Protocol #: 15-1309
Project Title: Bromocriptine QR as adjunct therapy in Type 1 Diabetes
Principal Investigator: Irene Schauer
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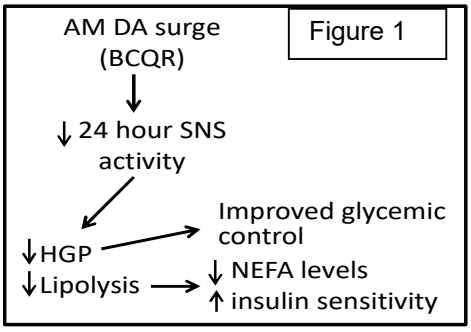
I. Hypotheses and Specific Aims:

Type 1 diabetes (T1D) continues to be a disease plagued by hyperglycemia, insulin resistance (IR), and increased cardiovascular disease (CVD) despite advances in insulin delivery and glucose monitoring. Therefore new approaches are needed. Bromocriptine (BC), a dopamine (DA) agonist, has long been widely used for treating Parkinson's disease and prolactinoma. Its recent approval in a quick release formulation, BCQR, for type 2 diabetes (T2D) is an exciting development, representing a novel mechanism for improving IR. A morning DA surge normally resets the circadian clock to a more insulin sensitive state, and stimulates the transition from fasting to the fed state. This dopaminergic signaling is attenuated or absent in T2D[1]. Morning BCQR is thought to mimic/restore this AM dopamine surge in T2D. In T2D, BCQR has been shown to cause persistent decreases throughout the day in sympathetic nervous system (SNS) activity, glucagon, hepatic glucose production (HGP) and lipolysis, which in turn lead to persistent lowering of glucose and fatty acid (FFA) levels (**Figure 1**)[1]. Increased SNS and renin-angiotensin system (RAS) activity likely play a key role in microvascular and macrovascular sequelae and both are decreased with BCQR. Thus BCQR may also decrease rates of complications independent of glycemic control. An initial study with BCQR, in fact, found a dramatic decrease in CVD events in T2D[1,2]. *BCQR has not been studied in T1D, but it's mechanism of action, mechanistic studies, and our preliminary data support our proposed study of possible benefits of BCQR on insulin action, glycemic control, and the vasculature in T1D.* **We hypothesize primarily that BCQR will improve insulin action, thus improving glycemic control and/or decreasing insulin requirements in T1D. We further hypothesize that (1) Subject characteristics such as age, BMI, sex, HbA1c, residual C-peptide, and/or sleep quality and duration may impact the response to BCQR, (2) BCQR will have additional benefits on vascular compliance and function that may translate to improved CVD outcomes and support the added cost of BCQR therapy in T1D, and (3) Decreased chronic central SNS activation will improve hypoglycemia awareness and thus hypoglycemia frequency and severity.**



Specific Aims:

SA#1: To test the hypothesis that BCQR will improve clinical parameters of insulin action in T1D (insulin dose, mean glucose and glycemic variability by continuous glucose monitoring [CGM], postprandial glucose excursion by glucose and insulin area under the curve during MMTT). We further hypothesize that (1) these benefits will incur due to improved postprandial suppression of glucagon and lipolysis and (2) the decrease in chronic SNS tone will lead to improved SNS responsiveness to hypoglycemia, with beneficial effects on hypoglycemia awareness and counter-regulatory response (hypoglycemia awareness questionnaire) and susceptibility to hypoglycemia (CGM).

SA#2: To investigate clinical parameters that may predict response to BCQR in order to allow targeted use of this intervention if appropriate. Subgroup analyses will be performed by age, BMI and estimated insulin sensitivity. Multivariate modeling will address other potential predictors including C-peptide, fasting NEFA, baseline HbA1c, sex, sleep characteristics, circadian rhythm delay.

SA#3: To test the hypothesis that BCQR-induced reduction in SNS activation, NEFA levels, and vascular IR will improve vascular function (arterial stiffness by Dynapulse and endothelial function by EndoPAT) and decrease blood pressure.

II. Background and Significance:

Novel approaches to the management of T1D are urgently needed to address the ongoing gap in therapeutic efficacy, to minimize complication risk and improve morbidity and mortality in T1D. For instance, only 25% of youth in our multi-center T1D EXCHANGE Registry were able to reach target A1c despite advances in insulin-based options[3]. Although T1D is primarily a disease of insulin deficiency, we and others have demonstrated IR in adolescents and adults with T1D[4-7] (preliminary data), even among subjects with normal body mass index (BMI). IR is associated with increased risk of CVD in type 2 diabetes (T2D), obesity, and metabolic syndrome[8-12]. In the Pittsburgh Epidemiology of Diabetes Complications (EDIC) and the Diabetes Control and Complications Trial (DCCT) adult T1D studies, IR estimated by non-clamp surrogate measures was worse than expected[13,14]. Moreover, in a study by Martin et al, IR predicted the progression of vascular disease in T1D adults, and in EDIC, estimated IR, not glycemia, predicted CVD events and mortality[13,15]. Further, a subset of participants in the DCCT with better glucose control experienced greater weight gain and worse CVD profiles, which suggests that lowering HbA1c with insulin alone may not decrease CVD risk[16]. In our recent study of 87 adults in the NIH Coronary Artery Calcification in T1D (CACTI) study, the T1D subjects were consistently and significantly more IR than the nondiabetic (nonDM) control group (preliminary data), and IR predicted increased coronary artery calcification[17,18]. In our recent adolescent JDRF-funded T1D studies, obese and normal weight T1D youth were more IR than control youth, which correlated with reduced exercise capacity, vascular dysfunction and more CVD risk factors (preliminary data) [18-20]. Moreover, obesity is increasingly seen in youth and adults with T1D (40% of T1D adolescents in the T1D EXCHANGE are overweight or obese[3]), and obesity super-imposed on T1D is likely to worsen IR and CVD complications. **Thus, IR is consistently present in T1D, and IR and its consequences present novel therapeutic targets to improve glycemic control and reduce complications.**

BromocriptineQR: Bromocriptine, a dopamine (DA) agonist, has long been widely used for treating Parkinson's disease and by endocrinologists for treating prolactinoma. Its recent approval in a quick release formulation, bromocriptine quick release (BCQR), for T2D is an exciting development, representing a novel mechanism for improving glycemic control and the metabolic disturbances associated with IR[1]. The normal physiological morning dopamine surge resets the circadian clock, and triggers the transition from the fasting to the fed state. This dopaminergic signaling is attenuated or absent in in insulin resistant states, including T2D[1]. Morning BCQR mimics/restores the AM dopamine surge in T2D, resulting in persistent improvements throughout the day including decreased sympathetic nervous system (SNS) activity and suppression of glucagon release, hepatic glucose production (HGP) and lipolysis (**Fig 1**). These changes then lead to the beneficial effects of persistent lowering of glucose and non-esterified fatty acid (NEFA) levels throughout the day, particularly postprandially, resulting in improved glucose tolerance[1] . In addition, BCQR results in persistently decreased SNS tone and decreased activation of the renin angiotensin system (RAS)[1,2]. The latter known effects likely explain the benefits of bromocriptine in congestive heart failure, an approach that has since been replaced by more specific adrenergic and RAS-targeted medications[21,22]. Finally, a randomized placebo-controlled clinical trial of BCQR in 3,070 adults with T2D found a significant 40% risk reduction in the pre-specified primary cardiovascular endpoint (ischemic and nonischemic events). A post-hoc analysis of the "hard" clinical CVD composite endpoint of MI, CVD death, and stroke also showed a significant 52% risk reduction[23].

