Glycemic control and the brain in children with type 1 diabetes

Protocol Identifying Number: NIH 5RO1 HD078463-04

Nelly Mauras, MD, Allan Reiss, MD

On Behalf of the Diabetes Research in Children Network (DirecNet)

Version Number: v. 2.0

15 of June 2018

KEY ROLES

Protocol Chair/Director	
Name, degree	Nelly Mauras, MD (contact) Allan Reiss, MD
Title	Co- Principal Investigators
Institution Name	Nemours Children's Health System – Jacksonville, FL Stanford University – Palo Alto, CA
JCHR Coordinating Center Director	
Name, degree	John Lum, MS
Title	Director, Artificial Pancreas Coordinating Center
Institution Name	Jaeb Center for Health Research
Medical Monitor	
Name, degree	Nelly Mauras, MD Kimberly Englert, RN
Institution Name	Nemours Children's Health System – Jacksonville, FL
Sponsor	National Institutes of Health, NICHD
Additional Funding	JDRF

TABLE OF CONTENTS

Chapter 1: Background Information ……11
1.1 Scientific Background ……..11
1.2 Preliminary Data ……....13
1.2.1 MRI …….... 13
1.2.2 Hybrid Closed-loop …… 15
1.2.3 Longitudinal study …… 15
1.2.4 Cognitive Testing ……..... 16
1.3 Innovation …… 17

Chapter 2: Specific Aims and Hypothesis ……...17

Chapter 3: Study Enrollment and Screening ……..18
3.1 Participant Recruitment and Enrollment ……18
3.2 Participant Inclusion Criteria ……18
3.3 Participant Exclusion Criteria **……..**18

Chapter 4: Study Procedures ……19
4.1 Data Collection and Testing ……19
4.2 Procedures at Screening Visit: Run-in phase….....19
4.3.1 Randomization……19
4.3 Baseline Visit ……20
4.4 Home Procedures …….....20

Chapter 5: Study Visits and phone/electronic Contact **……..**20
5.1 Study Visits Procedures **…….....**20
5.1.1 1-week visit (hybrid closed-loop arm only)……20
5.1.2 3-month visit (both groups)……..21
5.1.3 6-month visit (both groups)……..21
5.2 Phone/Electronic Contacts ……22
5.3 Early Termination Visit (if applicable). ……22
5.4 Unscheduled Visits…..……22
5.5 Access to Medtronic 670G CL insulin pump at Study Closure…….22

Chapter 6: PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES……..22

Chapter 7: Medtronic Minimed 670G Components ……22
7.1 Insulin pump ……22
7.2 Guardian Link (GST3C) Transmitter (MMT-7811 …….....22
7.3 Charger (MMT-7715) …….....23
7.4 Medtronic MiniMed Guardian (Enlite® 3)
Glucose Sensor (MMT-7020 ……23
7.5 One-press Serter ……23
7.6 Contour Next Link 2.4 Glucose Meter **…….....**23

Chapter 8: Continuous Glucose Monitoring……23

Chapter 9: Safety Measures ……...23
9.1 Ketone Strips ……23
9.2 CGM Calibration ……23
9.3 Pump Failure …….....23
9.4 Hypoglycemia Threshold alarm ……23
9.5 Hyperglycemia Threshold alarm ……24

Chapter 10: Structural and Functional Brain Testing procedures ……24
10.1 Brain MRI ……24
10.1.1 Subject preparation and testing schedule ……24
10.1.2 Scan sequences ……24
10.1.3 Multisite imaging ……24
10.1.4 Functional MRI tasks……24
10.1.5 Image processing and analysis ……25
10.2 Cognitive Testing……25
10.2.1 Cognitive testing procedures……26

Chapter 11: Questionnaires……27
11.1 Hypoglycemia Fear Survey……27
11.2 INSPIRE Survey……27

Chapter 12: Statistical Considerations……26
12.1 Power estimation……26
12.2 Statistical Analysis Plan……27
12.3 Safety Analysis……28
12.4 Subgroup Analyses……28

Chapter 13: Adverse Events ……28
13.1 Definitions……28
13.2 Reportable Adverse Events……29
13.2.1 Hypoglycemic Events……29
13.2.2 Hyperglycemic Events/DKA……29
13.2.3 Timing of Event Reporting……30

Chapter 14: Sources of Materials, Recruitment and Informed Consent Procedures……30
14.1 Research Material……30
14.2 Recruitment……30
14.3 Informed Consent Procedures…… 31

Chapter 15: Potential Risks of the Study……31
15.1 Risks of CGM and Insulin Infusion Sets……31
15.2 Risks of MRI……31
15.3 Risks of Neuropsychological Testing……31
15.4 Risks of Closed-Loop Studies……32
15.5 Risks Associated with Hypoglycemia……32
15.6 Risks Associated with Hyperglycemia……32
15.7 Risks Associated with DKA……32
15.8 Risks of Loss of Privacy and Confidentiality……32

Chapter 16: Miscellaneous Considerations……33
16.1 Alternative Treatments and Procedures……33
16.2 Participant Compensation……33
16.3 Withdrawal……33
16.4 Data collection and monitoring……33
16.5 Records Retention ……33
16.6 Quality Assurance and Monitoring……33
16.7 Protocol Deviations ……33
16.8 Data Safety Management Board……33
16.9 Criteria for Suspending Overall Study ……33
16.10 Institutional Review Boards……34
16.11 Future Use of Stored Specimens……34

Chapter 17: References..... 35

LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	Adverse Event
BG	Blood Glucose
BOLD	Blood Oxygenation Level Dependent
CGM	Continuous Glucose Monitor
DSMB	Data Safety & Monitoring Board
DTI	Diffusion Tensor Imaging
FDA	Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
FWE	Family-Wise Error
GM	Gray Matter
GMV	Gray Matter Volume
HbA1C	Hemoglobin A1C
HCL	Hybrid Closed-Loop
MRI	Magnetic Resonance Imaging
NIH	National Institutes of Health
SAE	Serious Adverse Event
SC	Standard Care
WM	White Matter
WMV	White Matter Volume

SITE PRINCIPAL INVESTIGATOR STATEMENT OF COMPLIANCE

Protocol Title: Glycemic control and the brain in children with type 1 diabetes

Protocol Version/Date: 6/15/2018; v2.0

I have read the protocol specified above. In my formal capacity as a Site Principal Investigator, my duties include ensuring the safety of the study participants enrolled under my supervision and providing the Nemours Clinical Coordinating Center and the Imaging & Data Coordinating Center, which serve as the complimentary Coordinating Centers for the protocol, as well as the Jaeb Center for Health Research, which will oversee all data and downloads regarding the hybrid closed-loop devices and continuous glucose monitors, with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site.

This trial will be carried out in accordance with ICH E6 Good Clinical Practice (GCP) and as required by the following (use applicable regulations depending on study location and sponsor requirements; examples follow): United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812).

As the Principal Investigator, I will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), or other approved Ethics Committee, except where necessary to eliminate an immediate hazard(s) to the trial participants.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Participants Protection Training and Good Clinical Practice Training. Further, I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Investigator's Signature _____Date: _____ / _____ / _____
dd mm yyyy

Investigator's Name: _____

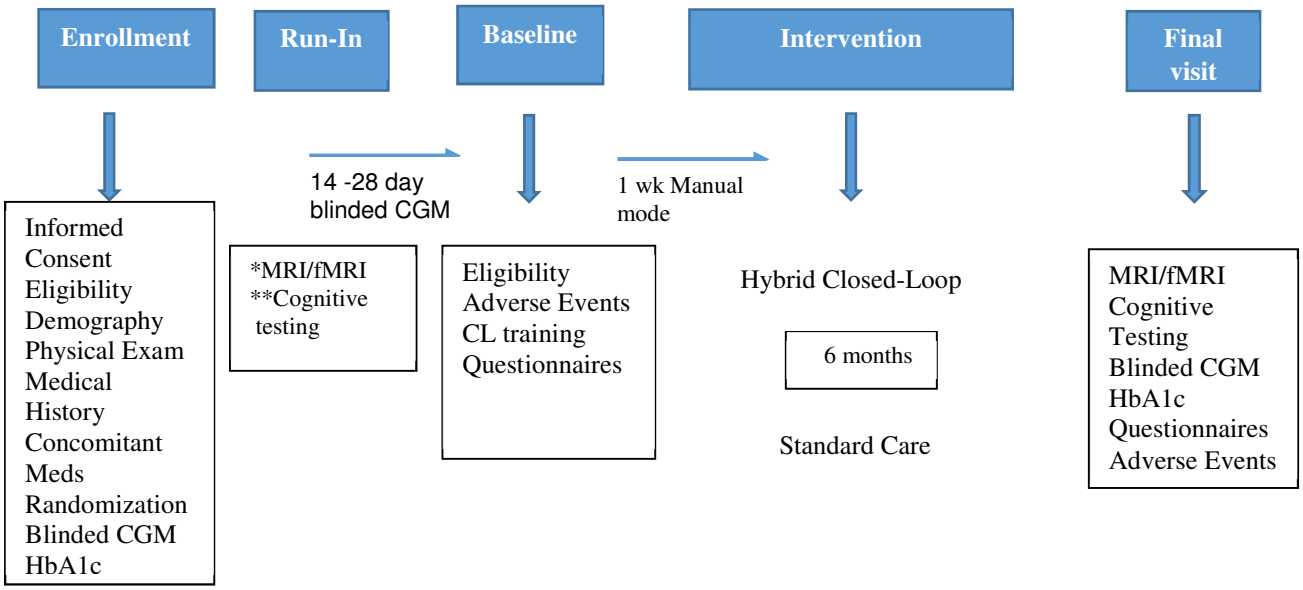
Site Name/Number: _____

PROTOCOL SUMMARY

PARTICIPANT AREA	DESCRIPTION
Title	Glycemic control and the brain in children with type 1 diabetes
Précis	This is a pilot, proof of concept study, on the impact of better normalization of the blood sugars in children with long-standing diabetes using hybrid closed-loop technology on specific metrics of brain structure and function and cognition.
Objectives	To determine if substantial improvement in glycemic control and reduced exposure to hyper- and hypoglycemia (using continuous hybrid closed-loop devices) (a) alters trajectories of total and regional gray and white matter volumes, white matter microstructure, and task- and resting-state blood oxygen level dependent (BOLD) activation, and (b) alters general and specific neurocognitive metrics in adolescents with long-standing T1D. We hypothesize that if the neuroimaging and cognitive differences observed in young children with T1D are due in part to repeated hypo- and hyperglycemic insults, that prolonged improvement of the blood sugars and decreased glycemic variability will create a neurodevelopmental trajectory towards normalization of total and regional brain volumes, white matter microstructure, specific neurocognitive functions, and BOLD-fMRI activation patterns. This is a pilot, proof of concept study to help us gather preliminary data to be used in a larger, more comprehensive study.
Study Design	Adolescents with T1D 14 years and older who wish to transition to hybrid closed-loop therapy who are willing to be randomized will be recruited. Subjects will be randomized to 6 months of continuous hybrid-CL therapy (N= 25, T1D hybrid closed-loop group), vs. Standard Care diabetes management (N=25, T1D controls) Unsedated MRI, DTI, fMRI and age-appropriate cognitive data will be collected, as well as demographics, CGM and HbA1c. Data from the final time-point assessment will be used for subjects enrolled in our current longitudinal study. The study will have 3 components**:** baseline data collection period, hybrid closed-loop phase, and final data

PARTICIPANT AREA	DESCRIPTION
	collection period. Imaging and cognitive data will be repeated after 6 months.
Number of Sites	There will be 5 centers participating in the study; Nemours Children's Health System – Jacksonville, FL, Stanford University – Stanford, CA, Yale University – New Haven, CT, University of Iowa– Iowa City, IO, Washington University – St Louis, MO
Endpoint	**Primary Efficacy Outcome:** Total and regional GM and WM volumes and white matter microstructure (fractional anisotropy) **Key Secondary Efficacy Outcomes:** Changes in BOLD-fMRI and neurocognitive metrics. **Key Safety Outcomes: %** time in target BG range, % time in hypoglycemia
Population	**Key Inclusion Criteria** Subjects with T1D for at least 1 year on stable insulin therapy who are age 14 years to less than 18 who have been diagnosed with T1D before the age of 8 years
Sample Size	50 subjects
Treatment Groups	A random assignment of study arm to either hybrid closed-loop group with 24- hour sensor-augmented therapy (Medtronics 670G insulin pump) or standard care group with use of blinded CGM or use of home CGM. Participants will be randomized 1:1 to the hybrid closed-loop group or the standard card group Random assignment 1:1
Participant Duration	6 months

SCHEMATIC OF STUDY DESIGN



SCHEDULE OF STUDY VISITS AND PROCEDURES

	Enrollment visit	Run-in 14- 28 days	Baseline visit	1 w visit	1m PC	2m PC	3m visit	4m PC	5m PC	6m visit	1 w PC
Informed consent	X										
Eligibility assessment	X										
Physical exam[1]	X						X			X	
Medical Conditions	X										
Concomitant Medications	X										
Urine pregnancy test[2]	X						X			X	
Psychosocial questionnaires[3]			X							X	
Insertion Blinded CGM[4]	X						X			X	
Blinded CGM wear		X									
Local HbA1c	X						X			X	
CGM compliance assessment			X								
Cognitive Testing[5]		X								X	
Brain MRI/fMRI[5]		X								X	
Glucose Data download				X	X	X	X	X	X	X	
Randomization	X										
Study pump training[6]			X	X			X				
Phone contact (2x/m)[6]					X	X		X	X		
Transition to Standard Care[7]										X	
Follow up patient contact[8]											X
Adverse Event Assessment			X	X	X	X	X	X	X	X	X

PC= patient contact (telephone or electronic)

1-Physical Exam to include Tanner staging at baseline and 6-month visit.
2-Urine pregnancy test for females post-menarche with childbearing potential
3-Quality of life questionnaires to be completed by participant and parent as appropriate
4-Insertion of Blinded CGM for participants who do not routinely wear a CGM (minimum six out of 14 days). Participants may repeat CGM insertion one time at baseline if sufficient data not obtained.. Timing of insertion of blinded CGM will depend on availability of the CL devices.
5- Some subjects who participated in longitudinal study may not need this at baseline
6- For study pump group only (phone or electronic)
7- For study pump group randomized to the CL arm, transition back to standard care will occur at six months
8-For study pump group

Chapter 1: Background Information

1:1 Scientific Background

Neuroanatomical and Cognitive Changes in Children with T1 diabetes

Human and experimental animal data suggest that both hyper- and hypoglycemia, depending on age and severity, can lead to altered brain structure and neurocognitive function [1, 2]. In early-onset diabetes, subjects may show altered brain morphology, and the young developing brain may be particularly vulnerable to neurodevelopmental insult associated with diabetes [3, 4]. An area especially sensitive to insult from hypoglycemia is the hippocampus [5-7], which could potentially explain in part the learning and memory dysfunction observed in some studies of children with diabetes [8-12]. Performing brain MRI in 62 children (mean age 9.8 years) with early-onset diabetes (~3 years) Ho et al. [13] showed a high prevalence of central nervous system structural abnormalities and mesial temporal sclerosis. Greater hyperglycemia was correlated with decreased white matter volumes in the parietal cortex in children [14] and with smaller cortical cells with reduced myelin content in rodents [15]. Prospective and cross-sectional cohort studies have examined cognitive functioning in youth with T1D vs. control subjects, and similar to adults, youth with diabetes tend to show lower intelligence quotients (IQs) and deficits in executive functioning, particularly attention, episodic and spatial working memory, and processing speed, as compared with control subjects [1, 3, 4, 9-12]. However, longitudinal studies of cognition in youth with T1D have shown more mixed results [16, 17].

