents fasting and protein-induced hypoglycemia, but it has no effect on hyperammonemia, seizures, or developmental delays (1). Furthermore, diazoxide causes significant side effects, including fluid retention, pulmonary hypertension, heart failure, and neutropenia (5; 6). **Thus, an alternative therapy for HI/HA syndrome, directly targeting GDH in all cell types to treat the disease's widespread pathophysiology, is warranted.**

Glutamate Dehydrogenase (GDH) Activating Mutations Cause HI/HA Syndrome

GDH is a mitochondrial matrix enzyme that is highly expressed in kidney, liver, brain, and beta cells (1). It is responsible for metabolizing glutamate into alpha-ketoglutarate and ammonia. In beta cells, elevated alpha-ketoglutarate leads to increased ATP production via the citric acid cycle, which stimulates insulin secretion. GDH activity is normally suppressed by binding of guanosine triphosphate (GTP), which alters conformation of the catalytic domain and prevents conversion of glutamate into alpha-ketoglutarate and ammonia (1). Dominant gain-of-function missense mutations in GDH interfere with GTP binding, impairing GTP-mediated inhibition and thus increasing GDH catalytic activity, leading to excess amino acid catabolism and ammonia production, which results in HI/HA syndrome.

1.6 Compliance Statement

This study will be conducted in full accordance of all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable federal and state laws and regulations including 45 CFR 46 and HIPPA. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others in accordance with the Children's Hospital of Philadelphia Institutional Review Board Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

The purpose of this pilot clinical study is to determine the feasibility and tolerability of alpha-tocopherol supplementation in subjects with HI/HA syndrome.

2.1 Primary Objective

To determine the tolerability of supplemental oral alpha-tocopherol in subjects with HI/HA syndrome as measured by responses to a subject/parent-reported tolerability questionnaire compared to baseline and the change in fasting plasma alpha-tocopherol concentration before versus after alpha-tocopherol supplementation.

2.2 Secondary Objectives

To assess the effects of Vitamin E supplements on plasma Vitamin E levels, on plasma glucose, insulin, and ammonia concentrations during fasting oral protein tolerance test (OPTT), and on hypoglycemic episodes in subjects with HI/HA syndrome.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This is a pilot and feasibility before-and-after study. Enrolled subjects will undergo lab draws at CHOP's outpatient Center for Human Phenomic Science (CHPS) prior to and after two weeks of daily oral Vitamin E supplementation. Fasting oral protein tolerance test (OPTT) will be performed prior to Vitamin E supplementation for all subjects and will be repeated after Vitamin E supplementation for subjects who do not who experience severe hypoglycemia, defined as in section 4.4, or severe hypoglycemic symptoms, including seizure or altered mental status, during the initial oral protein tolerance test. For each OPTT, the subjects will fast for 12 hours overnight at home, then will have fasting plasma glucose, insulin, alpha-tocopherol, and ammonia concentrations measured. Then the subjects will be given a weight-based oral protein drink, and subsequent plasma glucose, insulin, and ammonia concentrations will be measured. Subjects will continue diazoxide and other usual medications throughout the study period.

3.1.1 Screening Phase

- Subjects with HI/HA syndrome currently treated with diazoxide will be recruited from the Congenital Hyperinsulinism Center at the Children's Hospital of Philadelphia (CHOP).
- Informed consent and assent (for subjects ≥7 years of age) will be obtained.

3.1.2 Visit 1 (may occur on the same day as Screening Visit)

- Subject demographic and medical history will be reviewed, including prior and concomitant medications and supplements, and recent hypoglycemic events.
- A physical exam will be performed.
- Complete blood count with platelet count, fasting plasma glucose, insulin, alpha-tocopherol, and ammonia concentrations will be measured.
- Oral protein tolerance test will be performed with plasma glucose, insulin, and ammonia concentration measurements.
- Subjects/caregivers will be given the Vitamin E supplements, with instructions for administration over the next two weeks.

- Subject/parent will complete the baseline symptom questionnaire (Appendix 2).

3.1.3 Interim between Visit 1 and Visit 2

- Subjects will self-administer daily oral Vitamin E supplementation with a fat-containing meal at home.
- Phone interview with study staff on Day 4 (± 1 day) and Day 8 (± 1 day) to ensure study compliance, inquire about adverse events, and administer a verbal tolerability questionnaire. Study staff will use the script provided in Appendix 4, which includes administration of the tolerability questionnaire (Appendix 3).

3.1.4 Visit 2

- Recent medical history, hypoglycemic events, and concomitant medications and supplements will be reviewed.
- A physical exam will be performed.
- Fasting plasma glucose, insulin, alpha-tocopherol, and ammonia concentrations will be measured.
- Oral protein tolerance test will be performed with plasma glucose, insulin, and ammonia concentration measurements for subjects who do not experience severe hypoglycemia or hypoglycemic symptoms during Visit 1 OPTT.
- Subject/parent will complete the Vitamin E tolerability questionnaire (Appendix 3).
- Adverse events will be recorded.

