

**EMERALD: Effects of Metformin on  
Cardiovascular Function in Adolescents  
With Type 1 Diabetes**

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**Protocol**

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*COMIRB Protocol*

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Approval Stamp

**Project Title:** **EMERALD Study: Effects of Metformin on Cardiovascular Function in AdoLescents with Type 1 Dabetes**

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## **I. Hypotheses and Specific Aims**

Type one diabetes (T1D) and type 2 diabetes (T2D) clearly increase all-cause and cardiovascular disease (CVD)-related mortality. T1D and T2D are occurring increasingly in youth, forecasting earlier complications, despite modern advances in the control of glycemia and other known risk factors. Presently, there is a lack in appropriate and safe methods to ideally control glycemia and in fact, T1D CVD mortality in diabetes appears to increase independently of traditional CVD risk factors. Our overarching goal is to understand early CVD pathogenesis in diabetes to effectively prevent CVD-related morbidity and mortality. In order to reach this goal, unaddressed risk factors need to be identified and investigated. Insulin resistance (IR) contributes strongly to CVD in T2D; and increasing evidence implicates the importance of IR in T1D. Our novel preliminary data demonstrate that even reasonably well-controlled, non-obese T1D youth are IR, but with a phenotype distinct from that of youth with T2D. For example, they do not appear to have low HDL cholesterol or adiponectin, or high triglycerides, fasting free-fatty acids, muscle fat, liver fat or increased waist-to-hip circumference. Therefore, the mechanism of IR may differ in T1D compared to T2D. For this reason, typical therapies for IR may not be effective in T1D youth, and require study to better understand their effects in T1D and potentially aid in the development of better therapies.

Despite a unique phenotype, markers of early CVD and cardiovascular dysfunction correlate with IR in our T1D youth. IR is observed clinically in obese patients with T1D, but it is clear that obesity cannot account for all the IR of T1D. A few studies have attempted metformin interventions in T1D youth to improve glycemia, but disappointment over the relatively small impact on hemoglobin A1c (HbA1c) tempered enthusiasm. Few human studies have examined the non-glycemic benefits of metformin in T1D, nor metformin's mechanism of action in T1D. Thus, additional benefits of insulin sensitization may have previously been overlooked. We propose that improving IR with metformin will improve IR-associated abnormalities in T1D.

This proposal will provide novel data on tissue-specific response to insulin sensitization with metformin in T1D, cardiac, vascular, exercise and mitochondrial functional response to an insulin sensitizer, potential mechanisms of the response, as well as clarify the role of IR vs. glycemia in these abnormalities. Treating or ultimately preventing IR in all patients with T1D challenges our current approach to T1D care and assumptions about CVD pathophysiology in T1D. We are uniquely poised to address the nature and consequences of IR in diabetic youth. Our ability to perform 3-stage hyperinsulinemic clamps with isotopes in youth with T1D is a rare capability. Our use of non-invasive MRS imaging to assess mitochondrial function with exercise, sophisticated echocardiographic techniques in T1D youth, FPLC lipid subfraction and urine MMP's are all innovative and will generate new data for the field. Our data *will reframe how we think about* the traditional model of IR as synonymous with the metabolic syndrome phenotype and rather will focus on the unique phenotype of IR in T1D.

### **1. Hypotheses and Specific Aims**

#### **1. Hypotheses**

Hypothesis 1: Metformin will improve insulin sensitivity and mitochondrial function in T1D.

Hypothesis 2: Metformin will improve vascular and cardiac function in T1D.

#### **2. Specific Aims**

Specific Aim 1a: To assess if metformin will improve tissue-specific IR in T1D youth.

Rationale: Our preliminary data reveal muscle, hepatic, and adipose IR in normal-weight T1D youth. Super-imposed obesity, increasingly common in T1D, may also worsen the IR. Our data suggest that metformin

improves IR in T1D but by unclear mechanisms. Identifying metformin's site(s) of action in T1D is critical to revealing the cause of IR in T1D and guide future treatment targets(s).

1a Outcomes: Graded hyperinsulinemic euglycemic clamp with glucose and glycerol isotopes to assess metformin's impact on muscle (glucose infusion rate, GIR, 1° outcome), hepatic, and adipose IR, and HbA1c.

Specific Aim 1b: To assess if metformin will improve muscle mitochondrial and exercise function in T1D youth.

Rationale: Our preliminary data suggest reduced maximal exercise capacity (VO<sub>2peak</sub>), strongly correlated with IR and mitochondrial abnormalities in T1D youth. The literature suggests metformin improves mitochondrial function, biogenesis and reactive oxygen species (ROS) production, but effects in T1D humans are unknown.

5b Outcomes: Mitochondrial function (ADP t<sub>1/2</sub>, 1° outcome) by muscle exercise <sup>31</sup>P MRS and VO<sub>2peak</sub>.

Specific Aim 2a: To assess if metformin will improve vascular dysfunction in T1D youth.

Rationale: Our data suggest reduced blood flow related to IR and peripheral arterial stiffness in T1D youth.

Metformin improves arterial stiffness in other populations, but its vascular effects in T1D are unknown.

2a Outcomes: Peripheral arterial stiffness (BrachD, 1° outcome) by Dynapulse, central arterial stiffness by carotid ultrasound, endothelial function by brachial ultrasound (FMD) and endoPAT, aortic wall shear stress by MRI, and urinary vascular markers (matrix metalloproteins, MMP's).

Specific Aim 2b: To assess if metformin will improve cardiac dysfunction in T1D youth.

Rationale: Our preliminary data suggest the presence of clear functional cardiac abnormalities and reduced VO<sub>2peak</sub>, a marker of CV performance, in T1D youth. The literature suggests metformin improves cardiac function and hypertrophy in animal models of T1D and heart failure, but effects in human T1D are unknown.

2b Outcomes: Circumferential strain (CS, 1° outcome), resting and exercise-stressed cardiac structure and function by echocardiography; VO<sub>2peak</sub> from Aim 5.

All measures will be performed twice, before and after a 3 month placebo-controlled study where subjects are randomized to either metformin or placebo. The independent impact of IR as well as HbA1c, BMI, T1D duration, and gender on baseline outcomes and the impact of changes in IR, HbA1c and BMI on response to metformin will also be examined to help customize future CVD prevention strategies in T1D. This study will *advance the field* by providing new information about the role of IR in T1D CVD and whether improving IR in T1D is beneficial. *If a focus on directly treating IR in T1D youth is supported by our studies, the clinical approach to T1D management may significantly change.*

## II. Background and Significance

Identified CVD risk factors in patients with diabetes include hyperglycemia [1, 2], hypertension [3], dyslipidemia [4], albuminuria [5, 6], obesity [7], and adiponectin [8]. However, traditional CVD risk factors[9] do not fully explain the increased morbidity and mortality from CVD seen in individuals with diabetes [10-13]. Insulin resistance has a well-defined role in atherogenesis, but is more difficult to quantify in T1D subjects. Despite the relative lack of dyslipidemia and obesity in T1D patients, studies using a euglycemic clamp have demonstrated decreased global insulin sensitivity in T1D patients when compared to non-diabetic persons [14, 15] [12, 14].