We previously conducted a longitudinal study in young children with (n = 144) and without (n = 72) T1D (ages 4 to <10 years) to prospectively examine the impact of dysglycemia on brain and cognitive development. We used high-resolution structural and diffusion-weighted MRI (Siemens 3T, TimTrio), a comprehensive battery of cognitive metrics, and serial glycemic measures from continuous glucose monitoring (CGM) [18-20]. The MRI studies were performed unsedated using simple desensitization/training protocols with a 92% successful scan rate [21]. Baseline assessments showed that hyperglycemia was associated with lower scores for executive functions, intelligence, learning, and memory [19] as well as aberrant gray matter (GM) and white matter (WM) structural variations [18, 20]. Of note, although between-group cognitive differences detected at baseline were relatively subtle, neuroanatomical differences were substantial. Using voxel-based morphometry (VBM), we observed significant differences in specific brain regions in children with diabetes versus control subjects [20], in particular within areas involved in visual-spatial processing, executive functions and working memory. Greater hyperglycemia was associated with smaller GMV in medial-frontal and temporal-occipital regions and greater GMV in lateral prefrontal regions [18, 20]. We also found significant differences in WM microstructure, suggesting widespread aberrant fiber coherence in young children with diabetes; differences correlated with longer disease duration, increased glycated hemoglobin, and greater hyperglycemia [18]. These cross-sectional observations raised important questions concerning longitudinal trajectories of neurodevelopment in childhood-onset diabetes [22].

We repeated unsedated brain neuroimaging and cognitive testing after 18 months in these same children with and without diabetes to investigate whether brain differences would lessen, persist, or worsen over time. We also correlated these neuroanatomical changes with longer (18 months) exposure to hypo- and hyperglycemia and with targeted measures of neurocognitive function longitudinally [23]. Children with diabetes had slower total gray and white matter growth than control subjects. Particular gray matter regions (left precuneus, right temporal, frontal, and parietal lobes and right medial-frontal cortex) showed lesser growth in diabetes, as did white matter areas (splenium of the corpus callosum, bilateral superior-parietal lobe, bilateral anterior forceps, and inferior-frontal fasciculus). These changes were associated with higher cumulative hyperglycemia and glucose variability but not with hypoglycemia [23]. Young children with T1D had significant differences in total and regional gray and white matter growth in brain regions involved in complex sensorimotor processing and cognition compared with age-matched control subjects over 18 months, suggesting that chronic hyperglycemia may be detrimental to the developing brain. In this large cohort of young children (age 4 to <10 years) with and without T1D studied longitudinally, our studies demonstrate that early-onset diabetes significantly affects the development of total and regional gray and white matter volumes (WMV), with differences between groups enhanced over time. Importantly, in the diabetic group, the slower growth was most strongly associated with hyperglycemia and glycemic variability, as measured by several metrics, including glycated hemoglobin, and

extensive quarterly data from CGM. These findings were further supported by our recent paper showing that slower brain growth in T1D is identifiable with different, complementary image processing methods including results from the FreeSurfer analysis pipeline [24] [See *Preliminary Data* section] ***These studies provide strong evidence that the developing brain is a vulnerable target for diabetes complications*** [23].

We also repeated the cognitive testing performed at baseline and found that the cognitive domain scores in these children did not differ between groups at 18 months and did not change differently between groups over the follow-up period [25]. However, within the T1D group, a history of diabetic ketoacidosis (DKA) was correlated with lower verbal IQ and greater hyperglycemia exposure (HbA1c area under the curve since diagnosis) was inversely correlated to executive functions test performance. In addition, those with a history of both types of exposure performed most poorly on measures of executive function. This finding indicated that within the T1D group, associations between cognition (verbal IQ and executive functions) and glycemic variables (chronic hyperglycemia and DKA history) continued to be evident at 18 months in a manner similar to that observed at baseline. ***Our preliminary analysis of time 3 cognitive testing further suggest that verbal IQ is now significantly lower in the T1D group*** (see *Preliminary Data*)***.***

Because childhood is a period of rapid and dynamic brain development, including modification and pruning of synapses and increased myelination of white matter fiber tracts, it also is a time of increased vulnerability to brain insults [26, 27]. Hence, continued longitudinal study of this T1D cohort and their carefully matched healthy comparison group is the central focus of our current R01. We are presently in the 3rd year of the grant cycle with a cohort that includes 74% of the original children studied with T1D and their age-matched controls. Further, we are using identical protocols for acquisition of structural MRI (sMRI), diffusion tensor imaging (DTI), neurocognitive and glycemic data at 2 additional time points spread 2 years apart as the children grow and progress through puberty. We were also funded to include BOLD-fMRI (task and resting-state) studies during these later time points.

In the ongoing parent grant, we aim to determine over longitudinal follow up:

- If abnormalities in (1) total and regional gray and GMVs, and (2) white matter microstructure persist or worsen compared to age- and sex-matched, non-diabetic controls, and whether these changes are associated with measures of glycemic control and neurocognitive metrics as these children grow and progress through puberty;
- If children with T1D show abnormalities in BOLD-fMRI brain activity compared to controls in (1) frontal-parietal networks associated with visual-spatial working memory and response inhibition abilities, and (2) functional connectivity of resting state networks;
- If there are differences in key metrics reflecting general (e.g., IQ) and specific (e.g., executive, visual-spatial, memory) neurocognitive functions in the T1D group compared to controls, and whether these differences are associated with measures of glycemic control.

We are well on our way to accomplish these goals and, to-date, have recruited and re-studied all of the 214 targeted youth [see Preliminary Data section].

Closed-Loop Studies – towards the goal of normoglycemia

Despite the use of insulin infusion pumps and CGM, children with diabetes are still exposed to significant hyper- and hypoglycemia. Data in 8 to 17 year olds [28] indicated children spent over 40% of night and day with glucose values >180 mg/dl, and in our DirecNet study of 4 to 9 year olds even higher at ~ 50% [29]. Furthermore, hypoglycemia occurred in ~10% of nights, with mean duration over 80 minutes, and 23% of subjects had at least two hours of hypoglycemia per event [30]**.** Recent epidemiological data from a large cohort of patients followed in specialty endocrine clinics participating in the T1D Exchange network around the US confirms that adolescents (13-18yrs) continue to be the age group with the worst metabolic control (mean HbA1c 9.0%), despite access to the best available technologies, including insulin pumps and CGM [31]. When considering those children that developed diabetes at an early age, like the cohort we have been following for several years now, the potential impact of chronic exposure to abnormal blood glucose in the developing brain is very concerning.

Given our findings that the degree of hyperglycemia correlated positively with the observed neuroanatomical and neurocognitive changes, we posit that it may be possible to influence or modify the trajectory of these observed

changes if blood glucose can be significantly normalized and better controlled. Studies in either older or younger children with T1D [28, 29] have not demonstrated benefit from the use of CGM *per se* to lower glucose levels or the risk of severe hypoglycemia [28], suggesting a closed-loop feedback controlled insulin delivery system will be needed to achieve optimal metabolic control in youth. Over the last 2-3 years there has been an explosion of studies demonstrating the feasibility, safety and efficacy of closed-loop insulin delivery systems that could better accomplish metabolic control. Different from insulin pump therapy and threshold-suspend approaches, the artificial pancreas or closed-loop systems use a control algorithm that can increase and decrease subcutaneous insulin delivery based on sensor glucose levels in real-time autonomously and continually [32]. In both in-patient or controlled settings [33-37] and more recently also in outpatient settings (considered *transitional*), and using remote monitoring and supervision by investigators in hotels [38] or diabetes camps [39, 40], studies show that glucose control can improve, and reduction of hypoglycemia can be accomplished [39-42]. Three weeks to 3 months of at-home studies of overnight close-loop insulin delivery have been performed in adolescents and adults [43-46]. Home studies [46, 47] of unsupervised day-and-night closed-loop use have now been performed in adults, and recently, unsupervised day-and-night hybrid closed-loop insulin delivery in free-living settings in adolescents 10–18 years of age was reported with positive results [48]. In aggregate, these results and the preliminary data presented below strongly suggest that marked improvement of glycemic variability and better glycemic control are possible using these systems in the outpatient setting in children. One such hybrid closed-loop system, the MiniMed 670G Insulin Pump has recently received FDA approval for adults and children down to age 14 years [49] and is presently being tested in pivotal FDA-approved outpatient trials in over 300 patients as young as 7 years (Clinicaltrials.gov NCT02660827).

It is relatively easier to achieve good glycemic control at night with closed-loop systems because the effects of meal and exercise disturbances are less frequent. In studies in preadolescents using a bi-hormonal (insulin and glucagon) system at night for at least 5 nights in an outpatient setting [50] the mean overnight sensor glucose was 137 mg/dl and % of readings <60 mg/dl was 2%. In adults using a nighttime only closed-loop system the mean time spent in target range was higher with the use of the artificial pancreas [51]. Using insulin only closed-loop systems during both the day and night in adults and adolescents in an outpatient setting for at least five days [46, 47, 52, 53] the mean sensor glucose was 153 mg/dl and the %of glucose values <70 mg/dl was 2.9% (range 1.5-2.8%). The mean estimated HbA1c for these studies would be 7.0% based on average sensor glucose values. The longest published trials of hybrid closed-loop to date is of 3 months duration and achieved a mean HbA1c of 7.3% [46] and 6.9% [49]. ***We hence propose to study children with T1 diabetes who use a state-of-the-art hybrid closed-loop system continuously in order to assess the impact of improved glycemic control on brain and cognition.***

1.2 PRELIMINARY DATA

1.2.1 DirecNet Experience in structural MRI - Structural and diffusion-weighted imaging analyses have been a core component of our imaging-related work to date. These analyses, performed in the Center for Interdisciplinary Brain Sciences Research at Stanford, have utilized multiple, complementary image processing approaches including voxel-based morphometry (VBM), surface/vertex based analyses (with FreeSurfer) and state-of-the-art tools to assess DTI data (e.g., FSL TBSS, Tracula). In our most recent analysis of 214 children, we examined between-group differences in brain growth with VBM over 18 months and showed slower brain growth and smaller gray and GMVs at 18 months in the T1D group vs. controls (Figure 1) [23].

Using the FreeSurfer pipeline we recently re-analyzed our structural data and observed that, relative to controls, children with diabetes had significantly less growth of cortical GMV and cortical surface area, and significantly less growth of GMV throughout the cortex and cerebellum. For the diabetic population, the change across longitudinal time points of the blood glucose level at the time of scan was negatively correlated with the change in GMV. These results largely confirmed and extended our initial findings using VBM (Figure 2) [24].

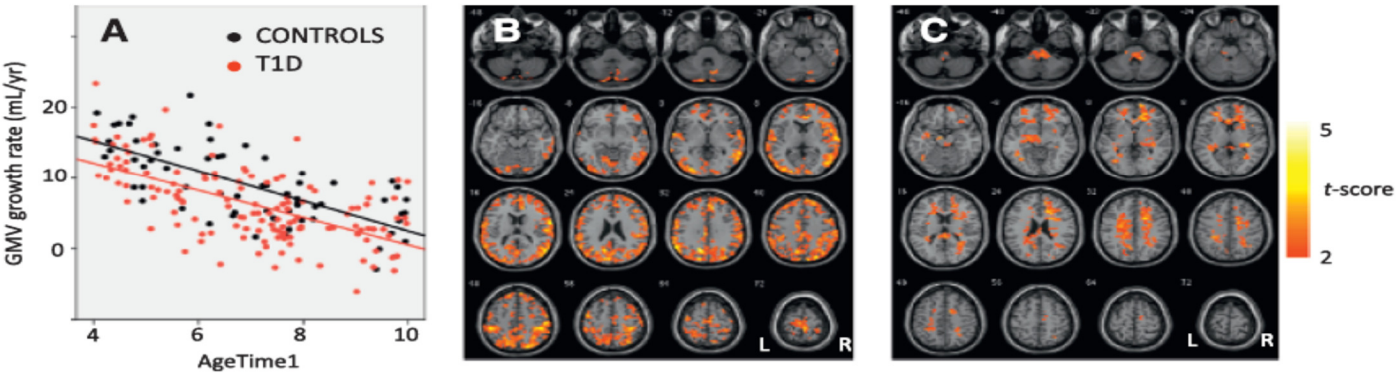


Figure 1: Brain regions where the T1D group (red dots) had significantly reduced growth compared to controls (black dots). (a) Growth rate of total GMV decreased with age (p<.001) and was significantly smaller for the diabetes group (p<.001). Regions of gray matter (b) and white matter (c) with significantly less growth in T1D than controls (p<0.001) (p<0.001) Fig. b & c are subtraction images, more orange in a region, the less growth in T1D compared to controls [23].

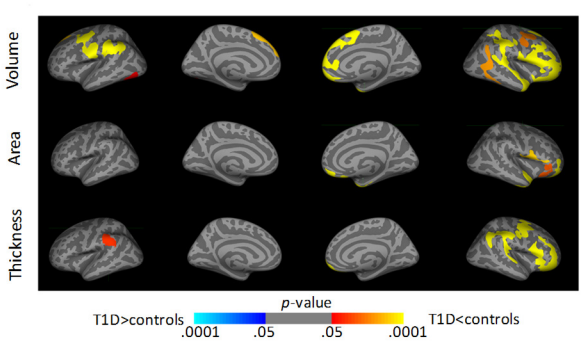


Figure 2: Cortical surface regions Indicated by colors with significantly reduced growth rates for T1D relative to controls; this included significantly less growth of cortical volume and surface area, and more cortical thinning. Regions are displayed on an inflated cortical surface with views of left lateral, left medial, right medial, and right lateral surfaces (gyri, light gray; sulci, dark gray) [24].

In our serial MRI images showed a correlation with the differences in the blood glucose at the time of the scan and changes in grey matter brain volume, left cerebral white matter, the corpus callosum, brain stem, mean cortical thickness, and ventricular volume changes [24].

Table 1: Correlations of changes in brain metrics with blood glucose [24].