3.2 Allocation to Experimental Groups and Blinding

All recruited subjects will be exposed to Vitamin E supplementation. Post-supplementation data will be compared to baseline data. There will be no blinding.

3.3 Study Duration, Enrollment and Number of Sites

3.3.1 Duration of Study Participation

The study duration per subject will be up to 15 days. Based upon the half-life of Vitamin E (approximately 73 hours), steady-state plasma levels are expected to be reached and maximal biological effect to occur within this timeframe.

3.3.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at the Children's Hospital of Philadelphia. Recruitment will stop when approximately 14 subjects are enrolled. It is expected that approximately 14 subjects will be enrolled to produce 10 evaluable subjects.

3.4 Study Population

3.4.1 Inclusion Criteria

1) Males or females age ≥1 year and ≤40 years.
2) Diagnosis of HI/HA syndrome.
3) Currently being treated with diazoxide.

4) Females ≥11 years of age or menstruating must have a negative urine/serum pregnancy test and must use an acceptable method of contraception, including abstinence, a barrier method (diaphragm or condom), Depo-Provera, or an oral contraceptive, for the duration of the study.

5) Informed consent for participants ≥18 years. Parental/guardian permission (informed consent) and, if appropriate, child assent for participants <18 years.

3.4.2 Exclusion Criteria

1) Males or females age <1 year or >40 years.

2) Individuals who have experienced an allergic reaction to Vitamin E.

3) Individuals with a known allergy to dairy, whey, or soy.

4) On concurrent therapy with a medication known to adversely interact with Vitamin E (refer to Appendix 1).

5) Known increased risk of bleeding (bleeding disorder or on antiplatelet or anticoagulation therapy).

6) Vitamin E supplementation within 30 days prior to enrollment, including multivitamins containing Vitamin E.

7) Severe hypoglycemia (plasma glucose <50 mg/dL on repeat checks using home glucose meter) more than once weekly within 30 days prior to enrollment.

8) Evidence of a medical condition that might alter results or compromise the interpretation of results, including active infection, kidney failure, severe liver dysfunction, severe respiratory or cardiac failure.

9) Evidence of severe hematologic abnormality including severe anemia and/or thrombocytopenia.

10) Any investigational drug use within 30 days prior to enrollment.

11) Pregnant or lactating females.

12) Parents/guardians or subjects who, in the opinion of the Investigator, may be non-compliant with study schedules or procedures.

13) Unable to provide informed consent (e.g. impaired cognition or judgment).

14) Parents/guardians or subjects with limited English proficiency.

Subjects that do not meet all of the enrollment criteria may not be enrolled. Subjects who have been screened and are later determined to be eligible can participate without having to be rescreened for a period of one year. The study staff will obtain verbal confirmation that there have been no health events that would make them ineligible if the interval between screening and participation is longer than 3 months. Any violations of these criteria must be reported in accordance with IRB Policies and Procedures.

4 STUDY PROCEDURES

All subjects will undergo an oral protein tolerance test (OPTT) at Visit 1. Subjects who do not experience severe hypoglycemia, defined as in section 4.4, or severe hypoglycemic symptoms, including seizure or altered mental status, during the Visit 1 OPTT will also undergo OPTT at Visit 2. OPTT is a standard test that patients with HI/HA undergo annually to evaluate efficacy of diazoxide treatment.

4.1 Screening Visit

- Review of inclusion/exclusion criteria
- Informed consent, assent
- Physical exam
- Medical record review
- Recent/current medication and supplement review
- Home glucose meter review
- Urine pregnancy test for females who are menstruating or ≥11 years old

4.2 Study Supplementation Phase

The supplementation period will span approximately 2 weeks (14 days ± 1 day) from Visit 1 to Visit 2.

4.2.1 Visit 1 (may be the same day as screening visit)

- Demographic/medical history review
- Physical exam
- Prior/concomitant medication and supplement review
- Home glucose meter review
- Subject/parent completion of the baseline symptom questionnaire (Appendix 2).
- Placement of peripheral intravenous (IV) line
- Fasting baseline plasma glucose, insulin, alpha-tocopherol, and ammonia concentrations, and complete blood count with platelet count drawn from IV line. Ammonia sample will be drawn from free-flowing IV line without tourniquet and placed immediately on ice.
- Oral protein tolerance test:
  - Enteral intake of Beneprotein (1.5 g/kg body weight protein, maximum 60 grams protein = 1.75g/kg body weight protein powder, maximum 70 grams powder) mixed with carbohydrate-free Crystal Light in 6 ounces of water.
  - Plasma glucose and insulin concentrations obtained from IV line will be measured at 15, 30, 45, 60, 90, 120, 150, and 180 minutes.
  - Plasma ammonia will also be measured at 60 minutes from free-flowing IV line without tourniquet, and sample will be placed immediately on ice.
  - Plasma glucose measurements will be repeated as needed (if <70 mg/dL).