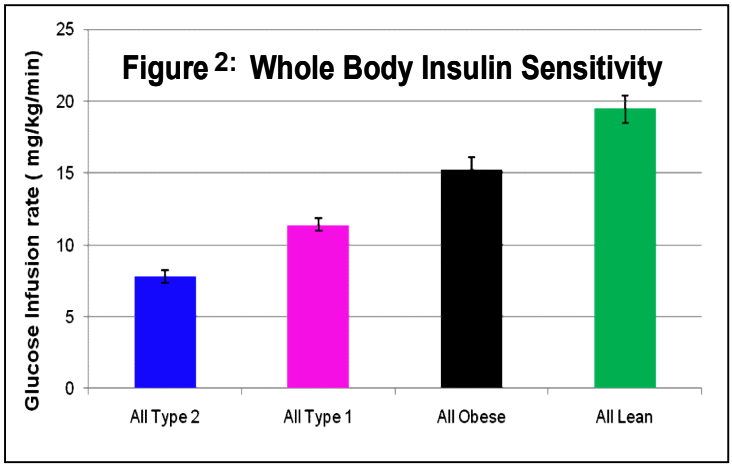
BCQR in T1D: *BCQR has not yet been studied in T1D, and its mechanism of action and recent literature support our proposed physiological studies of possible benefits of BCQR in T1D on insulin action and vascular function.* For example, studies in streptozotocin-induced diabetes in rats also implicate dopamine in the defects in central regulation of SNS activity in T1D[24]. Furthermore, BCQR reduces SNS activity in lean non-DM subjects, suggesting that its effect is not exclusive to obesity, and that BCQR could be expected to decrease SNS-triggered lipolysis and glucagon release in T1D[25,26]. Moreover, chronic SNS activation is known to occur in T1D and contributes to hypoglycemic unawareness, causing dangerous unrecognized hypoglycemia. We have demonstrated a significant degree of adipose, hepatic and muscle IR in normal weight and obese adolescents and adults with T1D[18,19], resulting in unchecked lipolysis, HGP and hyperglycemia similar to T2D. IR in T1D appears to involve the artificial nature of peripheral insulin delivery, a mechanism that cannot currently be directly targeted. This mechanism is supported by studies showing that IR is present early following diagnosis of T1D, but resolves transiently during a non-insulin-requiring "honeymoon" period[7]. Furthermore, pancreatic transplant improves IR[27,28] but only normalizes IR when the transplant is integrated into the portal circulation[29,30]. Non-physiological insulin delivery results in relative hepatic insulin deficiency and potentially glucagon dysregulation, both resulting in abnormal HGP[27-29,31]. Hepatic insulin deficiency may also lower insulin-like growth factor-1

(IGF-1), which subsequently increases pituitary growth hormone secretion, further inducing IR at multiple tissues[31]. New data from our fatty-acid targeted intervention demonstrate that IR in T1D correlates with NEFA levels and thus should improve with decreased SNS-triggered lipolysis. *BCQR's effects of reducing both HGP and NEFA levels would thus be expected to improve overall glycemia and postprandial glucose excursions, lower insulin dose by improving IR, and also reset the SNS system to respond more appropriately to hypoglycemia. In addition, SNS dysregulation likely also plays a key role in the macrovascular sequelae of diabetes. Our preliminary data show vascular dysfunction in T1D and increased CVD-risk markers associated with IR. The dramatic benefits in terms of CVD risk reduction in T2D are potentially related to decreased SNS activity and/or improvements in IR and therefore may translate to T1D[1,2].*

T1D and sleep: Poor sleep may contribute to the development of IR, either directly due to effects on glucose regulation or indirectly via dysregulation of appetite leading to weight gain. Alterations in sleep (time, disruption, architecture, and quality) are associated with neuroendocrine changes in appetite [32-34], SNS arousal[35], and metabolic dysregulation[35,36]. Reports of reduced sleep times of up to 64%[37] have been documented for children and adolescents with T1D. In adults with T1D, short sleep duration was associated with reduced insulin sensitivity[38], increased HbA1c and a greater prevalence of non-dipping BP patterns[39,40]. Additionally, two polysomnography studies revealed that those with T1D spent more sleep time in lighter stages of sleep (stages 1 and 2) and less time in restorative slow-wave sleep (stage 3) compared to healthy control subjects[41,42]. In one observational study in youth with T1D[43], the T1D cohort spent significantly more time in stage 2 and less time in stage 3 sleep than a matched control group. Within the T1D cohort, those who spent the most time in stage 2 sleep also had higher daily glucose and HbA1c levels suggesting metabolic dysregulation with lighter sleep[43]. Conversely, fluctuations in glucose levels (>25 mg/dl/hr), even when the blood glucose level remained within normal ranges, have been associated with sleep disruption[44]. Thus improving glycemic variability may help improve sleep and subsequently metabolic and CVD outcomes. In addition, all adolescents have a delayed phase circadian rhythm characterized by delayed melatonin release with later sleep onset and delayed melatonin offset with later waking[45,46]. As a result of this natural adolescent pattern, but artificially imposed early wake times for school, chronic short sleep is endemic in adolescence and sleep patterns and circadian rhythms are most likely to be abnormal in adolescents, arguing for their ultimate inclusion in our study [47]. By providing an AM dopamine surge, BCQR may help improve the circadian rhythm abnormalities expected in individuals with T1D, contributing to improved glycemic control and insulin action. Conversely, sleep quality and/or duration may help to determine and predict response to BCQR treatment. *This background supports our proposed inclusion of sleep duration, quality, and efficacy outcomes.*

Thus, the literature and our published studies support an expectation of improved insulin action, reduced insulin requirement, improved glycemic control and variability, and improved hypoglycemia awareness with BCQR treatment in T1D. In addition, the potential for reduction in macrovascular complications, even independent of glycemic control, and ease of administration makes BCQR a competitive candidate for adjunct oral therapy in T1D. We are singularly well positioned to perform this study with our large T1D population, existing pediatric and adult cohorts, and expertise in all proposed methods.

III. Preliminary Studies/Progress Report:



T1D subjects are markedly IR: Despite a lack of metabolic syndrome characteristics, hyperinsulinemic euglycemic clamps in our Coronary Artery Calcification in T1D (CACTI) study cohort reveal that T1D adults are significantly more IR than nonDM controls, with a mean glucose infusion rate (GIR) of only 44% of nonDM controls (GIR 5.8 ± 3.5 vs. 13.2 ± 5.7 mg/min/kgFFM, p<0.0001). Multiple adjustments, including age, fasting glucose, final clamp glucose and insulin, and BMI or visceral fat area attenuate the difference in IR only very slightly such that mean GIR for T1D remains 49% of that of nonDM[18]. T1D adults also fail to suppress HGP in response to insulin, evidence of significant hepatic IR[48]. Similarly, our JDRF-funded studies in T1D youth found a GIR that is 67% of age, BMI and pubertal stage-matched nonDM youth (13.3 ± 4.2 vs. 19.8 ± 4.6 mg/min/kgFFM, p<0.01)[19](**Fig 2**). T1D youth (pink bar) were even more IR than obese

nonDM (black bar). IR remained despite adjustment for BMI, age, pubertal stage, % body fat, activity, and post-clamp insulin levels. T1D youth also fail to suppress HGP (Glucose Ra), even at the highest dose of insulin (**Fig 3A**)[49]. *These data demonstrate muscle and hepatic IR across the lifespan in T1D and support the possibility of IR as a novel therapeutic target in T1D.*