Region	***p*-value**	**Regression coefficient***
Total gray matter	<.001	-93 (18)
Cortical gray matter	<.001	-76 (16)
Basal ganglia	0.001	-3.1 (.90)
Left hippocampus	0.04	-.24 (.12)
Right hippocampus	0.006	-.33 (.12)
Left cerebellar gray	<.001	-6.7 (1.4)
Right cerebellar gray	<.001	-6.9 (1.2)
Total white matter	0.06	-13 (7.0)
Left cerebral white matter	<.02	-6.9 (2.7)
Corpus callosum	0.006	-.18 (.06)
Brain stem	0.001	-1.2 (.34)
Total surface area	0.003	-6.1 (1.7) mm^2/(mg/dL)
Mean cortical thickness	<.001	-.00021 (.00006) mm/(mg/dL)
Ventricular volume	<.001	+6.1 (1.3)

*Mean (standard error). All units mm^3/(mg/dL) unless otherwise noted.

Resting state fMRI (rsfMRI) is increasingly being used as a sensitive and specific measure of the integrity of intrinsic brain connectivity. As such, rsfMRI data were acquired in a subset of our cohort consisting of 57 children with T1D (27F) and 26 age-matched non-diabetic controls (14F). Data were analyzed using both model-driven seed-based analysis and model-free independent component analysis, while controlling for age, sex and data acquisition site. Converging results were obtained across both methods indicating increased connectivity in children with T1D compared to non-diabetic controls. Further, connectivity was positively associated with cognitive functioning in T1D (Figure 3). The observed positive association of connectivity with cognitive functioning in childhood T1D, without group differences in cognitive function (at the time of the scan), suggests a putative compensatory role of hyper-intrinsic connectivity in the brain. This study aims to fill a critical knowledge gap on how dysglycemia in T1D might affect the brain's intrinsic connectivity at very young ages [54].

1.2.2 Preliminary Data – Using Hybrid Closed-Loop Devices to Improve Blood Glucose

Investigators in our consortium (Dr. Buckingham and Dr. Weinzimer) have been pioneers in the evaluation of closed-loop systems in the pediatric population, publishing the first studies in children in 2008, and characterizing the incremental improvements in the algorithms and hardware as these systems have moved from controlled inpatient studies to supervised outpatient studies in camp- and home-based environments.

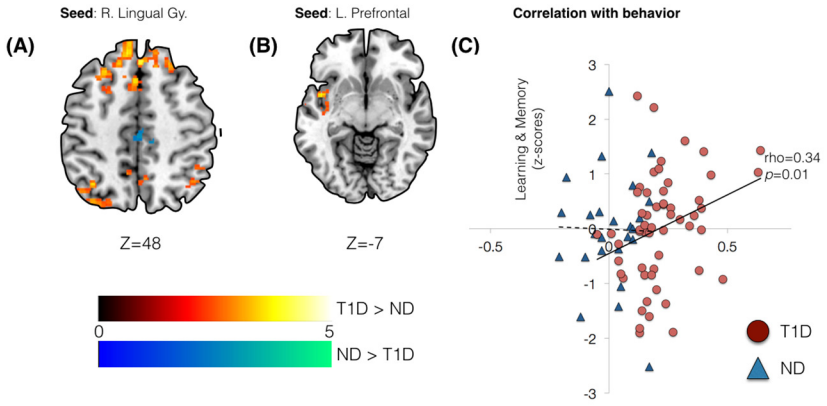
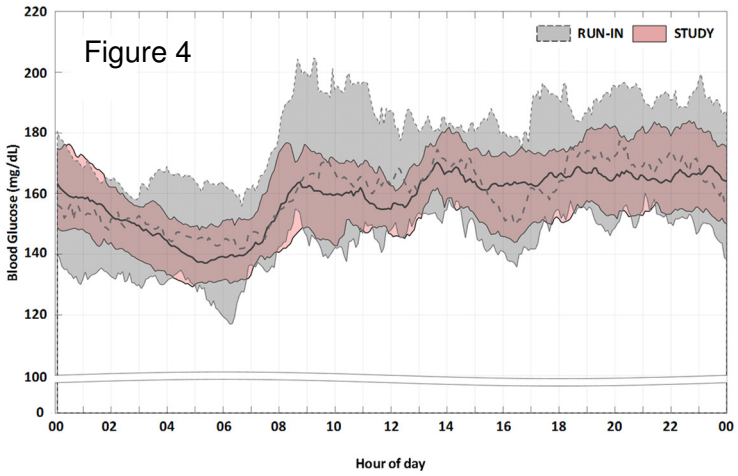


Figure 3: (A-B) Group differences in seed-based functional connectivity. (A) Seed-location: R. lingual gyrus and (B) seed-location: L. prefrontal cortex. (C) Increase in observed connectivity in the T1D group was positively related to cognitive functioning.

1) <u>Nocturnal predictive low-glucose suspend</u> – In two separate studies, Buckingham, et al randomized 45 adolescents/adults and 82 children to have the predictive suspend feature randomly engaged or disengaged for up to 6 weeks of wear. Results showed that episodes of sensor glucose levels < 60 mg/dL were lower on suspend vs. control nights (21% vs. 33%), and the median hypoglycemia area under the curve was reduced 81% on suspend nights [55]. Time spent with nighttime sensor glucose levels <70mg/dL was reduced from 10.1% to 4.6% in 11-14 year old and from 6.2% to 3.1% in 4-10 year olds (p<0.001) [56].

2) <u>Day and night hybrid closed-loop control</u> – After demonstrating feasibility over 6 days and nights in the challenging environment of a sleep-over camp [52], studies were conducted a multi-site hotel-based outpatient study in 15 adolescents over 5 days and nights using a hybrid day-and-night closed-loop system nearly identical to the system proposed in the current application (subjects continued to count carbohydrates and bolus for meals and snacks). Significant improvements were observed in mean glucose levels overall, and particularly overnight, even in the short duration of the study. Investigators at Stanford and Yale also participated in a pivotal study by Medtronic using the latest generation hybrid closed-loop system (670G pump), which is the system we propose to utilize in the present application. In this study of unsupervised home use of a hybrid closed-loop system in 124 adults and adolescents, we demonstrated significant improvement in A1c levels, increased time in target glucose levels, and reduced time in hypo- and hyperglycemia. In the adolescent subset, mean A1c levels dropped from 7.7± 0.8% to 7.1± 0.6% after 3 months on the system, with over 2/3 of glucose values in target range, <3% of glucose levels under 70 mg/dL, and no episodes of severe hypoglycemia [49] (Figure 4). The stabilization of overnight glucose values will also likely decrease large osmotic shifts prior to an MRI.



1.2.3 Preliminary Data From Current RO1 (Sept 2014 – May 2017)

In our current R01, we successfully established a new system of complementary coordinating centers, one clinical coordinating center at the Nemours Children's Health System in Jacksonville, Florida (Mauras – PI, contact PI) and a data and imaging coordinating center at Stanford (Allan Reiss – PI), as well as a new infrastructure for data capture and storage using the REDCap database. A neurocognitive core for centralized double scoring of the cognitive data was also established at Nemours. The 1st 6 months of the new cycle were spent organizing this infrastructure, securing IRB approvals for all 5 participating sites, establishing all subcontracts and securing all necessary support from needed partnerships for MRI scanning at the University of

Florida for the Nemours site and El Camino Hospital for the Stanford site. An NIH-designated data safety and monitoring board (DSMB) was reconvened from the same committee that monitored our grant in the previous cycle and protocol thoroughly reviewed and approved. Standard operating procedures were crafted for our Manual of Operations as well as governing structure with 2 co-principal investigators. Training of all participating MRI sites was performed and a traveling human phantom was scanned twice at all 5 sites before scanning the first subject to establish cross campus calibration. Functional MRI protocols have been standardized at all 5 sites. Training of all psychometricians and certification of proficiency was performed by the cognitive core. A face-to-face meeting was held in Bethesda to formally launch our study. The steering committee has been established with scheduled monthly or bi-monthly conference calls as well as bi-monthly study coordinators calls put in place by the coordinating centers. An investigator's website as well as a public site have been established (https://direcnet.stanford.edu). The study is listed in clinicaltrials.gov (NCT02351466).

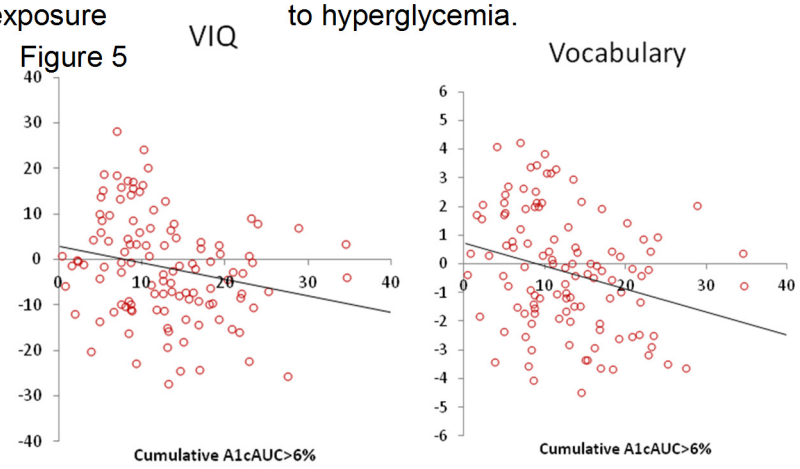
Table 2: Clinical Characteristics of Study Subjects Enrolled in Current RO1 to-date (mean ± SD) now at Time 3

	T1D	Controls
N	144	70
Age (yrs)	11.2 ± 1.9	11.6 ± 1.6
Male/Female	82/62	36/34
Race (%)		
Caucasian, AA, Other	88, 4, 8%	93, 4, 3%
Years with T1D	6.7 ± 2.7	NA
HbA1c (%)	8.0 ± 1.1	5.3 ± 0.3
%HbA1c>6%	1.9 ± 0.7	NA

In our current R01 grant we sought to recruit as many of the 144 children with T1D and 70 age-matched controls who participated in our original longitudinal 18 month study. We have successfully recruited the entire targeted cohort, 74% of the original children returning, 26% replaced; clinical characteristics are summarized in Table 2. All imaging and cognitive assessments have been performed as planned and subjects are followed longitudinally every 3 months and a CGM worn for 6 days and data downloaded. All time 3 (T3) data have been collected.

1.2.4 Preliminary Analysis of New Neurocognitive Data - We confined interim analysis of cognitive variables to IQ in the subset currently available (N=120 T1D, 69 controls). After controlling for Parent IQ, the T1D group had lower Verbal IQ (VIQ) and Full Scale IQ (FSIQ), but similar Performance IQ (PIQ) compared to the Control group (VIQ: Cohen's d=-0.46, p=.004; FSIQ: d=-0.33, p=.031; PIQ: d=-0.05, p=.651). Within the VIQ subtasks, groups differed most on Vocabulary (d=-0.43, p=.007). Within the T1D group, greater hyperglycemia exposure (measured by cumulative A1c AUC >6.0%) was associated with lower VIQ (partial r=-0.27, p=.006), and lower Vocabulary (partial r=-0.32, p=.001) after controlling for Parent IQ as shown in the scatterplots in Figure 5. These findings indicate that group differences in IQ are present in our cohort and lower IQ scores are associated with greater degree of chronic exposure to hyperglycemia.

Figure 5



We will use the same analytic methods utilized for the present grant (MRI, DTI, fMRI, cognitive testing, CGM and glycemic indices) in order to compare results after 6 months of better normalization of blood glucose using continuous hybrid closed-loop systems vs. standard care. This greatly economizes and leverages our resources by using our already established and efficient network infrastructure and assessment methodology.

I.3 INNOVATION: The brain as a target of diabetes complications has not received as much attention as other areas, particularly in children. Deleterious effects of severe hypoglycemia, particularly in the hippocampus and frontal cortex have been detected, and differences in brain volumes are evident in multiple brain regions in the diabetic state. Experimental evidence also suggests that chronic hyperglycemia may affect GMV, myelin glycosylation and cognitive processing speed in diabetes. Our data sugest that hyperglycemia may also be damaging to the developing brain. Tight glycemic control in very young children with T1D has been limited by the risks of hypoglycemia and the associated impairment in cognitive development, greatly limiting our ability to maintain near-normoglycemia in this age group.

This study will be the first to examine whether improved glycemic control and decreased glucose variability resulting from hybrid closed-loop control can improve brain and cognitive development. In support of the feasibility of this aspect of our study, it is clear from research in other clinical groups that significant changes in GMV, WMV, white matter microstructure and brain activation can occur in association with intervention within a 6-month period (or less). For example, short-term weight restoration for underweight patients with anorexia nervosa over a 50-day (average) period resulted in significant increases in gray and white matter volume relative to healthy controls [57]. In individuals with alcohol abuse, two weeks of supervised abstinence was associated with significant recovery of GMV in several brain regions including the cerebellum and parietal lobe [58]. In a separate study [59], individuals with alcohol addiction showed significant improvement in white matter measures after one month as reflected by either increased fractional anisotropy (FA) in the temporal lobe or increased WMV in the frontal and temporal lobes. In both of these disorders, alcohol abuse/addiction and anorexia, mitigation of an ongoing biological insult (alcohol or starvation) resulted in rapid changes in neuroimaging metrics towards normalization. There are additional examples of rapid changes in neuroimaging metrics in other clinical groups receiving disorder-focused intervention. Administration of risperidone and ziprasidone to patients with schizophrenia led to increased cerebral GMV after 28 days of treatment [60] and administration of citalopram to depressed patients increased hippocampal gray matter within 8 weeks [61]. Rapid changes have also been observed in white matter microstructure in other clinical groups. Patients with multiple sclerosis (MS) showed increases in FA within 2 months of facilitation of physiotherapy [62], depressed patients showed increased FA with 4 weeks from psychotherapy [63], and patients with obsessive-compulsive disorder showed a reversal of abnormal WM microstructure associated with a 12-week course of antidepressant (SSRI) treatment [64]. The feasibility and utility of utilizing functional neuroimaging changes as a putative biomarker of intervention has been demonstrated in hundreds of treatment studies, as described in recent reviews of pharmacology, psychotherapy, pain management and rehabilitation [65-71]. Thus, we predict that better normalization of blood glucose using hybrid closed-loop systems can improve/normalize brain structure and function, and neurocognitive performance in adolescents with T1D within a 6-month period, a time period of detectable change well-supported by scientific data. Results obtained from our study can also serve as the foundation for further investigations of brain development and function during full-time hybrid closed-loop control and over larger and more varied age groups, such as younger children in whom we have previously demonstrated blunted glucose counter-regulation [72].