- Dispensing of Vitamin E oral supplement with instructions for administration over the next two weeks and instructions to not take any other Vitamin E-containing supplements, including multivitamins containing Vitamin E, during the study period.

4.2.2 Interim between Visit 1 and Visit 2

- Daily Vitamin E oral supplementation taken with a fat-containing meal
- Phone interview with study staff on Day 4 (± 1 day) and Day 8 (± 1 day) to ensure study compliance, inquire about adverse events, and administer a verbal tolerability questionnaire. Study staff will use the script provided in Appendix 4, which includes administration of the tolerability questionnaire.

4.2.3 Visit 2

- Physical exam
- Prior/concomitant medication and supplement review
- Home glucose meter review
- Placement of peripheral intravenous (IV) line
- Fasting baseline plasma glucose, insulin, alpha-tocopherol, and ammonia concentrations drawn from IV line. Ammonia sample will be drawn from free-flowing IV line without tourniquet and placed immediately on ice.
- Oral protein tolerance test for subjects who do not experience severe hypoglycemia or hypoglycemic symptoms during Visit 1 oral protein tolerance test:
  - Enteral intake of Beneprotein (1.5 g/kg body weight protein, maximum 60 grams protein = 1.75g/kg body weight protein powder, maximum 70 grams powder) mixed with carbohydrate-free Crystal Light in 6 ounces of water.
  - Plasma glucose and insulin concentrations obtained from IV line will be measured at 15, 30, 45, 60, 90, 120, 150, and 180 minutes.
  - Plasma ammonia will also be measured at 60 minutes from free-flowing IV line without tourniquet, and sample will be placed immediately on ice.
  - Plasma glucose measurements will be repeated as needed (if <70 mg/dL).
- Subject/parent will complete the Vitamin E tolerability questionnaire (Appendix 3).
- Adverse event monitoring.
- Collection of any unused Vitamin E supplement.

4.3 Concomitant Medication

All prior and concomitant medications used to treat hypoglycemia within 30 days prior to the screening visit and through the end of the study will be recorded. All medications and supplements regardless of indication will be recorded during the study period. The dates of administration, dosage, and reason for use will be included. If a subject is taking a vitamin E supplement at the time of screening and then discontinues the supplement for 30 days, s/he can be eligible at that point for inclusion in the study. Subjects will be instructed to continue diazoxide per previously-prescribed dose throughout the study period. If hypoglycemia

(plasma glucose <70 mg/dl measured on twice repeated point-of-care glucose meter) is noted during Visit 1 or Visit 2, then the Investigator may recommend the individual consult with his/her physician regarding increasing the diazoxide dose. No medical management will be directed nor prescribed solely as means for study participation. If a subject later achieves better glucose control, s/he can be eligible at that point for inclusion in the study.

4.4 Rescue Medication Administration

Hypoglycemia will be treated per usual clinical regimen used by CHOP's Congenital Hyperinsulinism Center. During Visit 1 and Visit 2, if the subject has severe hypoglycemia, defined as plasma glucose <50 mg/dL measured on twice repeated point-of-care glucose meter, then after that time point blood sample is drawn, the subject will be given enteral fluids containing 15 grams carbohydrates, and the oral protein tolerance test will end. Plasma glucose will subsequently be measured with point-of-care glucose meter 20 minutes later to verify that plasma glucose has increased to >70 mg/dL. If plasma glucose is <70 mg/dL on repeat checks, then the subject will be given a second enteral dose of 15 grams carbohydrates, and plasma glucose will be measured 20 minutes later. If plasma glucose remains <70 mg/dL after two doses of enteral carbohydrates, or if subject is unable to tolerate enteral fluids, then 10% dextrose 2 mL/kg bolus will be administered via peripheral IV as needed until plasma glucose is ≥70 mg/dL.

4.5 Subject Completion/Withdrawal

Subjects who are found to have severe thrombocytopenia, defined as a platelet count <100,000 per microliter, during Visit 1 will be withdrawn from the study. Subjects may withdraw from the study at will at any time without prejudice to their care. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to study or visit schedules. The Investigator may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or not each subject completes the clinical study. If the Investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents and on the case report forms.