Figure 3A HGP in T1D youth

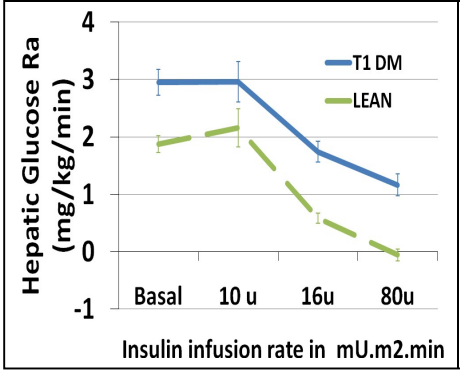
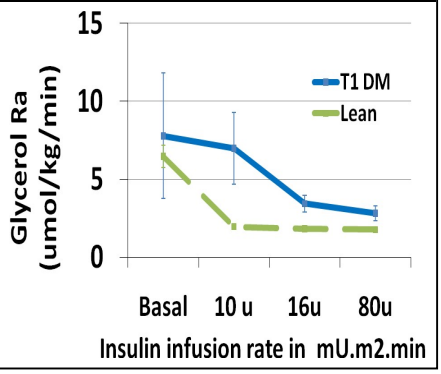


Figure 3B Adipose IR in T1D youth



IR in TID includes adipocyte IR: Low-dose insulin infusion reveals the novel finding of adipocyte IR in T1D relative to nonDM subjects[18,48,50]. At 8mU/m^2/min of insulin, glycerol rate of appearance (RA) and NEFA levels were more suppressed in nonDM than T1D adults (NEFA: 193 ± 24 vs 370 ± 26 μM, p<0.0001) (**Fig 4**). Similarly, NEFA levels and glycerol Ra failed to suppress in response to 10 mU/m^2/min in our JDRF-funded youth T1D studies, and even high dose insulin failed to completely suppress glycerol Ra (**Fig 3B**). NEFA's also correlate negatively with GIR (p=0.01) in our T1D youth, arguing that FFA's also impact systemic IR in T1D (ADA 2012). Finally, we found endothelial dysfunction in our T1D youth that correlated with NEFA's[19].

Figure 4

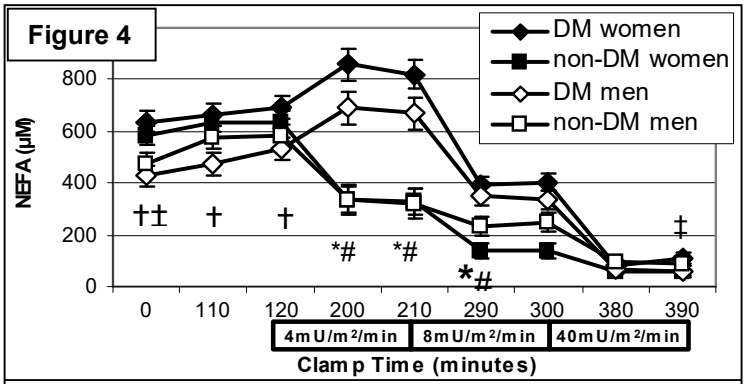


Figure 4: §Least square means adjusted for age, BMI (kg/m2), gender, starting glucose concentration, and time point insulin level. * p < 0.05 for difference by diabetes in men; # by diabetes in women; † p < 0.05 for sex difference in DM; ‡ in nonDM.

IR in T1D is improved with NEFA lowering: In an ongoing ADA-funded, double-blind, random order, placebo-controlled crossover IND-based pilot study, we are investigating the effects of acute NEFA lowering with acipimox in T1D. Acipimox inhibits lipolysis, lowers NEFA levels and improves IR acutely in T2D and in lean and obese nonDM controls[51,52]. Acipimox is not a viable clinical option for insulin sensitization because of tachyphylaxis and rebound. However, this proof of concept study examines whether NEFA lowering improves IR and vascular function in T1D. In 4 T1D and 2 controls to-date, lower pre-clamp NEFA levels were associated with lower IR (**Fig 5**).*These results suggest that failure to suppress lipolysis is a potential mechanism for IR and CVD in T1D, and support an intervention like BCQR that targets lipolysis will improve IR in T1D.*

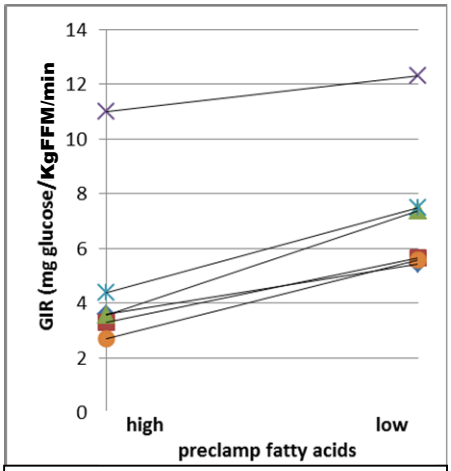


Figure 5: Insulin sensitivity (GIR) increases with lower fatty acid levels. X = nonDM; closed symbols = T1D.

IR in T1D is not solely due to poor glycemic control: Analyses of the role of glycemic control in IR in T1D suggest that residual hyperglycemia is not the sole cause of IR in T1D[18,53,54]. We found no correlation between fasting glucose, HbA1c or CGM measures of hyperglycemia, hypoglycemia or glycemic variability and GIR or failure of NEFA suppression in adolescents or adults. *These results suggest that in moderately well controlled T1D, level of glycemic control does not affect IR, arguing for additional therapies to specifically address the IR of T1D.*

Vasculopathy in T1D correlates with IR: Measurement of augmentation index (AI), a measure of arterial stiffness, was performed in a large subset of our CACTI population (n=385). After adjustment for age and sex, AI remains signi-ficantly greater in T1D than nonDM subjects (mean, 95% confidence interval: 19.5, 18.2 - 20.7 *vs*. 16.6, 15.4 - 17.7; p=0.0011). When estimated IR[5] is added to the model, the effect of T1D is completely lost indicating that AI is directly related to IR. We also found evidence of vascular stiffness in T1D youth in the SEARCH study using Brachial distensability (BrachD) by

Dynapulse[55,56] and endothelial dysfunction by venous plethysmography[19], both of which correlated with IR. We are currently assessing endothelial dysfunction with EndoPAT and arterial stiffness with Dynapulse in our JDRF- and ADA-funded metformin trials in lean and obese adolescents with T1D, and to-date approximately 60% of T1D adolescents overall (and 70% of obese) have endothelial dysfunction (AFMR/SPR abstract submitted). Thus, we are experienced with the use and interpretation of these measures and they are feasible in youth and adults. In particular, arterial stiffness and endothelial dysfunction seem to present early and persist[57], arguing for pediatric studies where prevention may still be possible. Moreover, three months of metformin was recently shown to improve arterial stiffness and endothelial function in 30 young women with IR related to PCOS[58], showing that short-term interventions are sufficient to impact these outcomes. *The associations with IR suggest that the increased arterial stiffness and endothelial dysfunction seen in T1D may be explained by IR and may improve with BCQR treatment*.