Chapter 2: Specific Aims and Hypothesis

This is a pilot, proof of concept study largely due to funding constraints, which we anticipate will provide critically important preliminary data for a larger and longer-term study. The overarching aims of this pilot study are to determine if substantial improvement in glycemic control and reduced exposure to hyper- and hypoglycemia (using hybrid closed-loop insulin delivery devices) (a) alters trajectories of total and regional gray and white matter volumes, white matter microstructure, and task- and resting-state blood oxygen level dependent (BOLD) activation, and (b) alters general and specific neurocognitive metrics in adolescents with long-standing T1D. We hypothesize that if the neuroimaging and cognitive abnormalities observed in young children with T1D are due in part to repeated hypo- and hyperglycemic insults, that prolonged improvement of the blood sugars and decreased glycemic variability will create a neurodevelopmental trajectory towards normalization of total and regional gray and white matter volumes, white matter microstructure, specific neurocognitive functions, and BOLD-fMRI activation patterns. To accomplish this we will recruit a convenience sample of 50 adolescents with T1D randomized to use either a hybrid closed-loop device or standard care for 6 months. Structural, diffusion-weighted and functional MRI data, as well as cognitive testing will be performed at baseline and 6 months afterwards. An FDA-approved hybrid closed-loop device will be used to better normalize blood glucose. The

device itself is not the subject of investigation, but rather the impact of better glycemic control on the brain in these children.

Chapter 3: Study Enrollment and Screening

3.1 Participant Recruitment and Enrollment

Fifty participants are expected to complete the trial, 60 individuals may be enrolled to achieve this goal. Subjects will be recruited from 5 clinical centers in the United States, 10 per center will be expected to complete. All eligible participants will be included without regard to gender, race, or ethnicity.

Informed Consent and Authorization Procedures: Potential eligibility will be assessed as part of a routine-care examination. Written informed consent will be obtained prior to any procedures. Since participants will be under 18 years of age at enrollment, a parent/legal guardian (referred to subsequently as "parent") will be provided with the Informed Consent Form to read and will be given the opportunity to ask questions. Age-appropriate Child Assent Forms will be provided to the participants as appropriate. A signed copy of the consent form will be provided to the participant and his/her parent and another copy will be added to the participant's study record. As part of the informed consent process, each participant will be asked for authorization for release of personal information to study-designated parties if needed. Questions will be answered about the details regarding authorization. A participant is considered enrolled when the informed consent form has been signed.

3.2 Participant Inclusion Criteria

Adolescents with T1D will be targeted for participation if they meet the following criteria:
1) Be at least 14 and not yet 18 years old
2) Must have been diagnosed with T1D prior to 8 years old but after 6 months
3) For those diagnosed prior to 1 year of age, a positive blood test for an antibody marker will be required
4) Have been born term or near term (≥34 weeks) and weighed more than≥ 2 kg (4.4lbs) at birth
5) Be in puberty

3.3 Participant Exclusion Criteria

1) History of intellectual disability, language or learning disability identified before diagnosis of diabetes, or enrollment in a self-contained special education program
2) ADD/ADHD and/or on stimulant medication (*** see below)
3) Any known genetic or medical problem that could impair brain development
4) Abnormalities of the brain/nervous system, visual or hearing problem
5) History of seizures not associated with fever before diabetes diagnosis
6) Previous inpatient psychiatric treatment
7) Unable to have a MRI of the head due to having metal: including metal ear tubes, full set of braces in mouth (retainer is acceptable), other appliances, or vascular clip

Subjects who participated in our ongoing longitudinal trial who are 14 years and older will be eligible for participation under either of the following two circumstances: 1) if within 2 months of their last MRI/cognitive testing – hence these results will be used as baseline in the new study; or 2) if > 6 months since the last MRI/cognitive test, in which case new baseline MRI/cognitive studies will be performed. These subjects will not be enrolled if it is between 2 and 6 months since their last MRI/cognitive studies because of an anticipating learning effect for some of the cognitive studies. They could be enrolled after a 6-month interval, however.
*** If study subject was a part of our ongoing longitudinal study, subject will not be excluded if he/she has an ADHD diagnosis and/or is on ADHD medications since this was not an exclusion criteria for our previous and ongoing study.

Chapter 4: Study Procedures

4.1 Data Collection and Testing

After informed consent has been signed, a medical history, anthropometric measures and physical examination will be performed by study personnel and local laboratory testing if needed to screen for exclusionary medical conditions. The following procedures will be performed/data collected/eligibility criteria checked and documented:

- Inclusion and exclusion criteria assessed
- Demographics (date of birth, sex, race and ethnicity)
- Contact information (retained at the site and not entered into study database)
- Medical history
- Concomitant medications
- Physical examination to include:
  - Weight, height
  - Blood pressure and pulse
  - Neurological exam
  - Tanner staging – pubic hair and breasts (females) or genitalia (males)
- Blood draw for:
  - Finger stick HbA1c
- Urine pregnancy test for all post menarcheal females
- Current insulin program, including type of insulin and doses, total daily dose and basal and bolus amounts will be collected and units/kg/day calculated. Type of insulin pump and/or CGM use will also be recorded
- Stored sera/plasma

4.2 Procedures at Enrollment Visit: Run-in phase

In addition to procedures noted above, during the screening visit, a CGM (iPro®, using Enlite sensor, Medtronic) will be inserted and worn blindly and continuously for 12 days, changed after 6 days. Subjects will be instructed on their use and care and calibration and participants will be instructed how to insert the Enlite sensor and connect the iPro2 recorder at home for an at home insertion after 6 days of wear. Device will be returned to medical center after completion of run in phase. A glucose meter will be used by the subject to calibrate the CGM device minimally every 12 hours during wear. Additional calibrations may be required. The meter and test strips and control solution will be provided by the study.

If the study subject is using a Dexcom G5 or equivalent CGM on a regular basis (at least 21 of 28 days prior), the blinded CGM step can be skipped. A cognitive test and MRI/fMRI will be scheduled and completed prior to the baseline visit (details below). Whenever possible cognitive and structural studies should be completed within 4 weeks of each other.

4.2.1 Randomization

Randomization visit can be conducted on the same day as the enrollment visit. Participants whose eligibility has been verified will be randomly assigned to one of 2 treatment arms:

- **The Standard Care arm** - Participants will continue in whatever insulin program they were prior to the study. They will be expected to count carbohydrates, monitor BG levels, take insulin before meals and as needed, wear home CGM (if applicable) and adjust basal rates and boluses doses as routine.

- **The Hybrid Closed-Loop arm** - Subjects randomized to the CL arm will be provided a Medtronic 670G® hybrid closed-loop insulin pump using a loaner system from Medtronic. This system can also be prescribed to be purchased as per their insurance if the family wishes. . The loaner devices will be provided through the 6 months of the study and the switch to the patient's

device (if purchased) can occur at the 6 month visit, unless device available before the study and family wishes to use it for the duration of the study.

4.3 Baseline Visit: Subjects will come back and have the CGM downloaded and compliance with all study procedures, MRI and cognition included, will be reviewed. A series of questionnaires including the INSPIRE study group survey, and Fear of Hypoglycemia Survey will be administered to the subject and/or parent as appropriate. If there has been more than 6 weeks between enrollment and baseline visits, a repeat HbA1C will be performed. Subjects randomized to hybrid closed loop will be thoroughly instructed on their use.

4.4 Home Procedures

The Standard Care arm - Participants will return for a 3 and 6-month visit for routine clinic visit, adverse event (AE) assessment and blinded CGM insertion (if a CGM device is not already used as standard care). Study subjects will be required to calibrate the blinded CGM minimally every 12 hours. More calibrations may be required. After 6 days of blinded CGM wear an overnight shipping envelope will be provided to return the CGM components and the study meter back to the clinical center. Once the CGM has downloaded, if the participant did not obtained sufficient data, they may be asked to return to the clinical center for an additional blinded CGM insertion and another 6 day wear. Phone/electronic contact will be as needed and determined clinically.

The Hybrid Closed-Loop arm - Participants will initially use the 670G in manual-mode with the sensor-augmented function-only activated for 7 days (without using the threshold suspend, predicted low glucose suspend or other hybrid closed-loop features of the pump). Subjects transitioning from multiple daily injections (MDI) will have an extra week of wear prior to activation of the auto mode to allow proficiency in pump use. This is a procedure recommended by the manufacturer (Medtronic) during routine initiation of the device. They will calibrate the sensor 3-4 times a day and continue to monitor BG levels as routine. Participants will receive the following recommendations for use of the 670G in ***manual-mode***:

- High Set up Limit recommend to be set at 300 mg/dL
- Low Set up Limit recommend to be set at 70 mg/dL (not lower than 65mg/dL). Alert Setting Options may be set per investigator discretion
- Predictive alerts and rate of change alerts are optional
- It is recommended (optional) to have the SmartGuard Low Management turned ON with suspend before low activated and a low limit set at 70 mg/dL
- Basal insulin rate setting in Manual mode may need to be adjusted due to changes in insulin sensitivity when using Auto Mode.

Study subjects will return to the clinical center after ~7-14 days of wear and have the 670G auto mode activated. After the visit in which the hybrid closed-loop system has been activated, participants will maintain once weekly phone contact for the next 2 weeks, then twice-monthly through the 3-months visit, and twice-monthly afterwards through the 6-months visit for the duration of the study. They will download their device prior to contact. A routine clinic visit will occur after 3 months visit and again in 6 months. Subjects and their parents will be instructed to contact Medtronic for technical support for the 607G hybrid closed-loop system as per routine.

Chapter 5: Study Visits and Phone/Electronic Contacts (see Schedule of Events above on page 10)
This study will last approximately 6 months and will include up to 7 scheduled clinic visits for the hybrid closed-loop group (including MRI and cognitive testing) and up to 11 scheduled telephone/electronic contacts. For the standard care group this will include up to 6 scheduled visits (including MRI and cognitive testing) and no scheduled phone contacts mandated by the study. Phone contacts may be substituted for electronic communication as appropriate.

5.1 Study Visits Procedures
Study visits will occur at Enrollment, Baseline (± 2 days), 1-week (± 1 day), 3-months (±5 days) and 6-months (± 5 days) (see schedule of events: study visits and phone contact). Additional office visits may occur as needed. ***The following procedures will be performed in both groups at each visit, unless otherwise specified***

5.1.1: 1-week visit: For participants randomized to the hybrid closed-loop arm only:

Participants will be allowed to enter auto-mode after 1 week of use in the manual mode. Additional training on the 670G in the ***auto-mode*** setting will be provided. The following auto-mode settings will be recommended:

- High BG Setup limit - 300 mg/dL
- Low Setup limit - 70 mg/dL (not lower than 65mg/Dl)
- Target BG for the hybrid closed-loop algorithm is set at 120 mg/dL
- A temporary target may be used when subject exercises; up to 150 mg/dL
- Alarm settings fixed into the system are: sensor glucose ≤50 mg/dL, ≥ 300mg/dL for 1 hour and/or ≥ 250 mg/dL for 3 hours; settings can be adjusted as needed at the discretion of the investigator

Follow up visits and phone/electronic (on line) contacts will be scheduled.

5.1.2: 3-month visit (both groups)

- HbA1c via finger stick using DCA 2000 or equivalent NGSP-certified point-of-care method or venous blood sample at a local laboratory (value within 2 weeks prior to enrollment acceptable)
- Collection of total daily insulin dose and basal/bolus amounts
- Physical exam Including anthropometrics and vital signs (blood pressure and pulse)
- Urine pregnancy test on females post menarche

For Standard Care participants only: Placement of blinded CGM. If participant is currently using a DexCom CGM as standard care and has collected at least 21 out of the last 28 days of data, this step can be skipped and the unblinded data from the participants CGM will be used for the 3-month CGM data collection.

For closed-loop arm participants only: The Medtronic 670G hybrid closed-loop insulin pump will be downloaded and data will be reviewed for auto-mode usages and glucose trends. Additional training may be provided as needed. The goal is to achieve at least 70% of time in range (70-180 mg/dl), less than 3% of values <70 mg/dl, and at least 70% of the time in automode.

5.1.3: 6-month visit (both groups)

Same as 3 month visit plus:
- Pertinent QoL questionnaires completed by participant and parent
- MRI, fMRI and cognitive testing will all be scheduled and repeated

For hybrid closed-loop arm participants only: The Medtronic 670G hybrid closed-loop insulin pump will be downloaded. If subjects wear a loaner pump, it will be returned to the study center and the subjects usual care (either insulin pump or MDI will be restarted). All 670G related supplies; Enlite-3 glucose sensors, Guardian transmitter, infusion sets and insulin cartridges will be returned if they were loaned to participants.

5.2 Phone/Electronic Contacts

Scheduled phone or electronic contact will only occur in the hybrid closed-loop group and at the following times:
- After baseline, once weekly for the next 2 weeks, then twice-monthly through the 3-months visit.

- After the 3-month visit, phone/electronic contacts are scheduled to occur twice-monthly for the duration of the study.
- One week after study termination to assess a smooth transition to subsequent diabetes care.

During these contacts for participants randomized to the hybrid closed-loop arm we will assess time in auto mode and trouble shoot technical problem that may have occurred. Trends for hypo and hyper glycemia and nocturnal hypoglycemia will also be assessed. Any AEs or serious AEs (SAE)s will also be assessed. Participants on standard care will be contacted as needed by the family as per routine care. Additional phone contacts in either group may be performed as needed.

5.3 Early Termination Visit (if applicable)
If a participant is withdrawn by a clinician or decides to withdraw from the study prior to the 6 month visit, he/she will be asked to return to the clinical center for a close out visit at which time all study device(s) or supplies will be returned. Repeat structural and functional studies will depend on time of the withdrawal and will require discussion with Nemours clinical coordinating center.

5.4 Unscheduled Visits
Unscheduled visits may occur as needed due to technical issues, additional supply request and/or unforeseen issues

5.5 Participant Access to Medtronic 670G hybrid closed-loop insulin pump at Study Closure
The Medtronic 670G hybrid closed-loop insulin pump is FDA approved for ages 14 and older. Participants who do not use the system during the study can obtain a prescription from their physician and submit to their insurance carrier for purchase of the device commercially.

Chapter 6: Prohibited Medications, Treatments, and Procedures

Acetaminophen use
Use of medications containing acetaminophen during the study period can affect sensor accuracy hence their use during the use of a closed-loop device discouraged. If medications containing acetaminophen are taken, participant should take additional BG meter readings to verify glucose levels, and exit Auto Mode for a period of time (at least 6 hours) at the discretion of the investigator/care giver.

Chapter 7: Medtronic MiniMed 670G System Components

7.1 Insulin pump: Medtronic MiniMed 670G Insulin Pump (MMT-1780): When used with the CGM components (Enlite 3 Sensor, GST3C), the pump system is capable of continuous or periodic monitoring of glucose levels in the subcutaneous interstitial fluid and detection of hypo- and hyperglycemia. When Auto Mode is enabled, the sensor glucose values received from the GST3C by the insulin pump will be used to automatically calculate the insulin dose delivered to the patient at 5min intervals to achieve target glycemic control. In both Auto-mode and open-loop (manual) modes, participants will need to bolus manually for all meals and snacks by counting carbohydrates and entering this value into the pump before meals.