Subjects who were enrolled in this study prior to Amendment 4 being approved and whom were withdrawn due to severe hypoglycemia during the Visit 1 OPTT may be re-enrolled in this study to complete Vitamin E supplementation at home for two weeks and Visit 2 procedures (with the exception of the repeat OPTT at Visit 2). This will allow for tolerability of the supplement to still be assessed in individuals for whom a second OPTT is not warranted.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Screening Visit Measurements

5.1.1 Inclusion/Exclusion Criteria Review

- Date of birth

- Relevant laboratory values for diagnosis of hyperinsulinism (including glucose, beta-hydroxybutyrate, non-esterified free fatty acids, insulin, c-peptide, insulin-like growth factor binding protein 1, ammonia, and genetic testing results, if available)
- Current use of diazoxide (including dosage and frequency)
- Review of prior and concomitant medications and supplements
- Review of allergies
- Medical history review, including any history of bleeding disorder or current use of anticoagulation therapy
- Review home glucose meter results for past 30 days
- Urine pregnancy test for females who are menstruating or ≥11 years old

5.2 Visit 1 Measurements

5.2.1 Demographic/Medical History Review

- Medical record number
- Sex
- Race and ethnicity
- Gestational age
- Birth weight and length
- Relevant co-morbidities
- Molecular genetic study results, if available
- Prior treatments for hyperinsulinism
- Current treatments for hyperinsulinism
- Review clinical care home glucose meter data over past 30 days
- Episodes of seizures over the past month and year
- Treatment for seizures
- Recent/concomitant medications and supplements

5.2.2 Physical Examination

Physical exam by medical professional to collect the following measurements:

- Length/Height (cm)
- Weight (kg)
- Vital signs:
  - heart rate [bpm] measured by automated device
  - blood pressure [mm Hg] measured by automated device in right upper arm while sitting

- respiratory rate [bpm]

- Skin exam (bruising)

5.2.3 Baseline Symptom Questionnaire (Appendix 2)

5.2.4 Laboratory Testing

- Urine pregnancy test for females that are menstruating or ≥11 years of age

- Point-of-care plasma glucose measurements from peripheral IV line at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes during OPTT
  - If plasma glucose is <70 mg/dL, then repeat point-of-care measurement will be obtained immediately.

- Blood tests from peripheral IV:
  - plasma glucose at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes during OPTT
  - plasma insulin at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes during OPTT
  - plasma alpha-tocopherol at 0 minutes (fasting)
  - plasma ammonia at 0 and 60 minutes during OPTT – sample to be drawn without tourniquet and placed immediately on ice
  - complete blood count with platelet count at 0 minutes

5.3 Interim Evaluations

- Phone interview with study staff on Day 4 (± 1 day) and Day 8 (± 1 day). Study staff will use the script provided in Appendix 4 which includes administration of the tolerability questionnaire.

5.4 Visit 2 Measurements

5.4.1 Physical Examination

- Vital signs:
  - heart rate [bpm] measured by automated device
  - blood pressure [mm Hg] measured by automated device in right upper arm while sitting
  - respiratory rate [bpm]

- Skin exam (bruising)

5.4.2 Laboratory Testing

For subjects completing Visit 2 OPTT

- Point-of-care plasma glucose measurements from peripheral IV line at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes during OPTT
  - If plasma glucose is <70 mg/dL, then repeat point-of-care measurement will

be obtained immediately.

- Blood tests from peripheral IV:
  - plasma glucose at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes during OPTT
  - plasma insulin at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes during OPTT
  - plasma alpha-tocopherol at 0 minutes (fasting)
  - plasma ammonia at 0 and 60 minutes during OPTT – sample to be drawn without tourniquet and placed immediately on ice

For subjects not completing Visit 2 OPTT

- Point-of-care plasma glucose measurement from peripheral IV line
- Fasting blood tests from peripheral IV:
  - plasma glucose
  - plasma insulin
  - plasma alpha-tocopherol
  - plasma ammonia – sample to be drawn without tourniquet and placed immediately on ice

5.4.3 Tolerability Questionnaire (Appendix 3)

5.4.4 Study Period Data Review

- Concomitant medications and supplements during the study period
- Hyperinsulinism treatment during the study period
- Episodes of documented symptomatic and asymptomatic hypoglycemia (meter plasma glucose <70 mg/dL and <50 mg/dL): number during the study period
- Review clinical care home glucose meter data during the study period
- Episodes of seizures during the study period
- Treatment for seizures during the study period
- All adverse events that occur during the study period will be documented and reported per regulatory requirements

6 STATISTICAL CONSIDERATIONS

6.1 Primary Endpoint

The primary endpoint will be tolerability as measured by responses to a subject/parent-reported tolerability questionnaire compared to baseline responses and change in fasting plasma alpha-tocopherol concentration before versus after alpha-tocopherol supplementation.