CAC presence and progression correlate with IR in T1D: In CACTI, presence and progression of CAC correlated inversely with GIR and directly with NEFA levels during clamp stage 2 for both T1D and nonDM adults after adjustment for age[18]. By logistic regression analysis the odds ratio for existence of CAC was 0.45 for every 1 SD increase in GIR and 2.4 for every 1 SD increase in stage 2 NEFA levels (**Table 1**)[18]. *These data suggest that BCQR may have benefit beyond glycemic control in T1D and support our proposed studies of vascular stiffness and function. CVD benefit would provide a powerful additional rationale to justify the cost of this additional intervention in T1D.*

Table 1: IR predicts CAC and correlates with CAC volume

	Spearman correlation coefficient IR vs CAC volume for total cohort (n=87)		
	V3 CAC volume	Baseline to V3 change in CAC	V2 to V3 change in CAC
GIR (mg/kg FFM/min)	-0.42 (p<0.0001)	-0.41 (p<0.0001)	-0.24 (p=0.028)
Stage 2 NEFA level (μM)	0.41 (p<0.0001)	0.40 (p=0.0001)	0.27 (p=0.01)

	Logistic regression analysis Odds ratio for any CAC at visit 3 (n=87)	
	OR per 1 SD change	p-value
GIR (mg/kg FFM/min)	0.45 (0.22-0.93)	0.03
Stage 2 NEFA level(μM)	2.40 (1.08-5.32)	0.032

Estimation of IR in T1D: We used the gold-standard hyperinsulinemic euglycemic clamp from our JDRF-funded study along with simple clinical variables (age, sex, race/ethnicity, BMI, WC, Tanner stage, HbA1c, lipids, blood pressure (BP) and %fat) in T1D and T2D adolescents to develop an estimate of IR[59,60 61]. Estimated IR correlated well with GIR from our hyperinsulinemic euglycemic clamps (R^2=0.74). We then applied this estimate of IR to a cohort of 290 T1D adolescents, and found that estimated IR was inversely related to CV risk factors (BP, LDL, hsCRP, p<0.0001). Our CACTI study similarly developed a predictive equation for T1D adults (eIS-CACTI)[18,62,63] which includes waist circumference, daily insulin dose per kg body weight, triglycerides and DBP: exp[4.1075 – 0.01299*waist(cm)–1.05819*insulin dose (daily units per kg) - 0.00354*triglycerides (mg/dL) - 0.00802*DBP (mm Hg)]. The eIS-CACTI explains 63% of the variance in the GDR in hyperinsulinemic-euglycemic clamp studies, and has now been validated in adolescent and adult cohorts with and without T1D[18,62,63]. We therefore can now make use of these equations to estimate IR in both youth and adults in the proposed study rather than performing clamps, saving cost and reducing subject burden.

IV. Research Methods

A. Outcome Measure(s):

Primary outcome measures are mean CGM glucose and insulin dose (SA#1), impact of age and BMI on response to therapy (SA#2) and improvement in brachial artery distensibilty and RH-PAT (SA#3). Insulin dose was chosen as a clinical primary outcome because of the short treatment duration and to allow insulin dose adjustments as needed to avoid hypoglycemia. Secondary outcomes (see detailed methods below) include measures of (1) glycemic control (CGM parameters (SD, %hyperglycemic)); (2) baseline/postprandial metabolic labs [area under the curve (AUC) for MMTT glucose, insulin, NEFA, triglycerides, glucagon, and glucagon-like peptide1 (GLP1); (3) hypoglycemia frequency and awareness (CGM %hypoglycemic, hypoglycemia symptoms scores (Gold method, Clarke method, and the McAuley score[64-67]); (4) SNS activity (heart rate variability); (5) other metabolic markers (HDL, LDL); (6) circadian rhythm/sleep effects (sleep quality scores, measured sleep with actiwatch sleep monitor); (7) CVD measures (arterial stiffness by Dynapulse, endothelial function by EndoPAT, and blood pressure), and (8), blood storage for future inflammatory (IL-6, TNF-α, hsCRP, adiponectin), and vascular (endothelin 1, PAI-1, ICAM, renin, angiotensin, aldosterone) markers.

B. Description of Population to be Enrolled:

Study population: Initially our goal is to enroll 60 T1D subjects (50% male) with the goal of completing 40 in the adult cohort. Ultimately, if the study drug is tolerated and safe in our adult cohort, we plan to amend the protocol to include adolescents, with involvement of Dr. Nadeau, the co-PI on the JDRF award. With the addition of the adolescent cohort, we plan to recruit 133 T1D subjects with the goal of completing 80. Submission of this amendment will occur in late year 1 ONLY IF there is no concerning safety signal in the adult cohort. Inclusion criteria: (1) T1D of >9 months duration based on a clinical course consistent with T1D and rapid conversion to insulin requirement after diagnosis (2) HbA1c 6.5-10% (adults) or any HbA1c up to 12% (pediatrics) and (3) age 12-60 years of age. Exclusion criteria include (1) any comorbid condition associated with inflammation, IR, or dyslipidemia including cancer(with the exception of skin cancer), heart failure, active or end stage liver disease, end stage kidney disease, inadequately treated thyroid disease, or rheumatologic disease; (2) tobacco or marijuana use; (3) pregnancy; (4) regular or frequent oral steroid use; (5) current use of insulin sensitizing medications, neuroleptics or ergot-related medications, or triptan medications for migraine, (6) diagnosis or history of psychosis and (7) diabetes of other cause such as MODY or cystic fibrosis-related diabetes. BMI will not be restricted in order allow secondary analysis for effects of BMI on responsiveness to BCQR. Postmenarchal premenopausal females will be tested in the early follicular phase (cycle d2-10) where possible.

C. Study Design and Research Methods

Research Design and Methods: We propose to address the study hypotheses and aims with a double-blinded, random-order, placebo-controlled cross-over design study in T1D. The intervention will consist of 4 weeks of treatment with BCQR (CyclosetTM) and 4 weeks of placebo separated by a ≥ 4 week washout. Interventions of this duration have been found to be adequate for detecting changes in vascular function, cardiac autonomic function, and glucose control for other anti-diabetic and fatty acid lowering agents (eg[68-72]). Most studies with BCQR in T2D have longer in duration. However, two studies demonstrated improved glucose and/or insulin levels after administration of a single dose[73] or 4-8 weeks of daily dosing[74]. In addition, the mechanism predicts acute changes in glucose control and vascular parameters. The shorter duration is proposed here to minimize the cost and subject burden in this pilot study.

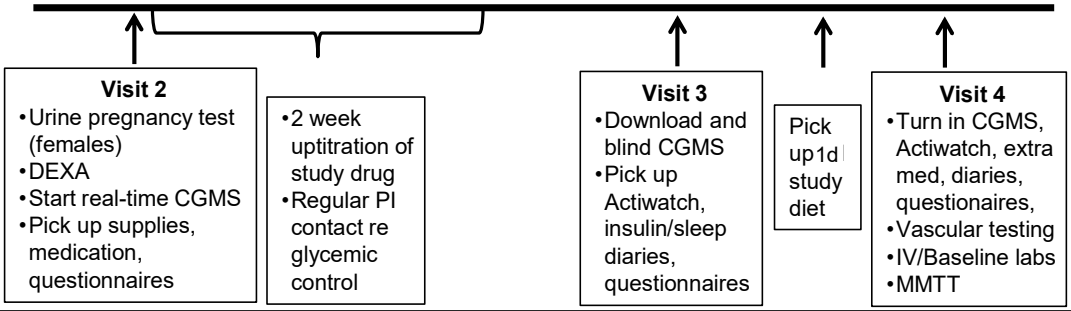
Recruitment: Ultimately 133 T1D subjects (50% male, 50% adolescent) will be enrolled with the goal of having 100 eligible and completing 80 (see power analysis). Recruitment will begin year 1 for the adult cohort and an adolescent cohort will be added in year 2. We will begin with a mass mailing to the established CACTI cohort of >600 adults with T1D mailing list established through Dr. Schauer's clinical relationship. In addition, a co-investigator from the Barbara Davis Center adult T1D clinic (Dr. Polsky) is included specifically for recruitment purposes. Through these approaches we hope to have the full adult cohort recruited, but not completed, by the end of year 1. Late in year 1, any adverse events will be thoroughly reviewed by the safety officer to assess for any safety concerns (in particular hypoglycemia) prior to submitting an amendment to broaden the age range to add the adolescent cohort. However, we do not expect to encounter significant safety issues since higher doses and much longer durations of standard release bromocriptine are widely used in adults and children for other medical conditions. Beginning year 2, adolescent recruitment will consist of patients from the Barbara Davis Center for Childhood Diabetes (BDC) where co-investigator Dr. Nadeau is a medical provider and has a weekly clinic. Dr. Nadeau has excelled when recruiting adolescent T1D patients as she currently recruits for multiple protocols researching the abnormalities associated with T1D. Furthermore, Dr. Nadeau holds a weekly Endocrine Clinic and is a provider in the Lifestyle Medicine Clinic at the Children's Hospital of Colorado where she has a clinical relationship with many patients with T1D and other endocrine disorders. Co-investigator Dr. Paul Wadwa is also a physician at the BDC and holds multiple weekly clinics with T1D patients. IRB approved flyers will be placed in private Endocrine clinics in the Denver Metro area. Through these approaches we hope to have the full adolescent cohort recruited. We include both lean and obese participants as BMI and age-based differences in IR, glycemic control, hormone levels, sleep patterns and diabetes duration may impact response to BCQR. This recruitment strategy has been used successfully for our similarly powered individual ongoing studies.