7.2 Guardian Link (GST3C) Transmitter (MMT-7811): This transmitter has a new internal electronics and firmware which reads the electronic signal generated by the sensor. The transmitter contains a custom Application Specific Integrated Circuit which enables Electrochemical Impedance Spectroscopy, the latter used as diagnostics for the sensor, which are incorporated into the sensor calibration logic. The transmitter also contains the sensor calibration algorithm that converts the sensor signal to a sensor glucose value using calibration glucose values from a meter relayed to the transmitter through the pump. The transmitter transmits the calculated glucose data to the pump via 2.4GHz RF technology. The new algorithm is designed to improve and optimize performance when paired with the Enlite 3 Sensor. Some elements of the new calibration logic include prompting the user to calibrate when needed, referred to as “Smart Cal,” instead of strictly scheduled time-based calibration requirements.

7.3 A charger (MMT-7715): This is used to recharge the GST3C Transmitter as needed providing up to 7 days of use. A battery charger will be used. The TST Tester (MMT-7726) operates as a sensor simulator creating signal current at a level that is within the range of an *in-vivo* sensor during normal operation.

7.4 Medtronic MiniMed Guardian 3 Glucose Sensor (MMT-7020*:* The Enlite 3 Sensor contains a microelectrode with a thin coating of glucose oxidase beneath several layers of biocompatible membrane, designed for improved accuracy. It penetrates the skin at a 90° angle using an introducer needle and is shorter and thinner than previous generations of sensors. The sensor continuously converts interstitial fluid glucose into an electronic signal received by a transmitter or recorder the strength of which is proportional to the blood glucose. The electrode is composed of embedding, signal-conducting and insulating layers.

7.5 One-press Serter: A Click Serter will be used for insertion and is intended as a single patient, non-sterile, multi-use device.

7.6 Contour Next Link 2.4 Glucose Meter: The Contour Next Link 2.4 Wireless Blood Glucose Monitoring System is an over the counter (OTC) device utilized by persons with diabetes in home settings for the measurement of glucose in whole blood, and is for single patient use only. The Contour Next Link 2.4 wirelessly transmits blood glucose values to the MiniMed 670G insulin pump and facilitate transfer of information to Medtronic CareLinkR Software through the use of radio frequency communication. The Contour Next Test Strips are intended for self-testing by persons with diabetes for the quantitative measurement of glucose in whole blood samples from 20 to 600 mg/dL. We may use either this meter or another FDA approved meter for BG checks.

Chapter 8: Continuous Glucose Monitoring The Study CGM will include the Medtronic iPro2 recorder and the Guardian 3 sensor. This is an FDA- approved device system with no changes to its hardware or firmware components. The CGM will be worn blinded at the Screening, 3-months and 6-month visit for approximately 6-14 days. Participants who wear a CGM as Standard care may use their home CGM in place of the study CGM. Participants using their own CGM must wear their CGM 21 out of 28 days daily.

Chapter 9: Safety Measures

9.1 Ketone Strips

Urine ketones will be measured using urine test strips as needed for persistent hyperglycemia and/or intercurrent illnesses as instructed by the study staff and in accordance with the manufacturer's labeling.

9.2 CGM Calibration

Throughout the study, participants will be instructed to calibrate the study CGM in accordance with manufacturer labelling.

9.3. Pump Failure

If there is a complete failure of the MiniMed 670G hybrid closed-loop insulin pump operations and it is anticipated it will take more than 1-2 hours to restore, participants may revert to usual care using their own insulin pump or insulin injections until the 670G can be brought back on line with the help of study staff and/or manufacturer's customer support. Every effort will be made to correct the problem as soon as possible.

9.4 Hypoglycemia Threshold Alarm and Safety Protocol

Study participants will be permitted to change the low glucose threshold alarm setting, but will be instructed to choose a value no less than 65 mg/dL. If a participant receives a CGM hypoglycemia threshold alarm or notes that the CGM glucose is below the hypoglycemia threshold alarm value, confirmatory fingerstick testing will be performed if required by CGM labelling and the participant will be instructed to treat hypoglycemia with ~15-30 grams of fast-acting oral glucose.

9.5 Hyperglycemia Threshold Alarm and Safety Protocol

This setting should not be >300 mg/dL. If a participant receives a CGM hyperglycemia threshold alarm or notes that the CGM glucose is above the hyperglycemia threshold alarm value, confirmatory fingerstick testing will be performed if required by CGM labelling.

If a participant's CGM reading is ≥300 mg/dL when fasting in the morning, >300 mg/dL for over 1 hour during the day, or ≥400 mg/dL at any point, he/she should take the following steps:

- Perform a blood glucose meter check.
- If BG >300 mg/dL, urine ketones should be measured and correction insulin bolus may be taken per the participant's usual routine.
- Participants may be instructed to change their pump site if appropriate

Chapter 10: Structural and Functional Brain Testing procedures
10.1 Brain MRI
Structural, DTI, and resting state fMRI scans will be identical to our ongoing DirecNet study in order to maintain backward compatibility of the new imaging data. Two task-based, and one resting state fMRI task are utilized to identify the neural substrates of cognitive differences between populations (see below).

10.1.1 Subject preparation and testing schedule: Subject preparation methods to be used in the proposed grant period have been highly successful in ≥90% of even younger children [21]**.** Those children recruited from the current study will have had 2-4 previous scans and will be very familiar with scanning procedures.

MRI will be scheduled in the morning after an overnight fast when possible. Research medical team will work closely with families the day before, and adjust insulin titration to achieve BG targets between 70 to <200mg/dL overnight if possible. The morning of the study a low carbohydrate breakfast will be recommended. BG target will be 70 to <200mg/dL at the initiation and duration of the scan. Participant BG can be titrated as needed to meet these targets at the discretion of the study staff and investigator.

10.1.2 Scan Sequences: Parallel imaging is used when appropriate to minimize scan duration and head movements. Structural scans: Sagittal MPRAGE (voxel size 1mm^3) and T2-FLAIR, voxel size 0.9x0.9x3 mm^3 (for possible white matter abnormalities); DTI: Axial EPI, 30 directions, voxel size 2mm^3; fMRI: BOLD-sensitive volumes are acquired with a gradient-echo imaging pulse sequence, 36 interleaved 4-mm thick slices, 3.0×3.0×4 mm^3 voxels. Cumulative imaging time for scans described above, including localizers and (if needed) repeated MPRAGE and DTI scans is 57 min, total session time of 75 min including set-up and breaks.

10.1.3 Multisite Imaging: All sites use a Siemens 3T Tim Trio scanner with identical 12 channel head coils and product sequences. A traveling human phantom is scanned at every site to confirm replicability of measurements across sites. Quarterly scans of a plastic phantom are used to monitor possible temporal variations in scan quality at each site. Functional MR image quality is measured with scans of an agar phantom [73]**,** and continuously monitored using subject fMRI data [74]**.** Human phantoms will be rescanned annually if quality monitoring detects significant changes in scanner performance. Image data will be transmitted to the IDCC using protocols to safeguard protected health information. Image data will be reviewed by a site neuroradiologist, and image quality noted within 48 hours by the IDCC.

10.1.4 functional MRI Tasks: Response inhibition (prefrontal function) will be measured in a rapid jittered event related Go-No-Go task featuring a series of letters [75]. Subjects push a button in response to all letters, except for the infrequent letter 'X'. This task is a classic test of one facet of executive function, requiring effortful inhibition of a prepotent response [76]**.** The task presents 300 Go and 75 NoGo stimuli (4:1 ratio) over the course of two runs with the inter-trial interval jittered from 2-12 seconds. Visual-spatial working memory (VSWM) (prefrontal-parietal) will be assessed with an N-back task suitable for children [77]. Briefly, the VSWM task consists of a single run of 6 alternating 35-s WM (3 1-back and 3 2-back) blocks and 6 35-s control blocks. During 1-back blocks, participants press a button if an "O" appears in the same position on 2 consecutive trials. During 2-back blocks (the WM condition of interest), button is pressed if "O" appeared in the same position 2 trials previously. During control blocks, button is pressed "O" appears at the center of the screen. In any block, subjects view the letter ''O'' once every 2s, at one of 9 screen locations, and 1/3 of the trials in each block require the subject's response. Stimuli are projected onto a screen and viewed through a mirror attached to the head coil, and subjects

respond using a handheld button box. All subjects will briefly practice the tasks before the scan E-Prime software (www.pstnet.com) will be used to present the task and collect responses. Preliminary review of fMRI scan quality collected from approximately one-half of our current cohort indicates ≥ 85% usable data.

10.1.5 Image Processing and Analysis: Primary image processing methods will be identical to those used for our current DirecNet analyses to enhance longitudinal inferences. Additional details/analyses are given below.

- ***Structural:*** MPRAGE data will be analyzed using longitudinal VBM and pre-processed using the VBM toolbox in FSL (v5.0, http://fsl.fmrib.ox.ac.uk). Following skull removal, images will be segmented into white matter, gray matter, and cerebrospinal fluid probability maps using FAST (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FAST). Segmented images are used to create a study-specific gray matter template. Individual subjects' gray matter maps are then non-linearly reregistered to the study-specific average template, modulated using the Jacobian of the warp field, and smoothed using a full-width half-maximum (FWHM) of 7 mm. Regional volumetric differences will be investigated based on the general linear model (GLM), and corrected for multiple comparisons using FWE. Cortical thickness, curvature, and regional surface area will be investigated using longitudinal FreeSurfer (v6.0), which reconstructs the gray/white and pial cortical surfaces and segments subcortical structures [78, 79]. Multivariate pattern analyses will be applied to VBM and Freesurfer data to identify global spatial characteristics (across voxel volumes or surface vertices) not apparent in mass univariate analyses [80, 81]**.** Extracted ROI data will be analyzed using linear mixed effects models in SPSS.
- ***DTI:*** Data will be preprocessed using DTIPrep to generate voxel-based maps of FA, RD, and AD over the whole brain. Resulting maps will be fed into FSL (www.fmrib.ox.ac.uk/fsl/) for voxelwise analyses using Tract-based Spatial Statistics (TBSS) [82]. TBSS data will be submitted to voxelwise regression statistics and corrected for multiple comparisons using permutation analysis implemented in FSL ("randomise") [83]. GLMs will be used to perform between-group comparisons, and for correlations between WM structure, clinical severity, and behavioral measures. Further, we will use GLM-based procedures and the FSL "randomise" function to investigate longitudinal changes in WM structure. A complementary *a priori* region-of interest analysis will be conducted using Tracts Constrained by Underlying Anatomy (TRACULA) software [84].
- ***fMRI:*** Data will be pre-processed in FSL using FEAT (FMRI Expert Analysis Tool), Version 4.1.4. Images will first be realigned and motion-adjusted to compensate for small head movements [85]. Data will be spatially smoothed using a 5 mm full-width-half-maximum Gaussian kernel, and band-pass filtered to correct for baseline drift and high frequency noise [86]. Functional datasets are individually registered to participants' T1-weighted structural image using a 6-parameter transformation, then to Montreal Neurological Institute (MNI) template space using FNIRT (FMRIB's Non-linear Image Registration Tool). Participant motion, estimated using MCFLIRT (fsl.fmrib.ox.ac.uk/fsl/fslwiki/MCFLIRT6D) and the "fsl_motion_outliers" program http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLMotionOutliers) will be entered as nuisance regressors for all fMRI analyses. *Resting-state functional connectivity* differences will be examined by two complementary methods in FSL: (1) hypothesis-driven seed-based analyses with locations defined by results from our structural data and previous studies in adult T1D [87], and (2) data-driven dual-regression and group independent component analyses (ICA) using temporally concatenated data obtained from both groups [88]. *Task-based functional activation* differences will also be examined using FSL. Because multiple runs are conducted for each participant for the Go-No-Go task, time-series statistical analyses will be carried out at a single-run intrasubject level using a generalized linear model that models the Go and No-Go epochs (binned separately according to accuracy) using a synthetic hemodynamic response function and its first derivative. To test our main hypotheses regarding an effect of intervention on BOLD responses of brain regions subserving response inhibition, a direct comparison of correct No-Go versus correct Go trials will be modeled at the single subject level. Single-run intrasubject maps will then be taken to a multiple-run fixed-effects intrasubject level to provide subject-specific summaries of activation maps which will then be carried to higher-level intergroup analyses using FMRIB's Local Analysis of Mixed Effects (FLAME; [86] to assess a main effect of group. Analyses of VSWM fMRI data will be carried out by modeling the instruction cues and the 1-and 2-back and match-center blocks using a synthetic hemodynamic response function and its first derivative at the single subject level. Comparisons of 1 and 2-back vs. match-center blocks will be done, and subject-specific activation summary maps will be carried to higher-level intergroup analyses using FLAME. All fMRI

analyses will control for participant age and gender and will include site to permit the estimation of scanner effects [89]. Repeated measures ANOVA will be used to assess longitudinal changes. Whole-brain statistical results will be corrected for multiple comparisons using FWE. Linear mixed effects models within SPSS will be used to investigate the association of cognitive scores with BOLD-fMRI metrics for *a priori* defined ROIs [90].

10.2 Neurocognitive Testing: All subjects will be administered a similar test battery to the one utilized in our current study. These specific measures align with hypothesized function of "vulnerable" brain regions (as suggested by cross-sectional anatomic findings in T1D) to as great a degree as possible, e.g., neurocognitive metrics reflecting frontal, parietal-occipital and hippocampal function). The Design Memory task from the WRAML-2 was selected to assess hippocampal function in light of the significantly smaller CA1-3 subfields (i.e., "deflation") found in T1D relative to controls. Cognitive domains of interest also include spatial cognition, episodic memory, executive function (e.g., response inhibition, working memory) and processing speed. Overall, cognitive tests for inclusion in the assessment battery were selected based on prior empirical evidence using the following criteria: **1)** strength of effects of T1D on the domain of cognitive function assessed by the instrument; **2)** strength of reliability/validity of the instrument and appropriateness for the targeted age range; **3)** sensitivity of the measure to cognitive functions subserved by affected neuroanatomical regions in T1D; **4)** other theoretical or conceptual reasons for anticipating an effect of T1D on the measured cognitive process; **5)** feasibility of test administration across the age range; and **6)** burden of measurement and ease of maintenance of procedural consistency across sites (see Table 3). Trained individuals who administer the neuropsychological tests at each site are already certified as proficient by the Neurocognitive core. Behavioral data from the fMRI tasks will provide additional cognitive data regarding spatial cognition and executive functions.