6.2 Secondary Endpoints

Secondary endpoints will include assessments of the effect of Vitamin E supplementation on the following measurements:

- fasting plasma ammonia
- delta-ammonia (plasma ammonia at 60 minutes during OPTT - plasma ammonia at 0 minutes)
- delta-glucose (fasting plasma glucose - nadir plasma glucose during OPTT)
- fasting and nadir plasma glucose
- fasting and peak plasma insulin
- delta-insulin (peak plasma insulin during OPTT - fasting plasma insulin)
- hypoglycemic episodes (plasma glucose <70 mg/dL) detected on clinical care home glucose meter
- adverse events, including bleeding and bruising

6.3 Control of Bias and Confounding

As a means to control bias and confounding variables, all patients with HI/HA syndrome aged 1 year to 40 years of age (inclusive) receiving ongoing treatment for recurring hypoglycemia followed at the Children's Hospital of Philadelphia's Congenital Hyperinsulinism Center (approximately 40 total) will be evaluated for eligibility for this study. Up to 14 eligible subjects whose parents consent to the study will be enrolled. Each subject serves as their own control.

6.4 Statistical Methods

Descriptive statistics will be used to characterize demographics and outcome measures in subjects. As a general strategy, continuous endpoints will be summarized using the five-number summary (mean, standard deviation, median, minimum, and maximum). Categorical endpoints will be summarized using frequency distributions. Paired t-tests (or if data are not normally distributed, Wilcoxon signed-rank tests) will be used to determine statistical significance of the primary and secondary outcomes, with alpha = 0.05. The number of subjects who experience any versus no adverse event will be tabulated, and the proportion will be calculated and reported, along with its corresponding 95% CI, based on all subjects in the study. The acceptable level of tolerability for this study is defined as if ≥75% of subjects find Vitamin E tolerable.

6.5 Sample Size and Power

The proposed sample size of 14 subjects for this pilot clinical study is a sample of convenience based on the number of subjects that we can feasibly study during one year. The data generated from this study will be used to examine the feasibility and tolerability of alpha-tocopherol in subjects with HI/HA syndrome, in order to design and optimize a phase II/III study within this population in terms of sample size and selecting appropriate endpoints.

7 STUDY SUPPLEMENT

7.1 Description

Vitamin E supplements are available over-the-counter in liquid and oral formulations. Vitamin E supplements are commonly used for anti-oxidant properties, although studies evaluating clinical benefit for various disorders show mixed results. Vitamin E is only synthesized by plants, and thus it is an essential nutrient for humans. The predominate bioavailable form of Vitamin E in humans is alpha-tocopherol. Approximately 90% of children and adults in the United States have inadequate intake of Vitamin E in their diet (11) and have insufficient plasma alpha-tocopherol concentrations (<30 μM) (12). The consequences of Vitamin E insufficiency are somewhat unclear, although true Vitamin E deficiency (plasma alpha-tocopherol concentration <12 μM) typically causes neurological deficits, although this only affects approximately 1% of the population (12; 13).

Alpha-tocopherol crosses the blood-brain barrier, and Vitamin E supplementation improves neurological deficits in patients with Vitamin E deficiency (13). The range of Vitamin E supplementation doses is quite broad (from 6 IU daily recommended for infants to 1,500 IU daily considered as tolerated in adults, see **Table 2**), which is due to the very low side effect profile of Vitamin E and low risk of Vitamin E intoxication. The only recurrently-described side effect of Vitamin E is increased risk of bleeding in patients with risk factors (such as a bleeding disorder or receiving anticoagulation therapy). In general, Vitamin E doses up to 400 IU daily are considered safe in adults and result in significant increases in plasma alpha-tocopherol concentrations. Lower and higher doses of Vitamin E have been used in children, without reported adverse events, although these were relatively small studies. No studies have evaluated Vitamin E supplementation in patients with HI/HA.

7.1.1 Dosing

Subjects will be given Vitamin E supplements and instructed to take their dose once daily with a fat-containing meal. Study dose will be below the upper acceptable limit for their age (**Table 3**), based on Institute of Medicine recommendations (**Table 2**). Capsules are available in the 200 IU dose, so can be used for subjects who will receive 600 IU daily. A liquid formulation (50 IU/mL) will be used for subjects who will receive lower doses, or any subjects who prefer liquid to capsules.

7.1.2 Supplement Accountability

Adequate records of study supplement receipt and disposition will be maintained by the study team and will be based upon the recommendations provided by the Investigational Drug Service. Investigational supplement orders, dispensing records, and disposition forms will be examined during the course of the study. The Vitamin E is to be provided by the Investigator or designee and may not be used for any purpose other than that described in this protocol. At study completion, all supplement supplies including partially used and empty containers must be returned to the Investigator so that they may be disposed of via manufacturer recommendations by the study team. Documentation of destruction will be captured via the Drug Accountability Log.

8 SAFETY MANAGEMENT

8.1 Clinical Adverse Events

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the supplement. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with an intervention, whether or not considered related to the supplement.

The occurrence of an AE will be determined on the basis of observed or volunteered signs and symptoms and changes in the subject's physical examination and laboratory test results. All AEs will be evaluated by the Investigator for seriousness, severity, relationship to study, and outcome.