Investigational product: Bromocriptine Quick Release (Cycloset™): Bromocriptine itself has been available and widely used for Parkinson's disease and prolactinoma for decades. It was also used for a time for congestive heart failure. BCQR is FDA approved for adult and T2D is currently undergoing safety and efficacy trials in T2D adolescents. BCQR has a similar side effect profile to standard formulations of bromocriptine. Common side effects include mild hypotension, mild fatigue/somnolence, headache, depressed mood, insomnia, nausea, and diarrhea. Less common and more serious side effects have been seen with BCQR including hypersensitivity reactions, hypoglycemia and syncope. Bromocriptine is

standardly used in young children, adolescents, and adults of all ages with hyperprolactinemia at higher does than proposed in our study and should be well tolerated. To assess the hypotensive side effect of Bromocriptine, blood pressure measurements, as outlined by the CDC to access orthostatic hypotension, will be collected at every study visit for the adolescent cohort only. Continuous glucose monitoring and contact with study staff will help avoid hypo- or hyperglycemia. Potential benefits include improved glycemic control and decreased insulin requirement, as well as metabolic and vascular benefits related to decreased chronic SNS activity, decreased renin angiotensin system activation, and resetting of the circadian rhythm.

Study design: Eligible subjects will be randomized to order of treatment (placebo followed by BCQR vs. BCQR followed by placebo). Belmar Pharmacy will be supplying the study drug (BCQR 0.8 mg and identical placebo tablets). The study timeline for Phase 1 is illustrated in **Fig 6.** Phase 2 consists of visits identical to Phase 1 numbered 5-7.

Figure 6: Study timeline (for each medication phase)



Visit 1: Screening visit: Prescreening will be done by phone or in conjunction with a clinical diabetes visit. The full screening visit will include the consent process, history, physical exam, and screening labs (HbA1c, comprehensive metabolic panel, TSH (adult participants only), urine microalbumin, lipids). All females will also have a pregnancy test and detailed menstrual history. Adult females will have FSH and estradiol to determine pre- or postmenopausal status.

Visit 2: Medication Start Visit: Qualifying subjects will return for a medication start visit and DEXA scan for body composition and estimation of daily caloric needs. Subjects will be trained in the use of a CGM system (Dexcom G4 Platinum). The monitor will be in real time unblinded mode throughout the uptitration portion of each study phase for safety purposes by guarding against hypoglycemia due to insulin sensitization and allowing informed insulin dose adjustment with assistance from study personnel if needed. Baseline insulin dosing totals and average blood glucose will be recorded by subjects 3 days prior to starting the study medication. Subjects will also complete hypoglycemia and sleep questionnaires to assess frequency and awareness of lows, sleep patterns and sleep adequacy. Medication will be dispensed by the UCH or CHCO pharmacy to blind subjects and study personnel. Subjects will uptitrate study medication as follows: BCQR (0.8 mg) or placebo- 1 pill QAM for one week, then 2 pills QAM for one week, then 4 pills QAM for the remainder of the 4 weeks. If an uptitration step is not tolerated, subjects will be returned to the last tolerated dose for a few days and uptitration will be attempted again. If again not tolerated, subjects will remain at the last tolerated dose as long as this is at least 1.6 mg QAM. If unable to reach minimum acceptable dose, subjects will be withdrawn from the study.

Visit 3: Pre-MMTT Visit: Subjects will return for provision of Actiwatch and review of CGM data from the past 3 weeks. There will be continued data collection regarding glycemic control, variability, frequency of hypoglycemia, physical activity, and sleep duration and quality. Detailed 7-day sleep and insulin diaries will again be provided for the last week. Prior to each post-medication metabolic study day, subjects will also be provided with a 1-day standardized, macronutrient-controlled, weight maintenance diet (45% carbohydrate, 20% protein, 35% fat) with caloric content based on prior DEXA lean body mass and an activity factor as in our previous studies. Depending on scheduling conflicts and the ability to pick up study diet, study staff may provide participants with food recommendations to eat 1 day prior to MMTT with the same macronutrient distribution listed above.

Optional combined Visit 1, 2, and 3: For subjects that might have long distances to travel or scheduling conflicts, a combined visit will be provided as an option. For adolescents, a point of care A1c will be completed at screening to satisfy inclusion/exclusion criteria in addition to the other procedures outlined under visit 1. If participant is eligible and enrolled, IDS pharmacy will dispense medication to the study team. The sleep and insulin diaries along with the Actiwatch will be given to the subject at Visit 1 or 2. A phone call from the study doctor will be made to the subject during week 3 of the

phase to review CGM data as well as discuss any issues that may have come up. Subjects will also be reminded at this time to complete the insulin and sleep diaries and wear the Actiwatch during the last week.

Visit 4: Mixed Meal Tolerance Test (MMTT) Visit: Subjects will present in the AM fasting following the 1-day study diet. Fasting Dynapulse measurements of vascular stiffness and central and peripheral blood pressure, EndoPAT measurements of endothelial function and augmentation index, and measurement of heart rate variability for autonomic tone will be performed. An IV will then be placed and baseline labs will be drawn, including hormonal and metabolic markers (described in MMTT detailed methods below). Additional blood will be drawn and stored for future vascular and inflammatory markers. Next, an MMTT will be performed as described below. Pills will be counted to assess compliance, and the hypoglycemic and sleep questionnaires will be repeated. An optional MRI will be performed of the chest and abdomen.

After at least a four week washout period, the full cycle will be repeated with the second blinded intervention, starting with Visit 5 (medication start visit as above, but without DEXA). Measurements following the placebo phase will serve as the untreated baseline.

Optional combined visits 5, 6 and 7 (adolescents only): For subjects that might have long distances to travel or scheduling conflicts a combined visit will be provided as an option. Activity watch, questionnaires, CGM and study medication will be mailed to participant following the 4-week washout period. In the event a participant does not feel comfortable placing CGM at home, they will be able to come to the CTRC for CGM placement assistance.