The DCCT emphasized that intensive glycemic control reduces, but does not eliminate complications in T1D, and was associated with weight gain in a subset of subjects, including women, along with worsening lipid profiles, BP, central obesity, IR and inflammation, all of which may negate positive effects of improved A1c[16]. IR clearly correlates with CVD risk factors and impaired exercise capacity in adults with obesity, pre-diabetes and T2D[17-23], and therefore may in T1D as well. For example, estimated IR, not glycemia, predicted CVD events and mortality in T1D adults[9, 24], predicted increased CVD risk-factors in our T1D youth, and clamp-derived IR, not glycemia was associated with CAC in CACTI[25]. T2D likely increases CVD through classic risk factors (BP, hyperlipidemia, etc), combined with adipose, hepatic and muscle IR, hyperglycemia, ectopic fat deposition, and mitochondrial dysfunction (MitoD). In contrast, the phenotype of IR in T1D appears atypical. For example, when compared to T2D, or obese and normal weight controls, hepatic fat content was unexpectedly lower [26] and adiponectin unexpectedly higher in IR adults with T1D. Also unexpectedly, intra-abdominal fat volume did not correlate with IR in women with T1D [27]. Similarly, HDL-cholesterol levels are generally more favorable in T1D patients than in non-diabetic controls [28]. In addition, IR in T1D co-exists with autoimmunity[29], peripherally injected insulin rather than regulated pancreatic insulin secretion, and hyperglycemia[30-33]. Each of these factors may uniquely influence IR in T1D.

The term IR implies global resistance, but the degree of IR varies from tissue to tissue and even between intra-

cellular post-receptor signaling pathways. Assessing tissue-specific IR will determine which tissue(s) respond to metformin in T1D, help reveal future intervention targets, and allow a better understanding of which components of IR influence CVD in T1D.

It has been proposed that this decreased insulin sensitivity may be due to the subcutaneous delivery of insulin in supraphysiologic doses in patients with T1D. As a possible explanation, peripheral insulin administration results in relative portal hypoinsulinemia, as does insulin omission. Portal hypoinsulinemia may lower hepatic IGF-1 secretion[34], as seen in our preliminary data, causing compensatory growth hormone (GH) increases, elevating FFA's, inducing muscle and hepatic IR[35-37]. Such events are exaggerated in puberty, when GH, IGF-1 secretion and physiologic IR peak[38] and compliance with insulin plummets, making youth important to study. In support of insulin deficiency as a culprit, hepatic IR[39], and lower IGF BP-1, implying hepatic IR despite equivalent serum insulin levels[34], were previously reported in only those T1D youth in poor control. However, hepatic IR, such as in the liver insulin receptor knockout (LIRKO) mice, is typically associated with marked dyslipidemia[40], not seen in most T1D youth. Hepatic IR may also occur mainly with poor glycemic control[39], and Hother-Nielsen et al reported peripheral IR, yet enhanced hepatic insulin sensitivity in T1D adults[41]. Results from our collaborators in the CACTI study of T1D adults suggest normal hepatic fat in T1D adults, also arguing against hepatic IR. Thus, hepatic IR and lipid require further evaluation. Metformin improves IR in T2D youth, is safe and well tolerated. Metformin's mechanism of action in T2D is typically described as decreasing hepatic glucose output, but effects in T1D are unknown. The proposed study allows further assessment of impact of HbA1c and IGF levels on hepatic IR in a larger sample size, and in response to metformin.

Suppression of lipolysis by insulin in adipose may also be impaired, especially with poor glycemic control, and decrease insulin-mediated glucose disposal. Jensen et al reported that poorly controlled T1D adults had adipose tissue IR [42]. Similarly, Heptulla et al reported that poorly controlled T1D adolescents had impaired suppression of lipolysis by microdialysis in muscle and adipose tissue; hypothesized to cause the peripheral IR they exhibited[43]. Children may also be under-insulinized to avoid hypoglycemia, minimize injections, or due to adolescent noncompliance. Such relative insulinopenia may elevate FFA, inducing muscle and/or hepatic IR. Alternatively, low IMCL in T1D youth argues against muscle IR or for non-IMCL mediated IR. Our preliminary data, supported by CACTI, demonstrate failure to suppress lipolysis at low, moderate and high-dose insulin. Elevated FFA's may contribute to hepatic and muscle IR[37, 44], as well as endothelial dysfunction, oxidative stress and other CVD-related pathology[45]. Metformin decreased lipolysis in vitro[46, 47], FFA's in some T2D studies[48], and peripheral IR and reactive oxygen species (ROS)[49] in FFA-induced models of IR[50], but effects in T1D are unknown. Unregulated lipolysis is likely important in accelerated atherosclerosis progression, but has not been extensively studied in T1D, especially in youth, and it is not known whether the lipolytic effect of insulin is suppressed in adipose and muscle tissue in well-controlled T1D.

Peripheral insulin administration may increase peripheral insulin resistance; there may be different effects of this peripheral hyperinsulinemia in the muscle, liver and adipose tissue in patients with T1D. Our preliminary T1D results, again supported by CACTI, demonstrate a novel finding: hepatic IR without steatosis. Data from our studies and CACTI both suggest that normal weight T1D subjects with reasonable glycemic control have significant muscle IR[51, 52]. Muscle IR could result from peripheral insulin administration. Rats lacking insulin delivery to the portal vein due to portal-caval vein transposition have reduced hepatic insulin clearance, peripheral hyperinsulinemia and whole body IR[53]. Alternatively, children may be under-insulinized to avoid hypoglycemia, minimize injections, or due to noncompliance typical of teens. Such relative insulinopenia may elevate FFAs (as would high fat diet or primary adipose IR), inducing muscle and/or hepatic IR. Our reported low IMCL in T1D youth argues for non-IMCL mediated mechanisms of muscle IR. Muscle blood flow, impaired in T1D (preliminary data, Aim 2a), is a large determinant of insulin-mediated muscle glucose uptake and thus impaired blood flow could cause IR[54, 55]. Muscle MitoD may also cause IR by increasing ROS and/or diacylglycerol, both of which may impair insulin signaling[56, 57]. Metformin was tested in several T1D youth trials[58, 59], and a recent meta-analysis including both adult and pediatric studies concluded that metformin in T1D significantly reduces insulin dose requirement, implying improved whole-body IR, but has no significant effect on A1c[60]. In 30 T1D youth, 3 months of metformin improved whole body IR measured by hyperinsulinemic euglycemic clamp[61]. Recent data indicates muscle effects of metformin[62], such as improved forearm muscle glucose uptake and blood flow in T2D[63], increased glucose disappearance during intralipid/heparin infusion, indicating protection against FFA-induced insulin resistance in extrahepatic tissues (i.e. muscle)[50] and improved muscle mitochondrial dysfunction (MitoD) in animal models[62]. MitoD is associated with IR in other populations, but it is unclear whether it is the cause[64] or result of IR[65, 66]. For example, T2D adults and their lean, nondiabetic, but IR adult offspring have evidence of skeletal muscle MitoD[64], arguing that MitoD causes IR, and that improving it might lessen IR. MitoD

may also be secondary to chronic gluco- and/or lipo-toxicity [65, 66] or lack of insulin effect. In a small adult T1D study, acute insulin deprivation decreased muscle mitochondrial ATP production rate and altered mitochondrial gene expression[24]. In another adult T1D study, muscle  $^{31}\text{P}$  MRS revealed alterations of the major pathways of ATP supply: higher glycolytic flux and lower oxidative capacity[67]. Regardless of its cause, MitoD increases production of damaging ROS, correlates with exercise dysfunction[68], and may contribute to dysfunction of many organs, including the heart, thereby contributing to decreased  $\text{VO}_{2\text{peak}}$ . Recent data indicates mitochondrial effects of metformin via AMP-kinase activation and other mechanisms. For example, 4 weeks of metformin normalized muscle mitochondrial  $\text{H}_2\text{O}_2$  emission in obese Zucker rats[62], and 2 weeks of metformin enhanced PGC-1 $\alpha$  expression and mitochondrial biogenesis in Wistar rats[69], arguing that metformin may improve muscle mitochondrial number and function and reduce ROS[70]. This proposal will generate new data on the impact of metformin on mitochondrial function and on  $\text{VO}_{2\text{peak}}$  in T1D. Muscle biopsy has been the most common approach to assess muscle mitochondrial function, however, a less invasive technique is highly desirable, especially in children. We have previously employed this technique with Dr. Mark Brown, a Ph.D. chemist who specializes in MRS spectroscopy in the Department of Radiology of the UCHSC and has extensive previous experience with in-vivo MRS to non-invasively evaluate muscle mitochondrial function via phosphorous MRS, [71-73].