Collection of clinical information will begin after the subject's written informed consent to participate in the study has been obtained. AEs may be either spontaneously reported or elicited during questioning and examination of the subject. All identified AEs must be recorded and described in the subject's source records. If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms.

8.2 Adverse Event Reporting

Unanticipated problems related to the research involving risks to subjects or others that occur during the course of this study (including SAEs) will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

8.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

8.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death,

- a life-threatening event (at risk of death at the time of the event),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity, or
- a congenital anomaly/birth defect in the offspring of a subject.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A distinction should be drawn between serious and severe AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

8.4.1 Relationship of SAE to study drug or other intervention

The relationship of each SAE to the study intervention should be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

8.5 IRB/IEC Notification of SAEs and Other Unanticipated Problems

The Investigator will promptly notify the IRB of all on-site unanticipated, serious Adverse Events that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly. Written reports will be filed using the eIRB system and in accordance with the timeline below. External SAEs that are both unexpected and related to the study intervention will be reported promptly after the investigator receives the report.

Type of Unanticipated Problem	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be

		reported at time of continuing review

8.5.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

8.6 Medical Emergencies

Medical emergencies during the course of the study will be managed by physician-level members of the study team as per standard of clinical care.

9 STUDY ADMINISTRATION

9.1 Data Collection and Management

1. Confidentiality and Security of Data. All information that is collected for this research protocol will be kept confidential. All subjects will be assigned a unique study identification number (study ID). This study ID will be used on all case report forms (CRFs). Data/CRFs will be stored in OnCore Clinical Trials Management System, a CHOP Research IS secured electronic data capture system. OnCore Clinical Trials Management System is a secure electronic data capture system with access controls and a data backup plan. The system is password protected. Only study team members will have access to subject data and case report forms stored in OnCore. Access to the system is monitored and logged for review if needed. Exports from OnCore will be stored on a secure CHOP server. Data may also be stored on a secure CHOP server with password-protected files when possible.
2. Anonymization, de-identification or destruction. All data and records generated during this study will be kept confidential in accordance with institutional policies and HIPAA on subject privacy. De-identified data will be retained for 10 years from date of last treatment if subject is age 18 or over or an emancipated minor, or if the subject is a minor the data will be retained for 10 years from earlier of age 18 or death. For subjects who consent to the use of their data for future research, PHI and the link between PHI and the study data will not be destroyed. A computer file, stored on one of CHOP's secure servers, will be the site of storage. Each folder will be labeled with a note to date after which to destroy the data. "Destroy by….," with the earliest dates at the front.
3. The CHOP PI will monitor and review the study progress and the accuracy and security of the emerging data.

9.2 Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy. The Investigators and other site

personnel will not use such data and records for any purpose other than conducting the study. Safeguards are described under Data Collection and Management.

9.3 Regulatory and Ethical Considerations

9.3.1 Data and Safety Monitoring Plan

There will be no added physical, psychological, economic, or societal risks due to this interventional study. The principal investigator will review the safety and progress of this study on a monthly basis. Data reviewed will include laboratory results, OPTT results, tolerability questionnaire responses, adverse events, and data collected as per Section 5.4.4. The principal investigator or a physician-level study team member will be available during the OPTT to monitor and treat subjects in the event of hypoglycemia. The primary risk stems from breach of confidentiality. The Principal Investigator will provide oversight for risks associated with management of confidential data (see Data Collection and Management).

9.3.2 Risk Assessment

This study poses a minor increase above minimal risk with no prospect for direct benefit to the subject, but is likely to yield generalizable knowledge about the condition. The study tests performed, including peripheral blood sampling and oral protein tolerance testing, are similar to those that HI/HA patients experience as part of their routine care. The Visit 1 OPTT can replace their annual OPTT. In the event that the Visit 1 OPTT cannot replace a subject's annual OPTT, then two OPTTs will be completed for this research study. Vitamin E is a nutritional supplement available over-the-counter that may be taken in the dose ranges used in this study by the general population. The potential risks associated with the use of the Vitamin E supplementation include bruising and bleeding. To minimize these risks, the dose of Vitamin E will be tailored to the subject's age, and baseline platelet counts will be measured. There is a potential risk of hypoglycemia during oral protein tolerance test. The frequent point-of-care glucose measurements, along with assessment of symptoms of hypoglycemia, during the OPTT will allow for rapid detection of hypoglycemia if it occurs. Potential hypoglycemia is readily reversible with oral carbohydrates or IV dextrose.

There is potential risk of breach of confidentiality. Measures will be taken to avoid breach of privacy and insure confidentiality (see Data Collection and Management).

9.3.3 Potential Benefits of Study Participation

There are no direct benefits to the study subjects. The knowledge gained from this study may lead to improved treatment of future patients with HI/HA syndrome.

9.3.4 Risk-Benefit Assessment

The potential benefits of advancement in medical knowledge outweigh the potential risk to subjects. Measures will be taken to minimize risks.