10.2.1 Cognitive Testing Procedures: Testing will occur when the child is rested, healthy and alert. Testing will be rescheduled if severe symptomatic hypoglycemia occurs the night prior. A fingerstick BG level will be obtained from subjects with T1D prior to testing using a home glucose monitor and titrated to be within 70-< 200 mg/dL within 60min of test initiation. Insulin or food will be given at the discretion of the investigator and parent/guardian to achieve this goal. Ketones will be evaluated if BG ≥200 mg/dL and if positive, the child will be treated and testing cancelled. If symptoms of hypoglycemia occur during testing, glucose levels will be tested and BG titrated again to 70 to <200mg/dL, then testing resumed. BG will be rechecked at the end of the session. If a parent/guardian has diabetes, a fingerstick BG will be obtained to verify it is within 70- <200 mg/dL and titrated by the adult with diabetes as needed to perform their testing, if out of range testing will be rescheduled. Testers will be blinded to the history of BG control of the subjects but not their diagnosis.

Table 3: Cognitive Tests for Follow up

Domain (Target brain region)	Test	Battery	Variable(s)	Minutes
Spatial Cognition (parietal-occipital)	*Spatial Span* *Spatial Relations* *VSWM Task*	*WISC-IV Integrated* *WJ-3 Cog (fMRI)*	*Scaled Score* *Standard Score* *Total Correct*	*5* *10* *--*
Episodic Memory (hippocampal)	*Design Memory, Delayed Recall* *Verbal Learning, Delayed Recall*	*WRAML-2*	*Scaled Score* *Scaled Score*	*25*
Executive Functions-Attention (prefrontal)	*Connor's CPT-2* *Concept Formation* *Go-No-Go Task*	*Connor's* *WJ-3 Cog (fMRI)*	*Detectability (D')* *Standard Score* *Total Correct*	*15* *10* *--*
Executive Functions-Working Memory (dorsolateral prefrontal)	*Digit Span, Letter Number* *VSWM Task*	*WISC-IV WMI (fMRI)*	*Standard Score* *Total Correct*	*10* *--*
Processing Speed (frontal white matter)	*Coding, Symbol Search*	*WISC-IV PSI*	*Standard Score*	*10*
P/G Executive Functions	*Global Executive Composite*	*BRIEF*	*T-Score*	*15*
P/G Mood/Behavior	*Internalizing Problems* *Externalizing Problems*	*BASC-2 PRS*	*T-score*	*15*
TOTAL Minutes*: Child=85, Parent/Guardian=30*				

Chapter 11: Questionnaires

11.1 Hypoglycemia Fear Survey [91] : The appropriate version of this questionnaire will be completed by subjects <18 yrs of age and their parents at baseline and the 6- month visit.

11.2 INSPIRE Survey: There are five versions of the INSPIRE; Adolescent, Adult, Child, Parent and Partner. The INSPIRE (Insulin Delivery Systems: Perceptions, Ideas, Reflections and Expectations) survey was developed to assess various aspects of a user's experience regarding automated insulin delivery for both

patients and family members. The surveys include various topics important to patients with type 1 diabetes and their family members based upon >200 hours of qualitative interviews and focus groups. The adolescent survey includes 28 items; and the parent survey includes 30 items. Response options for all surveys include a 5-point Likert scale from strongly agree to strongly disagree, along with an N/A option. Administration time is approximately 5 minutes.

Chapter 12: Statistical Considerations

12.1 Power Estimation

Due to funding limitations we are conducting this as a pilot, proof of concept study. Primary outcomes for this study are the trends in total and regional GM and WM volumes and white matter microstructure (FA). Secondary outcomes are the trends in changes in BOLD-fMRI and neurocognitive metrics. We employed mixed effects modeling for our power estimation considering the repeatedly measured outcomes (pre and post) with the projected missing data rate. We assumed ~15% attrition between the pre- (T4 from ongoing longitudinal study) and post-treatment (T5) assessments. Further, to maximize the statistical power given the moderate size of our sample, we will utilize the longitudinal data from the previous study, which will enable us to analyze five repeated measures instead of two (i.e., 3 from the previous study and 2 that will be measured in the proposed study). Given this richer longitudinal data, we conservatively assumed reliability of 0.75 for the structural measures (primary outcomes). As a lower bound of clinically meaningful outcome, we assumed an effect size of d=0.5 at post-treatment assessment. We will randomly assign 50 T1D subjects to the CL (N=25) and Standard Care (N=25) after the pre-treatment assessment (T4). Under this scenario, using the proposed mixed effects modeling using piecewise growth parametrization (i.e., 3 prior and 2 current measures), the estimated power to detect treatment effect (intention to treat) is 0.83 (two-tailed, α=.05). Given the preliminary nature of the proposed study, we did not adjust the significance level for multiple testing in our power estimation. However, in actual data analyses, if our primary outcome measures turn out to be highly correlated, we will formulate summary measures (either using average or using factor analytic methods) or adjust for multiple testing of interrelated outcomes. For the secondary outcomes, we will monitor both effect sizes and p-values, with more emphasis on gathering information on effect sizes.

12.2 Statistical Analysis Plan

Dependent variables include total and regional GM and WM volumes, WM microstructure, BOLD activation and selected neurocognitive metrics (executive function, spatial cognition, episodic memory, child IQ). Whole brain image analyses are carried out within the framework of specialized software (e.g., SPM, FSL) with stringent correction for multiple comparisons. Each neurocognitive domain is composed of a set of individual test variables (Table 3). For each domain, the scaled scores of the test variables will be averaged, creating a composite score. Univariate summaries of each dependent variable from imaging ROI and neurocognitive data will be given for the HCL and Standard conditions stratified by time from diagnosis and other risk factors (as sub-analysis). We will calculate a composite A1c history measure by the incremental area >6.0% and CGM glycemic indices. To reduce multiple comparisons, analysis of CGM indices will be restricted to one measure each of hypoglycemia (% CGM <70 mg/dL), hyperglycemia (%>250 mg/dL), euglycemia (% between 70-180 mg/dL) and glycemic variation (CV). Residual values will be analyzed for normal distribution; if highly skewed a transformation or nonparametric analysis will be used. Residual vs. predicted values plots will assess the assumption of common variance.

Our primary analysis strategy is longitudinal mixed effects modeling [92, 93], where each targeted brain ROI and neurocognitive metric is compared between the randomized groups (hybrid closed-loop and Standard) fully utilizing repeatedly measured outcomes (i.e., pre and post randomization plus three prior measures). Specifically, we will utilize a piecewise growth modeling strategy treating the previous study period (T1 to T3) and the proposed study period (T4 and T5, or pre/post) as two longitudinal stages. The analyses will be conducted in line with the intent-to-treat principle utilizing all available information based on maximum likelihood estimation.

Scores from the surveys and questionnaires will also be analyzed using mixed effects modeling, although only two repeated measures (i.e., pre and post randomization) will be available for these analyses. Given the

moderate sample size, our secondary analysis will require extra caution and will be limited in terms of power. Nonetheless, we will carefully conduct various secondary analyses, which may provide valuable information for hypothesis generation. We will estimate the effect of the CL use for those who would properly comply with the treatment assignment (compliers defined as ≥5d wear/week and 5h/night); we will employ a modern complier average causal effect (CACE) estimation [19, 21, 24, 47, 52], an approach embedded in our mixed effects longitudinal analysis using the maximum likelihood estimation via the EM algorithm [5]. The strength of this approach is that we analytically separate compliers from individuals who would insist on taking the hybrid closed-loop despite their assignment to the Standard condition (always-takers) and individuals who are offered the closed-loop but who do not wish to do it despite our best efforts (never-takers). Sensitivity analyses will be conducted to support validity of CACE estimates using alternative sets of identifying assumptions [28, 47]. We will conduct exploratory moderator analyses to examine the treatment effect heterogeneity. Treatment effect moderators and/or non-specific outcome predictors, we will use baseline age, total brain volume, and parental IQ and socioeconomic status. Sex differences will also be analyzed. We will conduct exploratory mediator analysis to examine the mechanism through which the intervention achieves its impact. As a key hypothesized mediator, we will focus on HbA1c. For moderator and mediator investigations, we will employ the MacArthur approach [94, 95] embedded in our mixed effects models. Finally, we will compare the longitudinal trajectories of closed-loop group and the non diabetic subjects, the latter will not be a part of our randomized study, although their longitudinal information will be collected (T1 to T4) in the core grant providing complete age overlap between the groups. This comparison will provide valuable information on whether and how T1D subjects who use closed-loop systems move towards the healthy control group.

12.3 Safety Analyses

Safety endpoints will be tabulated and analyzed as summary statistics during treatment and/or as change scores from baselines. AEs will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). Each AE will be counted per event, i.e., as often as a new episode occurs per participant and judged by severity and relationship of AEs to use of CL device or other study participation-related process. Information will also be reported about each AE including start date and stop date. The statistical procedure(s) that will be used to analyze the safety outcomes include: For binary variables, Fisher exact tests will be used to compare treatment groups. For counts, such as the number of severe hypoglycemic or diabetic ketoacidosis events per participant, data will be compared using robust Poisson regression or a Wilcoxon Rank-Sum test as indicated depending on the distribution, and the percentage of participants with at least one event will be compared using Fisher's exact test. All participants will be included in these analyses.

12.4 Sub-group analyses

Subgroup analyses/assessments of effect modification (interaction) will be conducted for the primary outcomes. These analyses will be considered exploratory. Additionally, interpretation of the analyses will depend on whether the overall analysis demonstrates a significant treatment group difference; in the absence of such an overall difference, subgroup analyses will be interpreted with caution. The general approach for these exploratory analyses are described above in the statistical analysis plan section, including complier average causal effect estimation and moderator/mediator analysis embedded in the mixed effects modeling framework for the primary analyses.

Chapter 13: Adverse Events

13.1 Definitions

Adverse Event (AE): Any untoward medical occurrence in a study participant, irrespective of the relationship between the adverse event and the device(s) used or study-related procedures.

Serious Adverse Event (SAE): Any untoward medical occurrence that:

- Results in death.
- Is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.

- Is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the participant or may require medical/surgical intervention to prevent one of the outcomes listed above).

Unanticipated Adverse Device Effect (UADE): Any SAE that was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with study participation that relates to the rights, safety, or welfare of participants (21 CFR 812.3(s)).

Adverse Device Effect (ADE): Any untoward medical occurrence in a study participant which the device may have caused or to which the device may have contributed.

Device Complaints: A device complication or complaint is something that happens to a device or related to device performance, whereas an AE happens to a participant. A device complaint may occur independently from an AE, or along with an AE. An AE may occur without a device complaint or there may be an AE related to a device complaint.

Device Malfunction: Any failure of a device to meet its performance specifications or otherwise perform as intended. Performance specifications include all claims made in the labeling for the device. The intended performance of a device refers to the intended use for which the device is labeled or marketed. (21 CFR 803.3).

13.2 Reportable AEs

These include: 1) SAEs, 2) Adverse Device Effects, 3) AEs occurring in association with a study procedure, 4) severe hypoglycemia as defined below, 5) diabetic ketoacidosis (DKA) as defined below. Episodes of hypoglycemia and hyperglycemia not meeting the criteria below will not be recorded as AEs unless associated with an Adverse Device Effect. Skin reactions from sensor placement are only reportable if severe and/or required treatment. Pregnancy occurring during the study will be recorded and patient discontinued from the study.

13.2.1 Hypoglycemic Events

Hypoglycemia not associated with an Adverse Device Effect is only reportable as an AE when considered severe as per the following definition for ***severe hypoglycemia***: the event required assistance of another person due to altered consciousness, and required another person to actively administer carbohydrate, glucagon, or other resuscitative actions. This means that the participant was impaired cognitively to the point that he/she was unable to treat himself/herself, was unable to verbalize his/ her needs, was incoherent, disoriented, and/or combative, or experienced seizure or coma. If plasma glucose measurements are not available during such an event, neurological recovery attributable to the restoration of plasma glucose to normal is considered sufficient evidence that the event was induced by a low plasma glucose concentration.

13.2.2 Hyperglycemic Events/DKA

Hyperglycemia not associated with an Adverse Device Effect is only reportable as an AE when one of the following criteria is met: (1) the event involved DKA, as defined by the Diabetes Control and Complications Trial (DCCT) and described below, or (2) in the absence of DKA if evaluation or treatment was obtained at a health care provider facility for an acute event involving hyperglycemia or ketosis.

DKA is defined if the following are present:

- Polyuria, polydipsia, accompanied by nausea, or vomiting;
- Serum ketones >1.5 mmol/L or large/moderate urine ketones;
- Either arterial blood pH <7.30 or venous pH <7.24 or serum bicarbonate <15; and
- Treatment provided in a health care facility for the above symptoms

All reportable AEs—whether volunteered by the participant, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means—will be reported on an AE form online.

13.2.3 Timing of Event Reporting

SAEs or unexpected device-related AEs must be reported to the Nemours Clinical Coordinating Center within 24 hours via completion of the online serious adverse event form. Other reportable AE and device malfunctions (with or without an AE) will be reported within 3 days of the investigator becoming aware of the event by completion of an electronic case report form. Device complaints not associated with device malfunction or an AE must be reported within 7 days of the investigator becoming aware of the event. The Nemours Coordinating Center will notify all participating investigators of any AE that is serious, related, and unexpected. Notification will be made within 10 days after the Nemours Coordinating Center becomes aware of the event. Each site PI is responsible for reporting serious study-related AEs and abiding by any other reporting requirements specific to the Network's Institutional Review Board.

In the case of a CGM device malfunction, the site's coordinator should be notified and information will be forwarded to the given company supplying the device.

Chapter 14: Sources of Materials, Recruitment and Informed Consent Procedures

14.1 Research material: It includes: 1) medical information about the subjects obtained from interviews, physical examinations, laboratory studies (e.g., HbA1c), and medical records; 2) information about blood and sensor glucose levels downloaded from the CGM devices and the home glucose meters as well as home glucose logs and diaries; 3) data from insulin infusion devices; 4) magnetic resonance imaging data and 4) neuropsychological study results from the parent grant longitudinal study. Subjects will be assigned unique identification numbers, and those participating in the parent grant will retain the same ID number, so that collected research data and specimens can be de-identified but still associated with the data from the parent grant if appropriate. All data connected to the studies will be collected in an electronic format accessible only by secure passwords to authorized study staff. Computers will be physically secured and hard copy data will be stored in locked cabinets in the office of the study personnel. All data analyses and reporting will be performed on de-identified data in accordance with the IRB and Health Insurance Portability and Accountability Act (HIPAA). Study information will be held in strict confidentiality, accessible only to study personnel, except as required by institutional IRB and federal guidelines. All glucose data will be stored by the Jaeb Center for Health Research servers and the imaging and cognitive data at the Stanford Imaging and Data Coordinating Center (IDDC); basic demographic information will be shared between the 2 data storage and analysis sites.