Hyperglycemia may also lead to IR by causing excess glucose entry into the hexosamine pathway and subsequent O-glycosylation of insulin signaling and gluconeogenic regulators (TORC2) [30, 31], or through reduced skeletal muscle glucose transport [32] or hyperglycemia-induced abnormalities in muscle diacylglycerol kinase delta[33]. Different categories of hyperglycemia (chronic, acute, mean glucose values vs. glycemic excursions, post prandial vs. fasting glycemia, etc) may differ in their impact on IR. Insulin transport across capillaries is important for insulin action[74], thus microvascular disease may also affect insulin action. IR in T1D could also be secondary to inflammation, due to its autoimmune nature[29]. The biggest contributors to IR are liver, muscle and adipose tissue, therefore our first goal is to examine hepatic, adipose and peripheral (muscle) IR via a 3-stage hyperinsulinemic euglycemic clamp. In adolescents without DM or with T2DM, IR is associated with many variables, including obesity [75], puberty, poor fitness [76], ectopic lipid deposition in muscle (IMCL) [77] and liver/viscera [75], and low adiponectin [78]. The relationship between these variables and IR are not well studied in youth with T1D. In addition, there is evidence of mitochondrial dysfunction in the muscle of adults with T2D and their insulin resistant relatives, which is thought to be related to the pathophysiology of insulin resistance [71-73]. The existence of mitochondrial defects in subjects with T1DM of any age has not been examined. Other factors affecting IR in subjects with DM uniquely may include degree of chronic glycemic control [39], current glucose levels [79] and route of insulin delivery [27].

The IR phenotype is atypical in lean T1D subjects, and obesity superimposed on T1D is likely to worsen IR and create a hybrid phenotype more like T2D. In support, in the small number of obese T1D youth we have studied, hepatic lipid, IMCL and triglycerides are higher and HDL lower than in lean T1D youth. Obesity is now increasingly seen in T1D youth. Thus, research on obesity in T1D and response to insulin sensitization is also required to prevent CVD in this very high-risk subgroup.

Since atherosclerosis and vascular dysfunction develop in a non-uniform fashion[80], multiple measures are needed to comprehensively evaluate vascular changes in youth with T1D. Abnormalities in endothelial dysfunction, vessel stiffness and atherosclerosis estimated by cIMT are reported in some pediatric T1D studies[81-84] [85, 86] but not in all[86, 87]. Our preliminary results with venous plethysmography indicate the novel findings of reduced exercise function and vascular reactivity in T1D. While venous plethysmography does not distinguish between endothelial dependent and endothelial independent dilation, and stiffness, it is a good indicator of blood flow overall. Brachial artery ultrasound can also be used to more specifically assess endothelial ultrasound. Therefore, venous plethysmography and brachial artery ultrasound will be used to assess endothelial dysfunction and blood flow.

Our collaborator in Gainesville used reactive hyperemia–peripheral artery tonometry (RH-PAT) by the EndoPat device (Itamar Medical Ltd., Caesarea, Israel) to assess endothelial function in T1D adolescents and found endothelial dysfunction as evidenced by lower mean RH-PAT scores ( $1.63 \pm 0.5$ ) when compared with children without diabetes ( $1.95 \pm 0.3$ ,  $p = 0.01$ ) compared to nondiabetic controls [88]. Children with T1D underwent a second RH-PAT study 4 wk after their initial study to determine the inpatient variability of the technique and repeat RH-PAT scores were predicted by initial RH-PAT scores ( $p = 0.0025$ ). Mean inpatient standard deviation of RH-PAT score was 0.261 and mean coefficient of variation was 14.8. Endothelial dysfunction can be measured by several techniques, including flow mediated dilation by brachial artery ultrasound and RH-PAT by Endo-PAT. While the brachial artery ultrasound is considered by some to be the gold standard technique, it

requires extensive training and experience to perform and analyze correctly, and its user-dependence introduces more variability for repeated measures. The Endo-PAT device is noninvasive, much simpler to use and is performed and analyzed in a standard way that allows comparability within subjects and therefore we will assess endothelial function with EndoPAT as an exploratory aim.

Additionally, our collaborators in the SEARCH study evaluated peripheral arterial stiffness in T1D vs. T2D youth noninvasively by Dynapulse. Their findings suggested differences between these two groups as well as impairments in T1D youth vs. controls[83]. In particular, stiffness seems to present early and persist[89], arguing for pediatric studies where prevention may still be possible. Increased peripheral stiffness appears more common than central stiffness in T1D youth[83], especially boys[83, 86]. Based on this evidence, peripheral stiffness as measured by Dynapulse will be a primary outcome and central stiffness with carotid ultrasound as a secondary outcome.

Endothelial dysfunction correlates with IR in T2D adults[90] and in obese youth[91], and we propose that IR is a similarly a major contributor to vascular abnormalities in T1D youth. Therefore, improving IR should improve these abnormalities, as our group's study of rosiglitazone in T2D, improved IR, endothelial function and  $\text{VO}_2\text{max}$ [92]. In addition, muscle blood flow is a large determinant of muscle glucose uptake in response to insulin, and therefore blood flow abnormalities and abnormal vaso-reactivity could lead to IR. Unregulated lipolysis likely also accelerates atherosclerosis, but little data exists in T1D. Three months of metformin was recently shown to improve arterial stiffness and endothelial function in 30 young women with IR related to PCOS[93], and metformin improved blood flow by plethysmography and exercise capacity in 11 adults with PAD[94], arguing for potential benefits of metformin on CV abnormalities which may be common to T1D.

Diabetes is associated with a cardiac defect, independent of CAD or hypertension, that in T2D is associated with IR [19, 95]. Adults with recently diagnosed, uncomplicated T2D have evidence of cardiac dysfunction and increased filling pressures during exercise, despite normal resting parameters[19]. Exercise stress echocardiography with tissue doppler and speckle tracking, newer and potentially more sensitive noninvasive echocardiographic techniques, may help further identify the mechanism of cardiac dysfunction in T1D[96-99], track response to treatment, and identify new treatment targets. Cardiac MitoD and lipid accumulation are reported in T2D, echoing skeletal muscle patterns. Similar cardiac findings are also reported in T1D [100], possibly secondary to dysfunctional mito-K(ATP) channels, impaired depolarization, superoxide production [101] or oxidative stress [100]. This sequence may limit cardiac efficiency, compromising ventricular function when oxygen demand is high or delivery is limited[102]. The heart's normal fuel switch between predominate fat utilization at rest, to glucose utilization during exercise, is insulin-mediated [103]. IR could prevent this adaptive switch, decreasing cardiac function and glycogen stores, further decreasing performance. Epicardial fat is also associated with IR in T2D and has not been evaluated in youth with T1D. Several reports show worse cardiac function in T1D girls than boys[104, 105], potentially due to differences in IR.