9.4 Recruitment Strategy

Subjects will be recruited prospectively through the Congenital Hyperinsulinism Center at CHOP. The Co-Investigator is the director of the center and the principal outpatient physician caring for these patients. The Primary Investigator, the Co-Investigator, or the study staff will approach the subjects in person, by telephone, mail, or email for enrollment.

An estimated 40 subjects over the course of 1 year will be eligible for inclusion based on clinical experience from recent years.

Informed consent will be obtained prior to completion of eligibility screening.

9.5 Informed Consent/Assent and HIPAA Authorization

The principal investigator or a physician-level investigator on the study team will obtain informed consent from the subject's family in person at the subject's bedside at CHOP or a private conference room at CHOP, or by telephone. If the consent discussion takes place via phone, then the physician investigators will obtain a signed consent form from the subject and their parent/guardian at the first in-person visit, prior to the start of any study procedures. Two parent/guardian signatures will be required for the enrollment of a minor in this research study. The study will be explained in full detail. The subject's family will have the opportunity to contact the staff at CHOP at any time to clarify any questions that they have. Subjects' family will be informed that their participation in the study is voluntary and will not affect the medical care the subject receives, that there will be no direct benefit of participation, and that the primary risk is breach of confidentiality. The informed consent form will be kept in the investigator's locked office cabinet. A combined consent-authorization document will be used.

9.6 Payment to Subjects/Families

9.6.1 Payments to the family for time and inconvenience, parking (i.e. compensation)

This study requires that subjects and their family must take time out of their schedules in order to participate. Therefore, time and inconvenience compensation of $50 per outpatient CHPS visit, and $25 for completing the whole study (maximum $125/subject) will be provided. For subjects <18 years of age, the subjects will receive one-third and the parents/legal guardians will receive two-thirds of the compensation. Subjects ≥18 years of age will receive the total compensation amount. Subjects/parents or legal guardians will receive this compensation only once (even if they must return to CHOP for further testing) at the completion of the study. If for some reason, the subject cannot complete the study at the time of study visit, they will still receive the time and inconvenience compensation. This compensation will be in the form of a Participant Reimbursement Card.

9.6.2 Payment to the family for transportation and lodging.

Subjects will be reimbursed for transportation and lodging up to a maximum of $1,500.00 for the entire study. Receipts for travel and lodging will be required for reimbursement. This reimbursement will be in the form of a check and will be paid at the end of their study participation. If a subject is under the age of 18, the compensation will be paid to the parent or legal guardian of the subject.

PUBLICATION

The investigators intend to present the results at national conferences and to publish the results in peer-reviewed medical journals.

10 REFERENCES

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11 APPENDIX 1

Medications Known to Adversely Interact With Vitamin E	
Orlistat	Risk Category D
Tipranavir	Risk Category D

*Note: As this list is subject to change over time, the Lexicomp database will be referenced for the most up-to-date drug interaction information during inclusion/exclusion screening. Medications known to adversely interact with Vitamin E are those listed as Risk Category D ("consider therapy modification") or Risk Category X ("avoid combination") in the Lexicomp database.

12 APPENDIX 2

Baseline Symptom Questionnaire

Event	Severity	Number of events in the past 14 days
Severity Definitions: **None** – event did not occur **Mild** – minimal symptoms, no treatment needed **Moderate** – symptoms requiring treatment at home or as an outpatient **Severe** – symptoms requiring hospitalization or emergency room visit *or* life-threatening or potentially life-threatening symptoms		
Seizure	□ None □ Mild □ Moderate □ Severe	
Headache	□ None □ Mild □ Moderate □ Severe	
Vision change/blurred vision	□ None □ Mild □ Moderate □ Severe	
Weakness	□ None □ Mild □ Moderate □ Severe	
Fatigue	□ None □ Mild □ Moderate □ Severe	

Nausea	□ None □ Mild □ Moderate □ Severe	
Vomiting	□ None □ Mild □ Moderate □ Severe	
Diarrhea	□ None □ Mild □ Moderate □ Severe	
Stomach pain	□ None □ Mild □ Moderate □ Severe	
Constipation	□ None □ Mild □ Moderate □ Severe	
Bruising	□ None □ Mild □ Moderate □ Severe	
Bleeding	□ None □ Mild □ Moderate □ Severe	
Rash	□ None	

	□ Mild □ Moderate □ Severe	
Itching	□ None □ Mild □ Moderate □ Severe	
LIST ANY OTHER SYMPTOMS YOU EXPERIENCED SINCE STARTING VITAMIN E *IN THE BOXES BELOW*		
	□ None □ Mild □ Moderate □ Severe	
	□ None □ Mild □ Moderate □ Severe	
	□ None □ Mild □ Moderate □ Severe	
	□ None □ Mild □ Moderate □ Severe	