Methods:

Mixed Meal Tolerance Test[75]: A standardized mixed meal (Boost +®, 45 grams of carbohydrate) meal tolerance test (MMTT) will take place at the end of each medication cycle as in our previous studies[61]. The subject will be confirmed to have arrived fasting, caffeine-free, and having taken the AM dose of the study medication. Daily insulin pump basal rate or basal long acting insulin injections should continue as usual. The subject will be instructed not to exercise throughout the MMTT. A point of care fasting glucose will be done prior to the MMTT. The MMTT may be cancelled and rescheduled at the investigator's discretion if this blood glucose measurement is below 50 mg/dl or above 300 mg/dl. An intravenous catheter (IV) will be inserted for multiple blood draws. At approximately -30 minutes, a fasting blood draw will occur, including the timed labs below, plus a fasting lipid panel, HbA1c, adiponectin, and progesterone (female only to document state of menstrual cycle). A urine sample will be collected for urine microalbumin:Cr ratio. At approximately -5 minutes, the "time 0" blood draw will occur. Immediately after the blood draw, the subject will subcutaneously inject a standard bolus of their usual short-acting mealtime insulin with their standard correction factor (CF) and carbohydrate:insulin ratio for ingestion of the liquid meal containing 45 grams of carbohydrate, 14 grams of fat, and 14 grams of protein. The phase 2 MMTT will be completed with the same bolus dose as the phase 1 MMTT unless the starting blood glucose requires a different CF dose. Immediately after bolusing with insulin, as close to the true "time 0" as possible, and to be completed within 5 minutes, the participant will drink the meal (Boost +®). Actual blood draw times will be recorded, as well as insulin dose and all glucose measurements taken throughout the MMTT. During the MMTT, blood is drawn for glucose, insulin, C-peptide, triglycerides, NEFA, GLP-1, glucagon levels at -30(approximately), 0, 30, 60, 120, 180 and 240 minutes and serum creatinine and cystatin C (adolescents only). If a subject experiences a hypoglycemic event or any other event compromising safety, the MMTT will be stopped at investigator discretion.

Dynapulse: Brachial artery distensibility (BrachD) will be measured with the DynaPulsePathway (PulseMetric, Inc., San Diego California) as in our previous studies. The Dynapulse is a noninvasive portable system utilizing a standard sphyg-momanometer cuff inflated in the same fashion as used to obtain blood pressure. The instrument derives BrachD using the technique of pulse waveform analysis of arterial pressure signals obtained from the sphygmomanometer. Brachial artery waveforms are uploaded to an online analysis site and analyzed for measures of vascular stiffness.

EndoPAT: Reactive hyperemia–peripheral artery tonometry (RH-PAT) by the EndoPat device (Itamar Medical Ltd., Caesarea, Israel) is a non-invasive technique that combines the traditional flow-mediated dilatation with pneumatic fingertip probes to measure arterial pulse wave amplitude and provide an objective measure of endothelial function (Reactive Hyperemia Index, RHI) and vascular stiffness (Augmentation index, AI). RH-PAT in T1D adolescents found endothelial dysfunction (lower mean RHI) (1.63 ± 0.5) when compared with children without diabetes (1.95 ± 0.3, p= 0.01) compared to nondiabetic controls [76]. Children with T1D underwent a second RH-PAT study 4 weeks after their initial study to determine the intrapatient variability of the technique and repeat RH-PAT scores were predicted by initial RH-PAT scores (p= 0.0025, mean intrapatient RHI SD=0.261, and coefficient of variation=14.8).

Briefly, the patient sits in a reclining chair with the hands at heart level and propped in a comfortable position such that the fingers are hanging freely. Fingertip probes are placed on both index fingers and pulse wave amplitudes are recorded for the duration of the study. After 5-6 min of baseline measurement, arterial flow to the nondominant arm is occluded for 5 min using a BP cuff inflated to 40 mmHg above systolic pressure. After the 5-min occlusion, the cuff is rapidly deflated to allow for reactive or flow-mediated hyperemia. Pulse wave amplitudes are recorded for at least 5 min after the cuff is deflated. An integrated software program compares the ratio of arterial pressure in the two fingers before and after the occlusion to calculate the RH-PAT score. The RH-PAT score is calculated as the ratio of the average pulse wave amplitude measured over 60 s starting 1 min after cuff deflation to the average pulse wave amplitude measured at baseline. This ratio is normalized to the concurrent signal from the contralateral finger to correct for changes in systemic vascular tone.

Heart Rate Variability: Heart rate variability (HRV) assessment provides a non-invasive and objective method for investigating autonomic input into the heart and measuring cardiac autonomic neuropathy (CAN). We have previously shown it is associated with subclinical atherosclerosis[77-79]. In addition, studies in T1D youth demonstrated reduction in HRV[80], mostly with vagal withdrawal early[81] and sympathetic dysfunction later in the disease process[82]. However, our large study found overall HRV reduction suggesting a more advanced state of cardiac autonomic neuropathy[77,78]. Therefore HRV analysis is expected to find abnormalities already occurring in T1D youth. The measurement quantifies the amount by which the R–R interval or heart rate changes from one cardiac cycle to the next, and can be assessed either by time-domain analysis or by spectral analysis (frequency-domain analysis) of ECG recordings. HRV will be assessed in supine subjects over a 5 minute period of rest, and then during 5 minutes of deep breathing. Following another 5 minute baseline period before each session, responses to standing and Valsalva breathing will be measured. Tachycardic and bradycardic extremes are identified and the difference used as a measure of CAN.

MRI: The MRI will be obtained during visits 4 and 7 at the UCD Brain Imaging Center on the Anschutz campus or at the CHC Radiology Department. The purpose of the thorax MRI is to obtain phase contrast imaging through several levels of the aorta, including the ascending aorta, the transverse arch, and the descending aorta and renal vessels, as well as the heart. MRI images will be taken supine of the thorax during normal breathing, as well as during inhalation and exhalation. These dicom images will be transferred from the MRI to an off-line processing system, MatLab, where the contours of the PC images will be carried out in order to determine the flow pattern and wall shear stress.

DEXA: Body composition will be measured using the DEXA technique on the adult and pediatric CTRCs and will be used to derive fat-free mass and % body fat. This technique relies on the absorption of dual electron wavelengths for the assessment of body fat, lean tissue, and bone mineral density.

Indirect calorimetry: For measurement of RQ fasting and during low infusion rate to assess insulin response of shift from fat to carbohydrate metabolism.

Waist Circumference: Measurements from phase 1 and 2 will be used in an insulin sensitivity prediction equation.[90]

Sleep questionnaires: The Epworth sleepiness scale for adults[83] and the adult Sleep Hygiene Scale[84,85] will be completed by adult participants to obtain information on behavioral sleep variables as in our ongoing sleep study in PCOS. Adolescent participants will complete the Cleveland Adolescent Sleepiness Questionnaire, the Sleep Disturbances Scale for Children, the Adolescent Sleep Hygiene Scale and the Behavioral Sleep Medicine Clinic Sleep Questionnaire. Through this study we will also collaborate with Ann Hallbower, MD, Pediatric Sleep expert (University of Colorado, Denver) who has extensive experience with these questionnaires and the Actiwatch sleep monitor, and is available for consultation on the proposed project as needed.

Sleep/wake and activity monitoring: Typical home sleep and activity patterns will be monitored for one week using actigraphy[86,87]. The Actiwatch Spectrum Plus (Philips Respironics, OR) will be worn on the non-dominant wrist and will collect data on movements and light exposure in 1-min bins. A concurrent sleep diary will be completed by participants to allow for removal of artifacts (e.g., failure to wear the actigraphy during a portion of the night). Average sleep start and end time, sleep duration, and sleep efficiency will be examined. Participants will be asked to refrain from caffeine, non-steroidal anti-inflammatory drugs, and alcohol during the sleep monitoring period.

Hypoglycemia questionnaires: Hypoglycemia awareness will be assessed using the Gold method (Question: “Do you know when your hypos are starting?” response on 7 point Likert scale), Clarke method (8 question questionnaire), and the McAuley score (list of symptoms with a 7 point Likert scale for each)[64-67].