Our preliminary data also suggests that adolescents with T1D have abnormal diastolic function by resting echocardiogram and abnormalities in circumferential strain. Our concerning preliminary cardiac findings in T1D youth argue for interventions aimed at improving cardiac function. The UKPS Diabetes Study reported that metformin was more effective than sulfonylureas or insulin in reducing CV mortality, despite similarly decreased A1c, and the HOME trial showed that metformin improves macrovascular outcomes in insulin-treated T2D patients, suggesting metformin provides CV protection via intrinsic (and possibly direct) AMPK activation effects independent of glucose lowering[106]. Metformin also improved cardiac function in several animal models of T1D[107, 108]. In particular, metformin improved left ventricular hypertrophy and function in heart failure via activation of AMPK and its downstream mediators, eNOS and PGC-1 alpha in cardiac myocytes, and significantly improved myocardial mitochondrial respiration and ATP synthesis [109]. Thus, the literature suggests beneficial effects of metformin on the heart, potentially similar to skeletal muscle effects, but no studies to date have assessed metformin's cardiac effects in T1D. Echocardiogram and ECG are non-invasive ways to assess cardiac function. Performing these tests before and after a  $\text{VO}_2$  max exercise test, will allow us to examine the heart under both resting and stressed conditions, at baseline and in response to metformin.

The demonstrated IR in T1D patients may also be related to decreased exercise capacity of these patients. We have found a correlation between  $\text{VO}_2/\text{kg}$  and IR in our previous studies. We suspect that the deterioration in exercise function may be due to endothelial and diastolic abnormalities associated with insulin resistance.

Increased levels of small dense LDL and decreased large HDL particles correlate with increased CVD risk[110] and lipid abnormalities affect vessel function and stiffness. Preliminary data from our collaborators in the CACTI

study of adults with T1D demonstrate higher VLDL, smaller LDL, and smaller HDL in T1D adults with coronary calcium, but lipid subfractions have not been similarly examined in diabetic youth. Under physiologic settings, insulin regulates lipid metabolism by inhibiting lipolysis of stored adipose triglyceride, inducing hepatic genes of de novo TG biosynthesis, increasing apo B degradation and decreasing hepatic VLDL release. IR would be expected to alter this regulation. Analysis of lipid subfractions with fast protein liquid chromatography (FPLC)[16] and apoB before and after insulin infusion will help determine the baseline atherogenicity of lipid profiles in T1D and T2D youth, and the effects of IR and DM on insulin-mediated lipid and lipoprotein metabolism. Using apo B in combination with VLDL TG will allow interpretation of the effect of insulin on particle composition vs. particle number. By examining hepatic insulin sensitivity, and body composition proposed in Aim 2, and the vascular measures proposed, the relationship between lipid profiles and these factors can also be examined. In addition, T1D adults consuming a high-fat diet had increased CVD risk factors, worse glycemic control and coronary artery calcification[111], and an intervention with higher carbohydrate intake improved IR in T1D, despite worsened HbA-1c, thus dietary intake will also be examined and a 3-day study diet provided prior to the visit.

Novel Cardiac Risk factors in T1D: MMP degradation of extracellular matrix is essential for vascular remodeling[112], processes that could be affected by IR or glycemia. In particular, MMP 2, 9, and 13 are associated with pathologic changes seen in the T1D arterial wall[113], and MMP activities correlate with stiffening, impaired angiogenesis, endothelial dysfunction<sup>189</sup> and reduced nitroglycerin-mediated dilation[114] in T1D patients. Therefore MMP's will be assessed for correlation to baseline IR, A1c, stiffness, FMD and cIMT, and changes in these variables in response to metformin. Recently, data has been published regarding the serum marker of cardiac function, N-terminal pro-brain natriuretic peptide (NT-pro-BNP), and its correlation to diastolic function in T1D youth. The Salem et al study found that NT-pro-BNP was significantly elevated in T1D youth (cut-off value = 62.5 Fmol/mL, sensitivity 82%, specificity 95%) and correlated with isolated diastolic dysfunction. NT-pro-BNP also correlated negatively with LV Em, Em/Am, and positively with Am. Since data suggests NT-pro-BNP could be a sensitive, specific and predictive marker for diastolic dysfunction, we will evaluate this serum marker and its response to metformin.

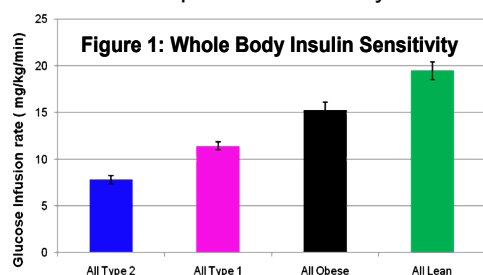
NMR of soleus muscle performed at UCHSC showing EMCL (extramyocellular) vs IMCL (intramyocellular) lipid content

### III. Preliminary Studies/Progress Report

#### A. Preliminary Data

##### 1. Presence of IR in T1D youth, unrelated to hyperglycemia

IR was reported historically in T1D adults and youth[29, 43, 115-118], but is frequently overlooked or



attributed to extreme hyperglycemia[43] or obesity. Using a controlled study diet, an overnight insulin infusion to normalize glycemia and hyperinsulinemic euglycemic clamps, we found that n=83 normal-weight T1D youth (Fig 1, pink bar), even if near-normal HbA1c, had a significantly lower glucose infusion rate (GIR) than n=32 pubertal stage, BMI, and activity-matched normal controls (green bar)[119].

T1D youth were even more IR than n=25 obese nondiabetic youth (black bar), but not as IR as n=38 T2D youth (blue bar) [120]. The mean HbA1c in our study (8.6%) was lower than previous

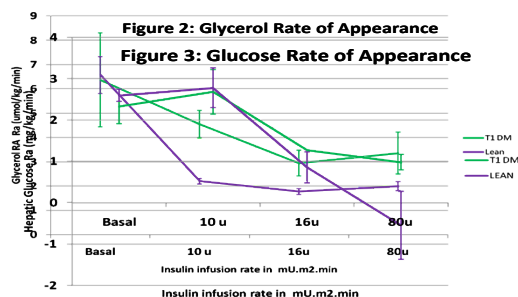
adolescent studies (9.6%-12.7%)[43, 117, 118, 121], and typical of contemporary data (mean adolescent A1c in the T1D EXCHANGE is 8.7%[122]). The CACTI study[25] in T1D adults also confirm our findings, at an even lower mean A1c (7.5%). Therefore, IR in T1D continues to occur despite post-DCCT advances in therapy and lower glycemic targets.

IR remained after adjustment for BMI, pubertal stage, % body fat, activity level, clamp insulin levels, and hepatic glucose production, and was not associated with acute (fasting glucose), chronic (HbA1c)[119, 123, 124] or variability in glycemia (continuous glucose monitoring, CGMS)[125]. Thus, neither glycemia nor BMI fully explain IR in T1D. Our novel preliminary findings argue that T1D patients are significantly more IR than controls, and that non-glycemic factors contribute to IR in T1D. Moreover, obesity is increasing in T1D youth, (40% of T1D adolescents in the T1D EXCHANGE are overweight or obese[122]), likely further worsening IR. Even among normal weight T1D youth, IR correlated with BMI, waist circumference (WC) and % fat[123]. *Accordingly, direct prevention and treatment of IR should be a priority in T1D research, yet few data exist.*



To date we have completed approximately 150 insulin clamps on the pediatric CTRC, on subjects age 12-21, thus are of the same age group as the proposed protocol. These subjects have included adolescents with T1D, T2D, controls with obesity without diabetes and normal weight controls. The insulin clamps have gone well, with no adverse events or safety concerns, including no hypoglycemia. The bedside glucose monitoring in our previous study was performed with a YSI (Yellow Springs Instruments) glucose analyzer, which is the same as in the proposed protocol. The YSI has performed accurately and without difficulties. Thus our insulin clamp technique appears to be working well, and can be performed properly and safely by our research team on adolescents with and without diabetes in the pediatric CTRC setting.