13 APPENDIX 3

Tolerability Questionnaire

Event	Severity	Number of events since starting Vitamin E
Severity Definitions: **None** – event did not occur **Mild** – minimal symptoms, no treatment needed **Moderate** – symptoms requiring treatment at home or as an outpatient **Severe** – symptoms requiring hospitalization or emergency room visit *or* life-threatening or potentially life-threatening symptoms		
Seizure	☐ None ☐ Mild ☐ Moderate ☐ Severe	
Headache	☐ None ☐ Mild ☐ Moderate ☐ Severe	
Vision change/blurred vision	☐ None ☐ Mild ☐ Moderate ☐ Severe	
Weakness	☐ None ☐ Mild ☐ Moderate ☐ Severe	
Fatigue	☐ None ☐ Mild ☐ Moderate ☐ Severe	

Nausea	□ None □ Mild □ Moderate □ Severe	
Vomiting	□ None □ Mild □ Moderate □ Severe	
Diarrhea	□ None □ Mild □ Moderate □ Severe	
Stomach pain	□ None □ Mild □ Moderate □ Severe	
Constipation	□ None □ Mild □ Moderate □ Severe	
Bruising	□ None □ Mild □ Moderate □ Severe	
Bleeding	□ None □ Mild □ Moderate □ Severe	
Rash	□ None	

	□ Mild □ Moderate □ Severe	
Itching	□ None □ Mild □ Moderate □ Severe	
LIST ANY OTHER SYMPTOMS YOU EXPERIENCED SINCE STARTING VITAMIN E *IN THE BOXES BELOW*		
	□ None □ Mild □ Moderate □ Severe	
	□ None □ Mild □ Moderate □ Severe	
	□ None □ Mild □ Moderate □ Severe	
	□ None □ Mild □ Moderate □ Severe	

14 APPENDIX 4

Study Staff Telephone Script

Hello [name of contact], I am [name of caller], calling from CHOP regarding you/your child's enrollment in the Vitamin E Supplementation in Hyperinsulinism/ Hyperammonemia Syndrome study. I am calling to check in and see how you are doing. The call will probably take around 15 minutes.

Have you/your child had any new medical problems since you last spoke with our team?

Have you/has your child been seen in the Emergency Department, Urgent Care Center, or by another health provider since you last spoke with our team?

If yes to either of the above, what has happened?

Have you/your child taken any new medications or supplements or stopped taking any previous medications or supplements since you last spoke with our team?

Sometimes it is hard to remember to take a new a new supplement. Since you were given vitamin E at the visit, how has it been going?

At what time of the day do you take the vitamin E?

How much do you take each time?

Do you take the vitamin E with meals or with any other medications or supplements?

Have you missed any doses of vitamin E?

If yes, how many?

Can you tell me about any new feeling or symptoms you/your child has experienced since beginning taking the vitamin E (or since our last call)? Are there any new feelings or symptoms you are/your child is experiencing that you think may be side effects from the vitamin E supplementation?

Have you/has your child had any of the following (if yes, was the severity mild, moderate, or severe, and, what is the number of events that have occurred since starting Vitamin E):

Event	Severity	Number of events since starting Vitamin E
Severity Definitions: **None** – event did not occur **Mild** – minimal symptoms, no treatment needed **Moderate** – symptoms requiring treatment at home or as an outpatient **Severe** – symptoms requiring hospitalization or emergency room visit *or* life-threatening or potentially life-threatening symptoms		
Seizure	□ None □ Mild □ Moderate □ Severe	
Headache	□ None □ Mild □ Moderate □ Severe	
Vision change/blurred vision	□ None □ Mild □ Moderate □ Severe	
Weakness	□ None □ Mild □ Moderate	

	□ Severe	
Fatigue	□ None □ Mild □ Moderate □ Severe	
Nausea	□ None □ Mild □ Moderate □ Severe	
Vomiting	□ None □ Mild □ Moderate □ Severe	
Diarrhea	□ None □ Mild □ Moderate □ Severe	
Stomach pain	□ None □ Mild □ Moderate □ Severe	
Constipation	□ None □ Mild □ Moderate □ Severe	
Bruising	□ None □ Mild □ Moderate □ Severe	

Bleeding	□ None □ Mild □ Moderate □ Severe	
Rash	□ None □ Mild □ Moderate □ Severe	
Itching	□ None □ Mild □ Moderate □ Severe	
HAVE THERE BEEN ANY OTHER SYMPTOMS YOU EXPERIENCED SINCE STARTING VITAMIN E? *(If yes, these should be recorded by the person administering the survey in the boxes below)*		
	□ None □ Mild □ Moderate □ Severe	
	□ None □ Mild □ Moderate □ Severe	
	□ None □ Mild □ Moderate □ Severe	

	□ None □ Mild □ Moderate □ Severe	