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Potential risks:

BCQR intervention: Bromocriptine itself has been available and widely used for Parkinson's Disease and prolactinoma for decades. It was also used for a time for congestive heart failure. The quick release formulation is so far apparently similar in safety and tolerability. Common side effects include mild hypotension, mild fatigue/somnolence, headache, depressed mood, insomnia, nausea, and diarrhea. Less common and more serious side effects have been seen with BCQR including hypersensitivity reactions, hypoglycemia and syncope. There is no increase in CVD. In fact studies so far indicate a protective effect. Bromocriptine is standardly used in young children, adolescents, and adults of all ages with hyperprolactinemia at higher does than proposed in our study and should be well tolerated. Frequent blood sugar monitoring and contact with study staff will help avoid hypo- or hyperglycemia. Potential benefits include improved glycemic control and decreased insulin requirement, as well as metabolic and vascular benefits related to decreased chronic SNS activity, decreased renin angiotensin system activation, and resetting of the circadian rhythm. Subjects will be withdrawn if they are unable to tolerate the minimum acceptable dose of BCQR (1.6mg QD) or if they experience a predefined serious adverse event (infection related to blood draw or IV placement; severe hypoglycemia during the mixed meal tolerance test (blood glucose < 45 mg/dl), more than one episode of severe hypoglycemia requiring assistance or diabetic ketoacidosis (DKA) while on study drug or other severe reactions possibly or probably related to the study drug). No further data will be collected from withdrawn subjects. All SAEs will be followed until resolution.

Blood Samples: The collection of blood samples may result in temporary discomfort, bruising, bleeding, and on rare occasions, infection. EMLA cream helps to prevent discomfort, and sterile technique helps to minimize these risks.

IV risks: There is temporary discomfort when the needle goes in and 10% of the time there is a small amount of bleeding under the skin that may produce a bruise. Rarely, there is a risk of a blood clot forming or infection. EMLA cream helps to prevent discomfort and sterile technique by experienced CTRC nurses helps to minimize these risks.

Study Diet: There is a small risk that blood sugars will be higher or lower than normal at home while eating the study diet as it may differ from the subject's usual diet. To avoid this risk, subjects will be asked to monitor their blood sugars at least four times a day while on the study diets and following procedures. Carbohydrate content will also be provided for each food item to assist with carbohydrate counting.

Continuous Glucose Monitoring (CGM): A continuous glucose sensor will be placed (Dexcom G4 Platinum, Dexcom, Inc., San Diego, CA) to assess home blood glucose values. The sensor is well tolerated with the only side effects being mild to moderate site irritation in 2% of patients, and in rare cases, skin infection.

DEXA Scan: There is no pain with this procedure. For females, a urine pregnancy test will be checked prior to the test to avoid any x-ray exposure to a pregnant person. This procedure involves the use of X-rays. The amount of radiation exposure during the DEXA test is approximately 15 mSv which is 7 times the level of background radiation in Colorado or approximately equal to the amount of radiation a subject would receive being outdoors in Denver for two days.

Mixed meal tolerance test: There is a small risk that blood sugars will be higher or lower than normal at home while eating the study diet and with the mixed meal tolerance test (MMTT) as it may differ from the subject's usual diet. To avoid this risk, blood sugars will be checked before and after the meal and additional carbohydrate or insulin given as indicated.

Ansar Heat rate variability, Endopat and Dynapulse: There are no significant risks associated with these procedures. There may be slight discomfort when the blood pressure cuff is inflated.

MRI: The MRI is a non-invasive scan. MRI uses a magnet and there is no radiation and no risk involved with the MRI. The scan may be loud, therefore participants may be provided with audio protection and optional television. Metal cannot enter the MRI area. Participants will be screened to assure no metal is present prior to MRI.

Indirect Calorimetry: The subject may feel claustrophobic.

Waist Circumference: There is no risk associated with measuring waist circumference

Data handling: Data will be examined for completeness and errors during data cleaning, in order to remove any spurious or incorrect data and complete any missing data in the database where possible. Protocol deviations will be reported to COMIRB and the study Safety Officer within 30 days of the deviation. Deviations from the original statistical analysis plan will be reported to COMIRB with the next continuing review and disclosed in any publication resulting from this study.

Assessment of Safety: Safety parameters will include monitoring of any adverse events (AEs) or serious adverse events (SAEs), including number of hypoglycemic episodes and percentage of time spent hypoglycemic during the last week of CGM data. Safety parameters will be obtained by questionnaire at each visit, and will include reports of any AEs or SAEs.

At each visit, study subjects will be asked if they have had any AE, including illness, injury or hospitalization since the last visit. If so, the onset date, resolution date, severity and outcome will be recorded. All AEs will be reviewed by the PIs and a determination of SAE or AE status will be made. All SAEs will be reported to the IRB, safety officer, and sponsor following regulations. Bromocriptine is less well studied in adolescents and therefore further assessment of Bromocriptine's hypotensive effect will be monitored at each visit for adolescents aged 12-17 via orthostatic measurements. Assessment of intercurrent illnesses will be made by asking study participants of any illnesses or symptoms they have had since the prior visit, onset date and resolution date, severity and outcome of the illness. Subjects with SAEs will be followed until resolution of the event. All other subjects with AEs will be followed until the end of the study.

Study stopping and subject discontinuation criteria: The trial will be stopped if >2 subjects meet criteria for individual subject discontinuation [≥2 hypoglycemic events requiring assistance or diabetic ketoacidosis (DKA) during a study phase] or if >2 subjects experience a serious adverse event (SAE) possibly or probably related to the study drug. Subjects will be withdrawn if they are unable to tolerate the minimum acceptable dose of BCQR (1.6mg QD) or if they experience a predefined SAE [infection related to blood draw or IV placement; severe hypoglycemia during the MMTT (blood glucose < 45 mg/dl), more than one episode of severe hypoglycemia requiring assistance or DKA while on study drug or other severe reactions possibly or probably related to the study drug]. No further data will be collected from withdrawn subjects. As some withdrawals are accounted for in the recruitment plan, there will be no specific replacement of withdrawn subjects. SAEs will be followed until resolution. Otherwise, withdrawn subjects will follow up with their care providers.

E. Potential Scientific Problems:

Interpretation of results: Based on the basic and clinical literature for BCQR, we expect that BCQR will reduce HGP and lipolysis throughout the day, effectively improving insulin action and reducing glycemic variability, improving glucose tolerance and control as evidenced by lower CGM-glucose and glucose AUC during MMTT, and/or decreasing insulin dose. Any of these results would support the use of BCQR as adjunct therapy in T1D. In addition, the presumed mechanism of BCQR supports an expectation of decreased vascular stiffness and improved endothelial function. These results would further support use of BCQR in T1D, even independent of glycemic benefits, as CVD risk reduction is an important goal in T1D with long term benefits in health outcomes, quality of life, and lifetime medical costs. We further speculate that the effects on sympathetic activation and circadian rhythm may improve sleep quality (thus further improving insulin sensitivity), blood pressure, hypoglycemia awareness, and thus hypoglycemia frequency and/or severity.

Pitfalls and limitations: This is a fairly ambitious study with a large planned cohort to allow subgroup analyses for potential targeting of this novel intervention. However, we have been successful individually and in collaborations in recruiting for T1D studies with far more burdensome and invasive outcomes, including OGTT, echocardiograms, exercise testing, 1-2 day overnight admission, overnight insulin drip (DM subjects), hyperinsulinemic insulin clamp with muscle biopsy, multiple study diets, medication interventions, brachial artery and carotid ultrasound, and hepatic MRI paired with muscle lipid MRS. Our preliminary studies used adolescents and adults and techniques similar to the proposed protocol, establishing the necessary complex procedures and collaborations to complete this study: (1) The PI's are experienced in randomized, double blinded clinical trials in T1D adolescents and adults. This depth of experience makes the interventional component likely to be highly successful. (2) The University of Colorado's recently re-funded Clinical and Translational Research Center has the facilities needed for all of the visits and the investigators own the equipment required. (3) Our preliminary data demonstrate the feasibility of the proposed studies in youth and adults, our ability to perform the proposed studies, compelling reasons for further study and the productivity of the PI's. (4) The Barbara Davis Center for Childhood Diabetes is a large, tertiary care center for type T1D, with approximately 3000 youth and 2500 adults with T1D. The PI's are able to recruit from all patients seen at the BDC, in addition to clinics at the University, and Children's Hospital making recruitment feasible. Likewise, we have previous experience recruiting from these demographics for similar studies. To limit drop-out, we maintain frequent contact with participants, use incentives for visits and adherence, and "surprise" incentives such as sports tickets and raffles to maintain contact with the study team. We find these strategies successful for maintaining compliance in our study participants. Due to our TODAY, RISE, and other metformin studies, our staff is experienced with long-term adolescent and adult diabetes studies, with excellent study retention for as long as ten years.