14.2 Recruitment

The 5 clinical centers submitting this proposal have a long history of recruiting and retaining children and adolescents in clinic research studies. The initial cohort included 144 children with T1D and 70 age-matched controls and to-date we have re-studied 74% of the original cohort and successfully replaced 26% of those lost by attrition. There is a great deal of interest in transitioning to closed-loop technologies so we expect no difficulty identifying the proposed number of subjects (20 per site) willing to transition to closed-loop pumps either at the beginning or after 6 months into the study. A summary of the number of ~patients with T1D followed at each site is in the table 4 below.

Table 4

Diabetes Center	~# T1D pts followed	# between 13-19yrs
Nemours Children's Health System-Jacksonville	937	593
Stanford University	885	470
Yale University	1000	400
University of Iowa	642	366
Washington University in St Louis	1546	670

14.3 Informed Consent Procedures
The principal investigators, co-investigators, or study coordinators at each center will introduce this study to eligible subjects and their caretakers during routine medical or research visits or in the form of a mailing or phone call. Prospective subjects/caretakers will have a face-to-face meeting with the investigator and/or study coordinator to address questions about the study rationale, procedures, risks and benefits. In order to identify and clarify any misconceptions, parents/caretakers will be encouraged to describe the research procedures and their associated risks in their own words, followed by correction of any errors by the investigator or study coordinator. All subjects will be between 14 and <18 years old at the time they are enrolled in this study. Written informed consent will be obtained from the parents/caretakers; assent will be obtained from minor subjects according to local IRB guidelines, the study will be explained to them in age-appropriate terms. The parents/caretakers of subjects will be encouraged to ask questions and will be clearly informed about the time required for these studies and their rights of non-participation and/or withdrawal. Caretakers/parents will be given copies of the signed consents to keep. Since personal health information will be collected as part of this research protocol, parents/caretakers will also be asked for authorization to collect this information in accordance with HIPAA and IRB guidelines. As mandated by new NIH guidelines the contact PI (N Mauras) will work with the Jaeb Center for Health Research (JCHR) to establish a single central IRB with all participating sites. All sites already have established reciprocity reliance agreements between the JCHR's central IRB and each participating site's IRB, hence a single centralized Human Subjects review informed consent process will be maintained. The Nemours Clinical Coordinating Center will be in charge of this process of submission. Each site PI will be responsible to upload needed materials to their own IRB as information and abide by the site-specific mandates of their reciprocity agreements.

Chapter 15: Potential Risks of the Study
Subjects in these studies have T1D and will therefore be at risk for hypoglycemia, hyperglycemia, and diabetic ketoacidosis as part of their ongoing disease state; these risks are already well known to these subjects and their families. Specific additional risks related to study participation are listed below.

15.1 Risks of Continuous Glucose Monitors (CGM) and insulin infusion sets: There is a low risk for developing a local skin infection at the site of the sensor needle or infusion set placement and itchiness, redness, bleeding, bruising and/or rash may occur, as well as local tape allergies. In our prior experience in DirecNet the incidence of infections related to sensor use was very low. Individual readings from CGMs are currently not approved for use in decisions regarding insulin dosing so subjects will be expected to continue performing conventional monitoring of the blood glucose tests at least four times daily. It should be noted that all subjects enrolled in this project will have had prior experience wearing CGM either as part of their routine diabetes care and/or as part of their participation in the parent project.

15.2 Risks of Magnetic Resonance Imaging: MRI is generally considered to be safe. MRI can be harmful if the patient's body contains any metal objects. Subjects will be screened for metallic objects and are excluded from participation if they have any contraindications for an MRI. Additionally, the MRI experience can be distressing to young patients if they are not adequately prepared. It should be noted that a % of subjects enrolled in this project will have had prior experience with MRI as part of their participation in the parent project.

15.3 Risks of Neuropsychological testing
The battery of tests selected for administration in this study is all in routine clinical use in pediatric neuropsychology and all have acceptable psychometric properties. The time to complete the entire testing battery is approximately 3-4hours for each subject, although this time may vary with the age, ability and cooperativeness of the subject. Breaks will be taken as needed, and the testing can be split into several testing sessions, if necessary. Some participants may find the duration of the evaluations to be somewhat taxing, but based on our prior experience in the parent project as well as other studies the great majority of subjects will be able to tolerate this. Some subjects or families may be unhappy if the subject doesn't score in the range they expected. There are no known lasting risks or discomforts from these testing procedures *per se*.

15.4 Risks of Hybrid Closed-Loop Studies
Hybrid Closed-Loop Insulin Delivery – As stated above under *Approach*, we will use the commercially available *Medtronic MiniMed 670G Insulin Pump (MMT-1780*) which when used with the CGM components (Guardian 3

Sensor, GST3C) continuously monitors interstitial glucose. When enabling Auto Mode (hybrid closed-loop control), the sensor glucose values received from the GST3C by the insulin pump are used to automatically calculate, at 5 minute intervals, the insulin dose delivered to the patient in order to achieve target glycemic control. This is considered a hybrid system as patients will still be required to bolus manually for all meals and snacks by counting carbohydrates and entering this value into the pump before ingestion of the meal.

The main potential study-related risks of using this hybrid closed-loop system are hypoglycemia, hyperglycemia and ketosis/diabetic ketoacidosis, related to performance properties of the system (inaccuracy of sensors, improper calibration) or malfunctions of the system (over-delivery or under delivery of insulin, loss of operation of the system). It should be noted that hypoglycemia, hyperglycemia, and ketosis/DKA are risks already familiar to all subjects/caretakers in the study, as they are risks of standard diabetes management with insulin pump therapy. Of note, in the recent Medtronic pivotal trial of this study in 124 subjects with type 1 diabetes, there were no episodes of severe hypoglycemia or diabetic ketoacidosis in over 12,000 subject-days of hybrid closed-loop use. This system was FDA approved October 2016 and is commercially available for adolescents 14 years and older. The devices will be used as clinically appropriate and our studies restricted to those willing to use the system.

15.5 Risks Associated with Hypoglycemia
Mild hypoglycemia may be associated with shakiness, sweating, dizziness, or hunger. Mild hypoglycemia is commonly experienced multiple times each week by people with T1 diabetes and will be well known and understood by these subjects and their families. Severe hypoglycemia may be associated with altered mental status, seizure, coma, loss of consciousness, and rarely death. The risk of severe hypoglycemia is less common than mild hypoglycemia, but is also well known and understood by these subjects and their families. All subjects will be instructed to have glucagon at home for the treatment of severe hypoglycemia and have been instructed on its use. The risks of hypoglycemia are potentially mitigated in the CL group by the use of alarms, alerts, and automated suspension of insulin by the CL system, and all subjects in both arms of the study will be required to test BG at least 4 times daily.

15.6 Risks Associated with Hyperglycemia
Risks associated with hyperglycemia include excess thirst or urination, dehydration and altered mental status. As with hypoglycemia, these symptoms are commonly experienced multiple times each week by people with T1 diabetes and will be well known and understood by these subjects and their families. In the absence of ketoacidosis (see below), symptoms of hyperglycemia pose no immediate risk. The risks of hyperglycemia are potentially mitigated in the CL group by the CL algorithms, which supplement insulin delivery in the setting of hyperglycemia, and all subjects in both arms will be required to test BG at least 4 times daily.

15.7 Risks Associated with Diabetic Ketoacidosis
The presence of ketones or diabetic ketoacidosis (DKA) is associated with the risk of dehydration, nausea, vomiting, abdominal pain, electrolyte imbalance and acidosis, shock, coma or death. DKA is uncommon is well-managed diabetes. All families are knowledgeable about prevention of DKA and when to call for assistance in avoiding DKA. This is a routine part of their clinical care.

15.8 Risks of loss of privacy and confidentiality: Collection of research data could pose threats to privacy and confidentiality, potentially jeopardizing patients' employability or insurability. Some parents/caregivers may be uncomfortable with the degree of scrutiny of their daily diabetes management consequent to use of the CGM sensor and frequent pump downloads. However, once again, it should be noted that all these subjects have T1D and most already have experience using insulin pumps and CGM.

Chapter 16: Miscellaneous Considerations

16.1 Alternative Treatments and Procedures
The proposed studies are optional and voluntary; the alternative to participating in them would be not to participate, and just continue routine diabetes care or start hybrid closed-loop pump treatment as part of their routine care. The decision not to participate will in no way affect the ability of the subject to continue to receive medical and diabetes care at our centers.

16.2 Participant compensation: This will be detailed in the informed consent document

16.3 Withdrawal: Participation in the study is voluntary, and a participant may withdraw at any time. For participants who withdraw, their data collected up until the time of withdrawal will be used.

16.4 Data Collection and monitoring: The main study data are collected through a combination of electronic case report forms (eCRFs) and electronic device data files obtained from the study software and individual hardware components and stored at the IDCC in the REDCap system at Stanford University. When data are directly collected in eCRFs, this will be considered the source data. Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of participants.

16.5 Records Retention: Study documents should be retained for a minimum of 2 years after study is completed and all principal analysis has been completed and data published. These documents should be retained for a longer period, however, if required by local regulations.

16.6 Quality Assurance and Monitoring: The Nemours Clinical Coordinating Center will be responsible for maintaining quality assurance (QA) and quality control (QC) systems to ensure that the clinical portion of the trial is conducted and data are generated, documented and reported in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements. Adverse events will be prioritized for monitoring. Stanford's IDCC will be responsible for maintaining QA and QC of the imaging data as well as the repository of all data in the study through REDCap. The JCHR will be responsible for collecting and maintaining all the glucose and insulin data generated by the hybrid closed-loop portion of the studies, as well as all glucose downloads in the entire cohort. Either or both of the clinical and data coordinating centers' representatives or their designees may visit the study facilities at any time in order to maintain current and personal knowledge of the study through review of the records, comparison with source documents, observation and discussion of the conduct and progress of the study.

16.7 Protocol deviations: A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or procedure requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. The site PI/study staff is responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the manual of operations.

16.8 Data Safety Management Board: A 5 members NIH-designated data safety and management board (DSMB) has been established (Dr. Mark Sperling – chair). Protocol will be thoroughly reviewed and approved prior to study initiation. Quarterly or as needed meetings will be established to monitor progress, AEs, SAEs, protocol deviations and any issues related to the human subjects participation in the studies. The committee has been actively functioning in the parent longitudinal RO1 for the last 3 years. They will have considerable latitude on the monitoring of the study progress.

16.9 Criteria for Suspending Overall Study: Study activities could be suspended if the manufacturer of any constituent study device requires stoppage of device use for safety reasons (e.g. product recall). The affected study activities may resume if the underlying problem can be corrected by a protocol or system modification that will not invalidate the results obtained prior to suspension. These decisions will be made in consultation with the DSMB and notification to the IRB as appropriate. If there is termination of funding the study may also be suspended.

16.10 Institutional Review Boards: The protocol, protocol amendments, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval prior to participant enrollment. If a central IRB is used, each individual center's IRB should be notified and pertinent materials shared locally as per their individual agreements.

16.11 Future use of stored specimens: Data collected for this study will be stored and analyzed at the Stanford IDCC. After the study is completed, the de-identified, archived data will be transmitted to and stored at the IDCC for use by other researchers including those outside of the study. Permission to transmit data to the IDCC will be included in the informed consent. With the participant's and IRB's approval, de-identified biological samples will be stored at the Nemours Children's Health System Clinic freezers in Jacksonville, FL until they are used for research assays. During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage will not be possible after the study is completed. When the study is completed, access to study data and/or samples will be provided through the Stanford IDCC.

Chapter 17: References

1. Bjorgaas, M.R., *Cerebral effects of severe hypoglycemia in young people with type 1 diabetes.* Pediatr Diabetes, 2012. **13**(1): p. 100-7.
2. Perantie, D.C., et al., *Prospectively determined impact of type 1 diabetes on brain volume during development.* Diabetes, 2011. **60**(11): p. 3006-14.
3. Blasetti, A., et al., *The effect of recurrent severe hypoglycemia on cognitive performance in children with type 1 diabetes: a meta-analysis.* J Child Neurol, 2011. **26**(11): p. 1383-91.
4. Ferguson, S.C., et al., *Influence of an early-onset age of type 1 diabetes on cerebral structure and cognitive function.* Diabetes Care, 2005. **28**(6): p. 1431-7.
5. Suh, S.W., et al., *Hypoglycemia induces transient neurogenesis and subsequent progenitor cell loss in the rat hippocampus.* Diabetes, 2005. **54**(2): p. 500-9.
6. Yamada, K.A., et al., *Repetitive hypoglycemia in young rats impairs hippocampal long-term potentiation.* Pediatr Res, 2004. **55**(3): p. 372-9.
7. Revsin, Y., et al., *Glucocorticoid receptor blockade normalizes hippocampal alterations and cognitive impairment in streptozotocin-induced type 1 diabetes mice.* Neuropsychopharmacology, 2009. **34**(3): p. 747-58.
8. Bober, E. and A. Buyukgebiz, *Hypoglycemia and its effects on the brain in children with type 1 diabetes mellitus.* Pediatr Endocrinol Rev, 2005. **2**(3): p. 378-82.
9. Flykanaka-Gantenbein, C., *Hypoglycemia in childhood: long-term effects.* Pediatr Endocrinol Rev, 2004. **1 Suppl 3**: p. 530-6.
10. Hershey, T., et al., *A prospective study of severe hypoglycemia and long-term spatial memory in children with type 1 diabetes.* Pediatr Diabetes, 2004. **5**(2): p. 63-71.
11. Kim, M., et al., *Susceptibility of the developing brain to acute hypoglycemia involving A1 adenosine receptor activation.* Am J Physiol Endocrinol Metab, 2005. **289**(4): p. E562-9.
12. Northam, E.A. and A. Lin, *Hypoglycaemia in childhood onset type 1 diabetes--part villain, but not the only one.* Pediatr Diabetes, 2010. **11**(2): p. 134-41.
13. Ho, M.S., et al., *Prevalence of structural central nervous system abnormalities in early-onset type 1 diabetes mellitus.* J Pediatr, 2008. **153**(3): p. 385-90.
14. Perantie, D.C., et al., *Regional brain volume differences associated with hyperglycemia and severe hypoglycemia in youth with type 1 diabetes.* Diabetes Care, 2007. **30**(9): p. 2331-7.
15. Malone, J.I., S.K. Hanna, and S. Saporta, *Hyperglycemic brain injury in the rat.* Brain Res, 2006. **1076**(1): p. 9-15.
16. Lin, A., et al., *Neuropsychological profiles of young people with type 1 diabetes 12 yr after disease onset.* Pediatr Diabetes, 2010. **11**(4): p. 235-43.
17. Ly, T.T., et al., *Neurocognitive outcomes in young adults with early-onset type 1 diabetes: a prospective follow-up study.* Diabetes Care, 2011. **34**(10): p. 2192-7.
18. Barnea-Goraly, N., et al., *Alterations in white matter structure in young children with type 1 diabetes.* Diabetes Care, 2014. **37**(2): p. 332-40.
19. Cato, M.A., et al., *Cognitive functioning in young children with type 1 diabetes.* J Int Neuropsychol Soc, 2014. **20**(2): p. 238-47.
20. Marzelli, M.J., et al., *Neuroanatomical correlates of dysglycemia in young children with type 1 diabetes.* Diabetes, 2014. **63**(1): p. 343-53.