## 2. Adipose and Hepatic IR in T1D youth



To date we have performed 51 3-stage hyperinsulinemic euglycemic clamps (10, 16, 80  $\mu\text{U}/\text{m}^2/\text{min}$  insulin) with glycerol and glucose isotopes to assess tissue-specific IR in T1D, T2D, obese and control youth. Insulin doses were chosen to mirror those in CACTI, to allow comparison to adults, as hormonal influences may result in unique findings in youth, as in our recent lipid publications[126-128]. In T1D (green line) vs. control youth (purple line) of similar BMI, pubertal stage, activity level and achieved insulin levels, T1D youth fail to suppress lipolysis (Fig 2), and hepatic glucose rate of appearance (Glucose Ra, Fig 3), even at the highest dose of insulin. IGF-1 was also significantly lower, and IGF BP-1 and 2 higher in

T1D youth, implying hepatic IR. In addition, for every 1  $\mu\text{M}$  increase in fasting free fatty acids (FFA) there was a 32  $\text{mg}/\text{kg}/\text{min}$  decrease in GIR ( $p=0.01$ ), arguing that FFA's impact systemic IR in T1D. These data show evidence of adipose and hepatic IR in T1D youth (confirmed by CACTI in adults[25, 42]), our unique expertise and feasibility of multi-stage clamps with isotopes in T1D youth. This technique is critical to determining which tissue(s) respond to our proposed intervention, and informing future intervention targets.

## 3. Preliminary data showing a divergent phenotype in T1D vs. T2D youth

Table 1	T2D	T1D	Control
Age (years)	15.4 $\pm$ 2	15 $\pm$ 2	15 $\pm$ 2
BMI percentile	96.5%*	56%	54%
A1c %	8%*	8.5%*	5.2%
Triglycerides	203 $\pm$ 250*	83 $\pm$ 36	83 $\pm$ 35
HDL	38 $\pm$ 11*	46 $\pm$ 8.5	48.6 $\pm$ 11
LDL	87 $\pm$ 25	82 $\pm$ 23	81 $\pm$ 22
Adiponectin	6.1 $\pm$ 2.9*	11.9 $\pm$ 6.2*	9 $\pm$ 3.2
IMCL (µg)	2649 $\pm$ 1109*	1489 $\pm$ 987	1379 $\pm$ 608
Visceral Fat (cm <sup>2</sup> )	78 $\pm$ 59*	18 $\pm$ 9	26 $\pm$ 15
Hepatic Fat	6.2 $\pm$ 5.5%*	0.6 $\pm$ 1.1%	0.2 $\pm$ 1.2%

Our T2D youth have a typical metabolic syndrome phenotype[120, 129-132]. In contrast, T1D youth paradoxically lack these abnormalities (Table 1), including IMCL by <sup>1</sup>H-MRS, hepatic and visceral fat by MRI[133], and low adiponectin[119, 120], despite having IR. No T1D subjects met clinical criteria for a fatty liver, and neither hepatic fat nor IMCL correlated with IR. These data are supported by similar IMCL findings in a small adult T1D study[134] and by liver data in CACTI[135], and are evidence of a potential dissociation of lipid deposition and IR in T1D[136]. *Thus, novel approaches to treating IR in T1D are likely required.*

## 4. Preliminary data showing IR is associated with CVD in T1D

We used simple clinical variables (age, sex, race/ethnicity, BMI, WC, Tanner stage, HbA1c, lipids, blood pressure (BP) and %fat) in T1D and T2D adolescents in collaboration with the SEARCH study to develop an estimate of IR[51, 137]. Estimated IR correlated well with GIR from hyperinsulinemic euglycemic clamp ( $R^2=0.74$ ). We then applied the estimate to a cohort of 290 T1D adolescents, and found that estimated IR was inversely related to CV risk factors (BP, LDL, highly sensitive c-reactive protein, hsCRP,  $p<0.0001$ ) (ADA abstract submitted). We will also apply this estimate to the T1D youth in the proposed study to test its future feasibility as a noninvasive method of detecting the change in IR as determined by hyperinsulinemic euglycemic clamp.

## 5. Preliminary data supporting the use of metformin in T1D

The PI was a Co-I on a previous adolescent fatty liver disease study[138], is a Co-I on the NIH TODAY T2D study[99, 139], and mentors an ADA Junior Faculty-funded obesity study; all randomized, double-blinded trials showing improved IR in adolescents with metformin. Thus, we are experienced in such trials and in the difficulties with recruiting and retaining adolescents. Our CO-I also performed a randomized, double blinded trial of "low dose" (500 mg BID) metformin in poorly controlled T1D adolescents, data from which we have now analyzed[140]. After 3 months, metformin significantly decreased HbA1c, weight, WC, and insulin dose, all of which except the effect on insulin dose waned at 6 months due to reduced medication adherence, suggesting sustained improvement in IR[59, 140]. Metformin, when blinded, was very well tolerated by youth in these studies, with no significant hypoglycemia. Based on this data, and its approval for use in pediatrics, we chose metformin to reduce CVD in the proposed study. We chose the 3-month time point and a full dose (1000 mg BID, note 500 mg



BID will be lowest dose allowed) where maximal effects would be expected. We did not choose a TZD for this study due to possible negative CVD effects, nor exercise, which has many confounding independent effects beyond improving IR, including changing IMCL<sup>190</sup>. *Using metformin will allow us to test mechanistically whether improving IR improves CVD in T1D, and whether metformin has other beneficial CVD-related effects in T1D.*

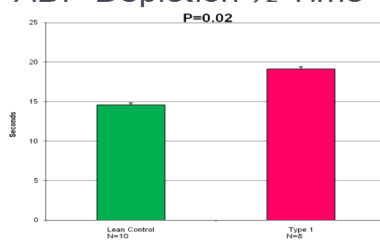
## 6. Evidence to support rationale and feasibility of MRS in youth

In the PI's NIH K23 and R56 projects, each subject underwent <sup>1</sup>H-MRS for IMCL, and MRI for hepatic and visceral lipid[133], performed by Co-I's Mark Brown, PhD and Anne Sherzinger, PhD (experienced in <sup>1</sup>H-MRS[119, 131, 133], <sup>31</sup>P-MRS[141-143] and <sup>13</sup>C-MRS[144-146]), in collaboration with Bradley Newcomer, PhD (extensive experience in <sup>1</sup>H- and <sup>31</sup>P-MRS acquisitions with exercise[68, 141-143, 147-154]), and the PI's mentee, Melanie Cree Green, MD, PhD, (PhD thesis on <sup>1</sup>H-, <sup>31</sup>P-MRS, and IR in youth[141-143, 155-159]). We found significantly increased IMCL, hepatic and visceral fat in T2D but not T1D youth[51, 120, 132]. IMCL, liver and visceral fat are not included in the current proposal as the low levels in T1D argue for non-lipid associated hepatic and muscle IR, but the same team will perform the proposed <sup>31</sup>P-MRS.