While the brachial artery ultrasound is considered by some to be the gold standard technique for endothelial function, its notorious user-dependence introduces more variability for repeated measures. The Endo-PAT device is also noninvasive, much simpler to use and is performed and analyzed in a standard way that allows comparability within subjects. Simultaneous assessment of the contralateral finger also allows an internal control not present with ultrasound, and therefore we will assess endothelial function with EndoPAT as in our most recent studies. In addition, while the hyperinsulinemic euglycemic clamp is the gold-standard measure of insulin sensitivity, our estimated measures correlate well with the gold standard and will help reduce invasiveness and cost and improve the feasibility and sample size of the study.

Multiple confounders can affect IR and glycemic control. We will control or adjust for these as possible using standard methods. Subject activity and diet that affect IR will be addressed by prescribing a standardized study diet (with carbohydrate content labeled for counting) and limiting strenuous exercise for 3 days prior to the MMTT visits. Stage of menstrual cycle will also be controlled for as hormonal status in females influences vascular measures, IR and circadian rhythms. Finally, use of CGM will help limit hypo- and hyperglycemia during the trial.

F. Data Analysis Plan:

Planned enrollment: 133 T1D subjects (50% male) will be enrolled with the goal of completing 80 (see power analysis below).

Power and Sample Size: Power analysis and sample size are based on expected changes from the literature and from preliminary data for the primary outcomes for each aim. The sample size for the entire study was based on the power analysis for the subgroup analyses by age, in order to ensure adequate power to detect clinically meaningful treatment effects within each subgroup. In addition, we plan to examine the results by other characteristics (e.g. by obesity level, positive c-peptide status, etc.) in exploratory analyses which are not fully powered, but which may provide insight into heterogeneity in response to the therapy, thus guiding a future clinical trial if results of this study are positive. Desired α is set at 0.05. Since data used for this analysis are from within-subjects comparisons, repeated measures analysis will be used to compare outcome variables after each treatment (placebo, BCQR), adjusting for the randomization assignment of treatment order.

Assessment of Efficacy

The primary efficacy parameters will be change in insulin dose and CGM mean glucose on treatment versus placebo. Secondary efficacy measures will include change in post-MMT glucose and insulin AUC, and change in other CGM parameters (SD, %hyperglycemic, % hypoglycemic). Total daily basal and bolus insulin dose will be obtained at screening, and then at time zero for each medication phase. Insulin diaries will be obtained during the last week of each phase. Change in total, basal, and bolus insulin dose in units per day and total insulin in units per kg per day will be examined between medication phases to determine whether insulin requirements were lower at the end of the BCQR phase than the end of the placebo phase. Similarly, change in mean CGM glucose from the last 2 weeks of each medication phase will be examined to determine if overall mean glucose is decreased while on treatment versus placebo.

Analysis Plan: All randomized subjects will be included in the analysis using an intention to treat analysis. The change in insulin dose, CGM-glucose and other metabolic parameters will be compared between the placebo and treatment visits in each subject using paired t-tests for univariate comparisons, and using repeated measures linear regression to adjust for covariates including age, diabetes duration, baseline HbA1c, and BMI. An interaction term will be entered into the model to examine whether the effect of treatment on either outcome differs among adults linearly by age, and if there is a significant interaction ($p<0.10$), results will be stratified by age group. We will also use interaction terms to assess whether the effect of treatment differs by BMI. We will also examine whether diabetes duration and initial HbA1c have an effect on outcomes by testing for an interaction between these continuous variables and the change in insulin dose and CGM-glucose. Change in vascular parameters will also be examined using paired t-test and repeated measures regression to determine if the treatment with BCQR affects vascular stiffness or function.

SA1: Effect on metabolic parameters (insulin dose, mean glucose and glycemic variability by CGM, postprandial glucose AUC by MMTT): While there are no published data on the effect of BCQR on insulin dose, prior data on the effect of Metformin[88] indicate that the change in daily insulin dose is normally distributed with standard error of 2.2 units (SD 7.6) and data from prior studies in adults with T1D performed at our center indicate that the change in total daily insulin dose

over 4 weeks is normally distributed with a standard deviation of 15 –18.8 units/day. Assuming a SD of 18.8 to be conservative, we will be able to detect a true change in the daily insulin dose of matched pairs of ± 5.9 units/day with 80% power in the overall group (n=80) and a change of 8.3 units/day within each age group (n=40). In a prior study of the efficacy of BCQR in adult patients with T2D, study participants treated with BCQR experienced a decrease in fasting plasma glucose level of 36.09 mg/dl and a decrease in post-prandial plasma glucose of 14.38 mg/dl after 12 weeks of treatment[89]. In the overall study group, we will be able to detect a change in CGM-glucose of 3.1 mg/dl with 80% power in the entire study group and a change in CGM-glucose of ± 4.4 mg/dl in each age group, assuming an SD of 10.03 mg/dl.

SA2: Effect in sub-groups: For comparisons of change in insulin dose and CGM-glucose by group in two-sample independent comparisons assuming equal variance and using estimates of SD as in SA1, we will have 80% power to detect a difference in the effect of BCQR treatment on daily insulin dose of ± 11.9 units/day, and a difference in change in CGM-glucose of ± 6.4 mg/dl. For evaluation of BMI and age as continuous variables, we will have 90% power to detect a correlation coefficient of 0.307 or greater in the overall group in univariate analysis, and a slope of 0.32 for daily insulin dose or 0.37 for BMI in linear regression analysis.

SA3: Effect on vascular measures: Change in measures of vascular health (BrachD and RH-PAT RHI as primary outcomes, AI and HRV as secondary outcomes) will be compared following placebo and treatment with BCQR. In a prior study of BrachD in healthy adolescents and young adults, BrachD was normally distributed with a SD ranging from 1.04 to 1.29. We will have 80% power to detect a difference in BrachD of 0.343 $mmHg^{-1}$ in the entire study group, and a difference of 0.60 $mmHg^{-1}$ in each age group[55]. A prior study in children with T1D found an intrapatient standard deviation of 0.261 for RH-PAT RHI. Assuming this intrapatient standard deviation, in the overall group we will have 80% power to detect a change in RH-PAT RHI of 0.10% between treatment and placebo visits, and 0.10% in each age subgroup (adolescents and adults).

G. Summarize Knowledge to be Gained: BCQR has shown promise in type 2 diabetes as an oral antiglycemic agent that is generally well tolerated and safe and has a novel mechanism with the potential to have significant independent benefit for vascular complications. Studies so far have shown a dramatic decrease in cardiovascular events in type 2 diabetes. The mechanism and our current understanding of T1D suggest that BCQR may also benefit individuals with T1D in terms of glycemic control, insulin resistance, and vascular complication risk. This study will provide preliminary data supporting or refuting the hypothesis that BCQR is a novel oral agent that will benefit glycemic control, vascular function and stiffness, and hypoglycemia awareness in T1D.

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