21. Barnea-Goraly, N., et al., *High success rates of sedation-free brain MRI scanning in young children using simple subject preparation protocols with and without a commercial mock scanner--the Diabetes Research in Children Network (DirecNet) experience.* Pediatr Radiol, 2014. **44**(2): p. 181-6.

22. Biessels, G.J. and Y.D. Reijmer, *Brain MRI in children with type 1 diabetes: snapshot or road map of developmental changes?* Diabetes, 2014. **63**(1): p. 62-4.

23. Mauras, N., et al., *Longitudinal assessment of neuroanatomical and cognitive differences in young children with type 1 diabetes: association with hyperglycemia.* Diabetes, 2015. **64**(5): p. 1770-9.

24. Mazaika, P.K., et al., *Variations in Brain Volume and Growth in Young Children With Type 1 Diabetes.* Diabetes, 2016. **65**(2): p. 476-85.

25. Cato, M.A., et al., *Longitudinal Evaluation of Cognitive Functioning in Young Children with Type 1 Diabetes over 18 Months.* J Int Neuropsychol Soc, 2016: p. 1-10.

26. Anderson, V., M. Spencer-Smith, and A. Wood, *Do children really recover better? Neurobehavioural plasticity after early brain insult.* Brain, 2011. **134**(Pt 8): p. 2197-221.

27. Anderson, V., et al., *Functional plasticity or vulnerability after early brain injury?* Pediatrics, 2005. **116**(6): p. 1374-82.

28. Tamborlane, W.V., et al., *Continuous glucose monitoring and intensive treatment of type 1 diabetes.* N Engl J Med, 2008. **359**(14): p. 1464-76.

29. Mauras, N., et al., *A randomized clinical trial to assess the efficacy and safety of real-time continuous glucose monitoring in the management of type 1 diabetes in young children aged 4 to <10 years.* Diabetes Care, 2012. **35**(2): p. 204-10.

30. Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study, G., *Prolonged nocturnal hypoglycemia is common during 12 months of continuous glucose monitoring in children and adults with type 1 diabetes.* Diabetes Care, 2010. **33**(5): p. 1004-8.

31. Miller, K.M., et al., *Current state of type 1 diabetes treatment in the U.S.: updated data from the T1D Exchange clinic registry.* Diabetes Care, 2015. **38**(6): p. 971-8.

32. Hovorka, R., *Closed-loop insulin delivery: from bench to clinical practice.* Nat Rev Endocrinol, 2011. **7**(7): p. 385-95.

33. Breton, M., et al., *Fully integrated artificial pancreas in type 1 diabetes: modular closed-loop glucose control maintains near normoglycemia.* Diabetes, 2012. **61**(9): p. 2230-7.

34. Atlas, E., et al., *MD-logic artificial pancreas system: a pilot study in adults with type 1 diabetes.* Diabetes Care, 2010. **33**(5): p. 1072-6.

35. Hovorka, R., *Artificial Pancreas Project at Cambridge 2013.* Diabet Med, 2015. **32**(8): p. 987-92.

36. Castle, J.R., et al., *Novel use of glucagon in a closed-loop system for prevention of hypoglycemia in type 1 diabetes.* Diabetes Care, 2010. **33**(6): p. 1282-7.

37. Russell, S.J., et al., *Blood glucose control in type 1 diabetes with a bihormonal bionic endocrine pancreas.* Diabetes Care, 2012. **35**(11): p. 2148-55.

38. Kovatchev, B.P., et al., *Feasibility of outpatient fully integrated closed-loop control: first studies of wearable artificial pancreas.* Diabetes Care, 2013. **36**(7): p. 1851-8.

39. Phillip, M., et al., *Nocturnal glucose control with an artificial pancreas at a diabetes camp.* N Engl J Med, 2013. **368**(9): p. 824-33.

40. Ly, T.T., et al., *Overnight glucose control with an automated, unified safety system in children and adolescents with type 1 diabetes at diabetes camp.* Diabetes Care, 2014. **37**(8): p. 2310-6.

41. Russell, S.J., et al., *Outpatient glycemic control with a bionic pancreas in type 1 diabetes.* N Engl J Med, 2014. **371**(4): p. 313-25.

42. Haidar, A., et al., *Outpatient overnight glucose control with dual-hormone artificial pancreas, single-hormone artificial pancreas, or conventional insulin pump therapy in children and adolescents with type 1 diabetes: an open-label, randomised controlled trial.* Lancet Diabetes Endocrinol, 2015. **3**(8): p. 595-604.

43. Nimri, R., et al., *MD-Logic overnight control for 6 weeks of home use in patients with type 1 diabetes: randomized crossover trial.* Diabetes Care, 2014. **37**(11): p. 3025-32.

44. Thabit, H., et al., *Home use of closed-loop insulin delivery for overnight glucose control in adults with type 1 diabetes: a 4-week, multicentre, randomised crossover study.* Lancet Diabetes Endocrinol, 2014. **2**(9): p. 701-9.

45. Hovorka, R., et al., *Overnight closed-loop insulin delivery in young people with type 1 diabetes: a free-living, randomized clinical trial.* Diabetes Care, 2014. **37**(5): p. 1204-11.

46. Thabit, H., et al., *Home Use of an Artificial Beta Cell in Type 1 Diabetes.* N Engl J Med, 2015. **373**(22): p. 2129-40.

47. Leelarathna, L., et al., *Day and night home closed-loop insulin delivery in adults with type 1 diabetes: three-center randomized crossover study.* Diabetes Care, 2014. **37**(7): p. 1931-7.

48. Tauschmann, M., et al., *Day-and-Night Hybrid Closed-Loop Insulin Delivery in Adolescents With Type 1 Diabetes: A Free-Living, Randomized Clinical Trial.* Diabetes Care, 2016. **39**(7): p. 1168-74.

49. Bergenstal, R.M., et al., *Safety of a Hybrid Closed-Loop Insulin Delivery System in Patients With Type 1 Diabetes.* JAMA, 2016. **316**(13): p. 1407-1408.

50. Russell, S.J., et al., *Day and night glycaemic control with a bionic pancreas versus conventional insulin pump therapy in preadolescent children with type 1 diabetes: a randomised crossover trial.* Lancet Diabetes Endocrinol, 2016. **4**(3): p. 233-43.

51. Kropff, J., et al., *2 month evening and night closed-loop glucose control in patients with type 1 diabetes under free-living conditions: a randomised crossover trial.* Lancet Diabetes Endocrinol, 2015. **3**(12): p. 939-47.

52. Ly, T.T., et al., *Day and Night Closed-Loop Control Using the Integrated Medtronic Hybrid Closed-Loop System in Type 1 Diabetes at Diabetes Camp.* Diabetes Care, 2015. **38**(7): p. 1205-11.

53. de Bock, M.I., et al., *Feasibility of Outpatient 24-Hour Closed-Loop Insulin Delivery.* Diabetes Care, 2015. **38**(11): p. e186-7.

54. Saggar, M., et al., *Compensatory Hyper-Connectivity in Developing Brains of Young Children with Type 1 Diabetes.* Diabetes, 2016. **66**(3): p. 754-62.

55. Maahs, D.M., et al., *A randomized trial of a home system to reduce nocturnal hypoglycemia in type 1 diabetes.* Diabetes Care, 2014. **37**(7): p. 1885-91.

56. Buckingham, B.A., et al., *Predictive Low-Glucose Insulin Suspension Reduces Duration of Nocturnal Hypoglycemia in Children Without Increasing Ketosis.* Diabetes Care, 2015. **38**(7): p. 1197-204.

57. Roberto, C.A., et al., *Brain tissue volume changes following weight gain in adults with anorexia nervosa.* Int J Eat Disord, 2011. **44**(5): p. 406-11.

58. van Eijk, J., et al., *Rapid partial regeneration of brain volume during the first 14 days of abstinence from alcohol.* Alcohol Clin Exp Res, 2013. **37**(1): p. 67-74.

59. Gazdzinski, S., et al., *Cerebral white matter recovery in abstinent alcoholics--a multimodality magnetic resonance study.* Brain, 2010. **133**(Pt 4): p. 1043-53.

60. Garver, D.L., J.A. Holcomb, and J.D. Christensen, *Cerebral cortical gray expansion associated with two second-generation antipsychotics.* Biol Psychiatry, 2005. **58**(1): p. 62-6.

61. Arnone, D., et al., *State-dependent changes in hippocampal grey matter in depression.* Mol Psychiatry, 2013. **18**(12): p. 1265-72.

62. Ibrahim, I., et al., *Fractional anisotropy and mean diffusivity in the corpus callosum of patients with multiple sclerosis: the effect of physiotherapy.* Neuroradiology, 2011. **53**(11): p. 917-26.

63. Wang, T., et al., *Early-stage psychotherapy produces elevated frontal white matter integrity in adult major depressive disorder.* PLoS One, 2013. **8**(4): p. e63081.

64. Fan, Q., et al., *Abnormalities of white matter microstructure in unmedicated obsessive-compulsive disorder and changes after medication.* PLoS One, 2012. **7**(4): p. e35889.

65. Gudayol-Ferre, E., et al., *Changes in brain connectivity related to the treatment of depression measured through fMRI: a systematic review.* Front Hum Neurosci, 2015. **9**: p. 582.

66. Beauregard, M., *Functional neuroimaging studies of the effects of psychotherapy.* Dialogues Clin Neurosci, 2014. **16**(1): p. 75-81.

67. Singh, M.K. and K.D. Chang, *The neural effects of psychotropic medications in children and adolescents.* Child Adolesc Psychiatr Clin N Am, 2012. **21**(4): p. 753-71.

68. Lukas, S.E., *New perspectives on using brain imaging to study CNS stimulants.* Neuropharmacology, 2014. **87**: p. 104-14.

69. Chapin, H., E. Bagarinao, and S. Mackey, *Real-time fMRI applied to pain management.* Neurosci Lett, 2012. **520**(2): p. 174-81.

70. Matthews, P.M., G.D. Honey, and E.T. Bullmore, *Applications of fMRI in translational medicine and clinical practice.* Nat Rev Neurosci, 2006. **7**(9): p. 732-44.

71. Reid, L.B., et al., *Interpreting Intervention Induced Neuroplasticity with fMRI: The Case for Multimodal Imaging Strategies.* Neural Plast, 2016. **2016**: p. 2643491.

72. Tsalikian, E., et al., *Blunted counterregulatory hormone responses to hypoglycemia in young children and adolescents with well-controlled type 1 diabetes.* Diabetes Care, 2009. **32**(11): p. 1954-9.

73. Friedman, L. and G.H. Glover, *Report on a multicenter fMRI quality assurance protocol.* J Magn Reson Imaging, 2006. **23**(6): p. 827-39.

74. Brown, G.G., et al., *Multisite reliability of cognitive BOLD data.* Neuroimage, 2011. **54**(3): p. 2163-75.

75. Casey, B.J., et al., *A developmental functional MRI study of prefrontal activation during performance of a Go-No-G0 task.* J Cogn Neurosci, 1997. **9**(6): p. 853-47.

76. Lock, J., et al., *Aberrant brain activation during a response inhibition task in adolescent eating disorder subtypes.* Am J Psychiatry, 2011. **168**(1): p. 55-64.

77. Kwon, H., A.L. Reiss, and V. Menon, *Neural basis of protracted developmental changes in visuo-spatial working memory.* Proc Natl Acad Sci U S A, 2002. **99**(20): p. 13336-41.

78. Dale, A.M., B. Fischl, and M.I. Sereno, *Cortical surface-based analysis. I. Segmentation and surface reconstruction.* Neuroimage, 1999. **9**(2): p. 179-94.

79. Fischl, B., M.I. Sereno, and A.M. Dale, *Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system.* Neuroimage, 1999. **9**(2): p. 195-207.

80. Fan, Y., et al., *Multivariate examination of brain abnormality using both structural and functional MRI.* Neuroimage, 2007. **36**(4): p. 1189-99.

81. Uddin, L.Q., et al., *Multivariate searchlight classification of structural magnetic resonance imaging in children and adolescents with autism.* Biol Psychiatry, 2011. **70**(9): p. 833-41.

82. Smith, S.M., et al., *Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data.* Neuroimage, 2006. **31**(4): p. 1487-505.

83. Nichols, T.E. and A.P. Holmes, *Nonparametric permutation tests for functional neuroimaging: a primer with examples.* Hum Brain Mapp, 2002. **15**(1): p. 1-25.

84. Yendiki, A., et al., *Automated probabilistic reconstruction of white-matter pathways in health and disease using an atlas of the underlying anatomy.* Front Neuroinform, 2011. **5**: p. 23.

85. Jenkinson, M., et al., *Improved optimization for the robust and accurate linear registration and motion correction of brain images.* Neuroimage, 2002. **17**(2): p. 825-41.

86. Woolrich, M.W., et al., *Temporal autocorrelation in univariate linear modeling of FMRI data.* Neuroimage, 2001. **14**(6): p. 1370-86.

87. van Duinkerken, E., et al., *Resting-state brain networks in type 1 diabetic patients with and without microangiopathy and their relation to cognitive functions and disease variables.* Diabetes, 2012. **61**(7): p. 1814-21.

88. Filippini, N., et al., *Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele.* Proc Natl Acad Sci U S A, 2009. **106**(17): p. 7209-14.

89. Yendiki, A., et al., *Multi-site characterization of an fMRI working memory paradigm: reliability of activation indices.* Neuroimage, 2010. **53**(1): p. 119-31.

90. Laird, N.M. and J.H. Ware, *Random-effects models for longitudinal data.* Biometrics, 1982. **38**(4): p. 963-74.

91. Gonder-Frederick, L.A., et al., *Psychometric properties of the hypoglycemia fear survey-ii for adults with type 1 diabetes.* Diabetes Care, 2011. **34**(4): p. 801-6.

92. Raudenbush, S.W. and A.S. Bryk, *Hierarchical linear models : applications and data analysis methods*. 2nd ed. ed. Advanced quantitative techniques in the social sciences ; 1. 2002, Thousand Oaks: Sage Publications.

93. Singer, J.D. and J.B. Willett, *Applied longitudinal data analysis : modeling change and event occurrence*. 2002, Oxford ; New York: Oxford University Press.

94. Kraemer, H.C., et al., *Mediators and moderators of treatment effects in randomized clinical trials.* Arch Gen Psychiatry, 2002. **59**(10): p. 877-83.

95. Kraemer, H.C., et al., *How and why criteria defining moderators and mediators differ between the Baron & Kenny and MacArthur approaches.* Health Psychol, 2008. **27**(2 Suppl): p. S101-8.