MitoD is associated with IR in T2D adults[64], but its role in T1D is unclear. Our preliminary data in rodents treated with streptozotocin, show abnormal expression of mitochondrial respiratory chain proteins by western blot (Mitocomplex by MitoScience, Inc), and led us to examine MitoD in T1D humans. Using the NIH R56 funding, we established <sup>31</sup>P-MRS methodology, well correlated with biopsy measures[153] and ideal in youth to noninvasively assess muscle MitoD. We use a customized MRI-compatible exercise bench, incorporating a load cell and force transducer to digitally measure force output during in-magnet plantar flexion, while a time series of <sup>31</sup>P MRS spectra are acquired at rest, during exercise, and during recovery[142, 143, 160]. Measurement of maximal volitional contraction (MVC) is subjective, especially in youth. Therefore, we also developed a method to predict MVC to assure a reproducible metabolic perturbation to the muscle. Data was collected from n=10 healthy adults, and n=10 control, T1D and T2D adolescents. Volitional MVC was assessed with the exercise board outside of the magnet to allow visual verification of proper technique. Maximal cross-sectional area of the gastrocnemius and soleus was assessed from MRI images in at least 4 discrete areas and used to calculate expected MCV. The predicted and actual MVC force output were similar (actual MVC mean  $43.13 \pm 2.2$  kg vs. predicted  $43.17 \pm 1.0$  kg) and significantly correlated ( $R=0.46$  and  $P=0.039$ )[141-143](manuscript under review). In subsequent studies, we now measure predicted and actual MCV and use the higher of the two for the in-MRI exercise.

We next assessed mitochondrial function in 14 T1D (Figure 3, red bar) and 10 lean control youth (green bar),

ADP Depletion  $\frac{1}{2}$  Time



and similar numbers of T2D and obese youth, with the <sup>31</sup>P-MRS exercise protocol described. ADP depletion half-time (ADP  $t_{1/2}$ , seconds) following exercise was significantly longer in T1D, despite smaller increases in ADP concentration above pre-exercise concentrations than controls ( $18 \pm 0.4$  vs.  $29 \pm 0.5$  mmol/l;  $p < 0.05$ )[161]. VPCr, the initial rate of phosphocreatine (PCr) resynthesis following exercise, ( $0.2 \pm 0.04$  vs.  $0.3 \pm 0.05$ ) and oxidative phosphorylation ( $0.09 \pm 0.03$  vs.  $0.16 \pm 0.04$ ) were also significantly lower in T1D. In summary, T1D youth performing exercise at equal workloads had less conversion of ATP to ADP, and exhibited delayed post-exercise ATP synthesis. In common with T2D youth[131], T1D youth also had significantly

reduced  $VO_{2peak}$ [119] that independently correlated with IR ( $r = -0.82$ ,  $p < 0.0001$ ) in multivariate analysis[119]. HbA1c did not correlate with  $VO_{2peak}$ , suggesting exercise dysfunction is not primarily a function of hyperglycemia. In addition, MRS PCr/Pi post-exercise ratio correlated well with  $VO_{2peak}$  ( $R = 0.60$ ,  $P = 0.04$ ), indicating muscle MitoD and CV performance are related in T1D. *Our preliminary data suggest muscle MitoD in T1D youth that correlates with IR and decreased  $VO_{2peak}$ , and provides evidence the feasibility of MRS in youth.*

## 7. Preliminary data showing vascular dysfunction in T1D and T2D youth

Our preliminary results with venous plethysmography show reduced forearm reactive blood flow in T1D and T2D youth[162]. Plethysmography correlated with IR, not A1c, thus non-glycemic factors contribute to vascular function. T1D youth also had significantly reduced  $VO_{2peak}$ [119] that independently correlated with vascular reactivity in multivariate analysis[119, 120]. We previously detected endothelial dysfunction using flow mediated dilation (FMD) in T2D adults[90], one potential cause of the reduced vascular reactivity we report in youth. Vessel stiffness or reduced capillary density may also contribute to abnormal vascular reactivity. Our SEARCH collaborators found peripheral vascular stiffness (BrachD), in T1D youth, especially boys, by Dynapulse[163, 164].

We are also currently assessing baseline endothelial function by FMD, peripheral arterial stiffness with Dynapulse, and central arterial stiffness and atherosclerosis (cIMT) by carotid ultrasound in T1D, T2D, obese and lean nondiabetic youth in our JDRF study. The endo-PAT will measure endothelial function as another non-invasive measurement for comparison. *These studies demonstrate vascular dysfunction related to IR in T1D, our expertise in vascular assessment and the feasibility of these techniques in youth.*

A growing body of literature in the adult population indicated that vascular wall shear stress is significantly higher in vessels with atherosclerotic changes and is an early predictor of atherosclerotic changes. Our hypothesis is that even as young as adolescence, early cardiovascular changes can be detected in youth with diabetes as reflected by abnormal wall shear stress. Aortic wall shear stress can be assessed noninvasively by MRI.

#### **8. Preliminary Evidence supporting relationship between adverse lipids and IR in T1D**

Despite finding overall relatively “normal” lipid profiles in T1D[165, 166], we determined that IR predicted a more atherogenic lipid profile in T1D youth and adults of both genders[126, 127]. In CACTI, those with CAC had significantly higher IR and VLDL and smaller LDL and HDL by FPLC. T1D women in CACTI also appeared to have a lipid profile more typical of males[25, 127, 167]. LDL also independently predicted VO<sub>2peak</sub> in our T1D youth[119]. Thus, plasma lipids appear related to IR and effect CAC and cardiopulmonary function, possibly via vascular dysfunction.

#### **9. Preliminary Evidence to support Matrix metalloproteinase (MMP) evaluation**

Diabetes is associated with dysregulated angiogenesis and vascular remodeling, which may lead to renal and vascular disease. MMP's are a family of endopeptidases that degrade extracellular matrix (ECM) components and are essential for vascular remodeling. Together with our collaborator Dr. Karen Moulton, we demonstrated that activities of MMP-9, MMP-2, and the complex of NGAL/MMP-9 correlated with accelerated atherosclerosis, plaque neovascularization and nephropathy in rodent models of T1D complications prior to albuminuria being detectable[168, 169]. Urinary activities were also increased and more prevalent in our T1D youth vs. controls<sup>143</sup>. We are also evaluating urinary MMP activities in an RC4 Challenge grant of T2D adolescents in the TODAY study, to determine whether these vascular biomarkers correlate with IR at baseline and after treatment with metformin alone or together with lifestyle or rosiglitazone. These results will provide comparisons with T1D subjects of similar age range as the proposed study. *Thus, urinary MMP activities appear to be a sensitive, non-invasive, early biomarker for predicting atherosclerosis, vascular remodeling, and nephropathy in T1D[168]. We also propose to evaluate a relation between IR and atherogenic lipid profiles and MMP activities we previously report.*

#### **10. Preliminary data on relationship between IR, exercise and cardiac dysfunction in T1D**

In common with T2D youth[131], we found that T1D youth have significantly reduced VO<sub>2peak</sub> and left ventricular hypertrophy [119, 120]. Unlike T2D youth, T1D youth already had resting echocardiographic evidence of diastolic dysfunction, confirming recent findings of Suys et al[104]. We also found that mitral valve E:E'lateral, a measure of diastolic dysfunction, correlated inversely with IR in T1D youth. Based on these concerning initial findings, we next studied left ventricular mechanics in T1D youth using speckle tracking. Speckle tracking is a novel echo technique where the location of echo patterns is tracked from frame to frame, to obtain global circumferential strain (CS) and longitudinal strain (LS). We performed tissue tracking on 54 sedentary adolescents (mean age 15.8±2.3 years) (20 controls, 34 T1D, mean T1D A1c 8.4±1.5%). T1D adolescents had significantly lower CS, fractional shortening and higher LVID than controls[170]. Among T1D adolescents, there was a correlation between CS and HbA1c (R=0.35, p=0.04)[170]. The poor diabetes control typical of adolescence may contribute to the unexpected severity of myocardial dysfunction, and deserves further research. Echo parameters were otherwise unrelated to HbA1c or T1D duration. This evidence of significant left ventricular dysfunction in T1D youth suggests that damage to midwall fibers mediate the myocardial dysfunction, a concerning pattern typically seen in adults with more severe disease and higher incidence of overt diabetic cardiomyopathy. *These data show our ability to detect early significant disease in youth, not easily seen with traditional 2D echo and pulse wave tissue Doppler.*

In summary, we demonstrate that T1D youth have significant muscle, hepatic and adipose IR, not explainable by typical risk factors, but likely to contribute to CVD. Improving IR may help decrease CVD in T1D.

### **IV. Research Methods**

Insulin resistance is known to be associated with risk for CVD among patients with obesity, metabolic syndrome and T2D. However, more recently, there has been increasing interest in the role of IR in CVD risk in patients with

T1D, particularly as the population of children with T1D becomes more obese. However, research in this area has been limited by technical problems. Surrogate estimates of insulin sensitivity used commonly for studies in adults with metabolic syndrome and T2D are not useable in patients with T1D, as they assume intact insulin and c-peptide secretion. These estimates also do not allow differentiation of site of insulin resistance (muscle vs. liver vs. adipose). Therefore, multi-stage hyperinsulinemic euglycemic clamps are required to assess tissue-specific insulin sensitivity in youth with type 1 diabetes.

### **1. Outcome Measure(s)**

#### **a) Primary Outcome Measures**

- Graded hyperinsulinemic euglycemic clamp with glucose and glycerol isotopes to assess metformin's impact on muscle IR via glucose infusion rate (GIR).
- Mitochondrial function (ADP  $t_{1/2}$ ) by muscle exercise  $^{31}\text{P}$  MRS
- Peripheral arterial stiffness (BrachD) by Dynapulse
- Circumferential strain by echocardiography

#### **b) Secondary Outcome Measures**

- Graded hyperinsulinemic euglycemic clamp with glucose and glycerol isotopes to assess metformin's impact on hepatic and adipose IR, HbA1c.
- Central arterial stiffness by carotid ultrasound
- Endothelial function by brachial ultrasound and endo-PAT (FMD)
- Aortic shear stress by MRI
- Resting and exercise-stressed cardiac structure and function
- Urinary vascular markers (matrix metalloproteins, MMP's)
- $\text{VO}_2$  peak from maximal exercise bicycle test

### **2. Enrollment**

The recruitment goal is to screen 100 T1D subjects to obtain 60 completers. This number was determined on the basis of power calculations and the number of available subjects likely to participate.

### **3. Eligibility**

#### **a) Inclusion Criteria**

1. Adolescents 12-21 years of age with type 1 diabetes (defined as having positive GAD, ICA and/or IA2A antibodies as well as insulin requirement)
2. Willing to consent for participation in study
3. Both normal weight (BMI 5-85%) and overweight/obese T1D subjects (BMI > 85%) will be enrolled to allow sub-analysis of the effects of BMI on IR, CVD and response to metformin in T1D.

#### **b) Exclusion Criteria**

1. Current use of medications known to affect insulin sensitivity: oral glucocorticoids within 10 days, atypical antipsychotics, immunosuppressant agents, metformin or TZD
2. Currently pregnant or breastfeeding women
3. Use of a thiazolidinedione within 12 weeks
4. Severe illness or DKA within 60 days
5. Macroalbuminuria
6. Hemoglobin A1C > 12%
7. Weight > 136.4 kg or < 26 kg, BMI < 5%
8. Creatinine > 1.2
9. Hemoglobin < 9
10. Major psychiatric or developmental disorder limiting informed consent
11. Implanted metal devices
12. Inability to tolerate  $\geq 500\text{mg}$  BID of metformin

#### 4. Study Visits

**Table 2: Study Timeline**

	V 1 Screen	BASELINE ASSESSMENTS				TREATMENT ASSESSMENTS			
		V2 MRS 1	V3 CV1	V4 Clamp 1		V5 PC	V6 MRS 2	V7 CV 2	V8* Clamp 2
Serum Cr, AST, ALT History & Physical	X					X			
Hemoglobin	X					X			
Accelerometer	X								
Height, Weight	X	X	X	X		X	X	X	X
Waist Circumference	X			X		X			X
BP	X		X	X		X		X	X
HbA1c, Insulin Dose	X			X		X			X
Pregnancy Test				X					X
DEXA Scan				X					X
Insulin Clamp				X					X
Urine MMPs				X					X
Brachial and Carotid Artery Ultrasounds			X					X	
Dynapulse/ endoPAT			X					X	
Rest/Exercise Echocardiogram, VO <sub>2</sub> max			X					X	
Muscle MRS		X					X		
Aortic MRI		X					X		
Randomization				X					
Medication Dispense				X		X			
Pill Counts						X			X

After an initial screening visit (Table 2 above, Visit 1), 100 T1D adolescents will be asked to come in for initial measurements to obtain baseline measurements. . All participants will undergo the following assessments prior to randomization: a, muscle/aortic MRS assessment (Visit 2, MRS 1) and a cardiovascular and VO<sub>2</sub>peak assessment (Visit 3, CV 1 ) and hyperinsulinemic euglycemic clamp and Dexa Scan (Visit 4, Clamp 1). After completion of the baseline assessment visits, subjects will then be randomized to 3 months of double-blinded metformin or placebo,. They will have an interim brief visit (Visit 5) to assess blood sugars, insulin dose, compliance and side effects. A phone call will also be done twice in between the baseline and pill count visit and between the pill count and final outcomes visits. At completion of the treatment phase, all participants will undergo repeat outcomes assessments (identical to baseline assessments): a muscle/aortic MRS assessment (Visit 6, MRS 2), a cardiovascular and VO<sub>2</sub>peak assessment (Visit 7, CV 2 ), and a hyperinsulinemic euglycemic clamp and Dexa Scan (Visit 8, Clamp 2). The treated and untreated Assessments will then be compared to determine the impact of metformin vs. placebo on the outcomes.

**Intervention:** T1D subjects will be randomized by our CTRC pharmacist in a double-blinded fashion at the end of Visit 4 to either active metformin or identical-appearing placebo. Randomization is stratified by: 1) HbA1c above or below 8.5% (mean HbA1c in our preliminary studies) 2) BMI above or below the 85<sup>th</sup> percentile. The metformin and placebo randomization and dispensing will be done as in our ongoing obesity study (PI Kelsey, COMIRB# 07-0988).

Each participant is provided with a pill dispenser, a calendar to record doses, a study glucose meter and strips, and a log sheet to record glucose levels and insulin dose changes. Participants are asked to check their blood sugar ≥4 times a day, check urine ketones if blood sugar is >than 300mg/dl for more than two readings, and contact the study staff if they have large urine ketones, 2 or more blood sugars <65 mg/dl in any week or any hypoglycemia requiring assistance, any of which will trigger blood sugar adjustment. Subjects will otherwise adjust their insulin as routinely recommended at our center, and are asked not to make changes to the type or

delivery mode (shots vs. pump) of insulin they are on during the study, and not to make any significant diet or activity changes during the intervention period. Metformin will be titrated to a goal final dose of 1000 mg BID, over a period of 4 weeks. The minimum tolerated dose allowed will be 500 mg BID.

Participants are telephoned during this period to assist with dose titration and monitor possible side effects. Patients are also seen once for pill counts to monitor adherence, medication side effects, height, weight, WC, HbA1c and BP. Participants are given a point each time they bring their pill bottle and their log sheet to visits, whether or not they have been adherent, and will be eligible for an extra gift card for every 2 points obtained to encourage compliance. Random “surprise” incentives such as sports tickets and raffles will also occur to maintain contact with the study team as well as text-message reminders, popular with teens.

**Subject Preparation:** Starred visits\*(Table 2) will be performed in AM fasting, in early follicular phase for menstruating females where possible, preceded by 3 days of no strenuous physical activity and the insulin clamp visit will be preceded by a fixed macronutrient (55% carbohydrate, 30% fat, 15% protein, no caffeine, no regular soda or candy), weight-maintenance meal provided and labeled for carbohydrate counting by the CCTSI metabolic kitchen (as in our previous studies[119, 131]), as diet, activity[171] and circadian rhythms affect IR and CV function(119, 113). MRS visits will also be fasting and preceded by limited physical activity. Subjects will communicate with study personnel to adjust insulin doses to gradually achieve a goal arrival glucose of 90-150 mg/dl on study days, as in our previous studies [119, 131].

### **Visit 1 (Screen Visit)**

Pediatric CTRC Outpatient unit or Endocrinology clinic: Participants will begin with a screening and randomization visit. During this visit, patients will review and complete consent documents, have demographics and medical history confirmed, assess allergies and inclusion/exclusion criteria, have blood samples drawn, and have anthropometrics completed. HbA1c, Cr, ALT, AST and Hb samples will be drawn at the beginning of the visit after consent. At the completion of the screening visit, the subject will be given an accelerometer to be worn for the following seven days to measure level of habitual physical activity.

In addition, subjects will be educated on checking blood glucose frequently (minimum of 4 times a day), and on parameters for contacting study staff.

If subjects meet entry criteria, they will be invited to come in for the baseline assessments as soon as feasible.

### **Visit 2 (MRI Visit)**

UCD Research MRI (Brain Imaging Center) or CHC Radiology: Subjects will be asked to fast for 4-6 hours prior to this visit. An MRI will be performed of the aorta to assess resting wall shear stress. An MRI will also be performed of the calf muscle to measure calf muscle metabolites at rest and during contraction. The subject will press his or her foot against a pedal for one or two 90 second periods, separated by a resting period if pressing twice, with calf muscle imaging before, during and after the calf exercise. Oxygen may also be given through the nose.

### **Visit 3 (Exercise Visit)**

UCD Exercise Lab (12<sup>th</sup> floor inpatient University Hospital): Subjects will be asked not to have caffeine or exercise strenuously for 3 days prior to this visit. They will be asked to continue to check their blood sugars at least 4 times a day (as we normally recommend) during this time. Study personnel will call the subject to discuss the numbers, and adjust insulin if needed. Subjects will be studied in the AM fasting. Subject will be questioned regarding compliance, adverse events, changes in concomitant medications and medical history, height, weight, BP, and blood sugars.

Patients will undergo brachial artery ultrasound with 5 minute blood pressure cuff occlusion and endoPAT to measure endothelial function. They will also undergo carotid artery ultrasound and Dynapulse in order to measure arterial stiffness. They will then have a resting ECG and echocardiogram performed by an echocardiographer. Following resting measurements, subjects will complete a VO<sub>2</sub>max exercise test on a stationary bicycle. During and immediately following the completion of an exercise test, they will undergo another brief echocardiogram.

### **Visit 4 (Baseline Insulin Clamp Visit)**

Pediatric CTRC Inpatient unit: Subjects will be asked not to have caffeine or exercise for 3 days prior to this visit and will be provided with a study diet dinner. They will be asked to continue to check their blood sugars at least 4 times a day (as we normally recommend) during this time. Study personnel will call the subject to discuss the numbers and adjust insulin if needed. Patients will be admitted to the Pediatric CTRC for a monitored overnight fast and stabilization of blood glucose by overnight intravenous infusion of insulin and glucose. Subject will be questioned regarding compliance, adverse events, changes in concomitant medications and medical history,

height, weight, BP, and blood sugars. A DEXA scan will be performed to assess body composition. A urine pregnancy test will be done on all female subjects prior to the DEXA scan. If a female subject is confirmed to be pregnant, she will be withdrawn from the study and referred to her primary diabetes physician for follow-up.

The following morning, a HbA1c and clamp studies will be completed. Using a metabolic cart and hood, **resting** VO<sub>2</sub> (ml/kg/min) and VCO<sub>2</sub> (ml/kg/min) measurements will be collected the morning of the clamp prior to the start of the insulin clamp, as well as, during the last 30 min of each stage of the clamp. This will help distinguish between rates of oxidative and non-oxidative glucose disposal. Metformin or placebo will be started at dinner following the insulin clamp.

Following the clamp procedure, the participant will be randomized by the pediatric CTRC pharmacist in a double blinded manner, such that the investigators and subjects are blinded. 6 weeks of metformin or identical appearing placebo will be dispensed by the pediatric CTRC pharmacist. The metformin and placebo will be obtained by the pediatric CTRC pharmacist from Belmar Pharmacy, a local compounding pharmacy, as in our currently approved CTRC study (Megan Kelsey PI, COMIRB# 07-0988). Metformin or placebo will be titrated as follows: 500 mg po qd with dinner x 1 week, increase to 500 mg po BID with breakfast/dinner x 1 week, increase to 500 mg po with breakfast/1000 mg with dinner x 1 week, and then final dose to remain at 1000 mg po with breakfast/1000 mg po with dinner. If subjects cannot tolerate the metformin due to GI side effects, the dose will be lowered to the lowest tolerated dose for another week, and a second attempt at increasing the dose will be made. The goal will be 1000 mg po BID, but the minimum dose required will be 500 mg po BID. If this minimum dose cannot be tolerated, the subject will be withdrawn.

#### **Phone Interview 1**

Subject will be contacted via phone 2 weeks after Visit 4 (+/- 3 days) to assess compliance, adverse events, changes in concomitant medications and medical history, blood sugars. The subjects will be instructed to call sooner if GI side effects are significant, or blood sugars are problematic.

#### **Visit 5 (Pill Count Verification)**

Subject will come back in 45 days (+/- 5 days) from Visit 4 with all opened and unopened pill bottles to assess compliance, GI side effects, adverse events, changes in concomitant medications and medical history, height, weight, BP, WC, HC, HbA1c, AST, ALT, serum Cr, Hb and blood sugars. An additional 8 weeks of metformin or placebo will be dispensed. The final assessment visits, if not already scheduled, will be finalized. The subject will be given an accelerometer to be worn again for the following seven days to measure level of habitual physical activity.

#### **Phone Interview 2**

Subject will be contacted via phone 3 weeks after Visit 5 (+/- 3 days) to assess compliance, GI side effects, adverse events, changes in concomitant medications and medical history, blood sugars. Reminders will be given about the upcoming assessment visits and to return the accelerometer if it has not yet been returned. Subjects will remain on metformin or placebo until Visit 8 has been completed.

#### **Visit 6 (Repeat MRI Visit)**

This visit will follow the same procedures as the Baseline MRI Visit (Visit 2). Please refer to the description above for procedure details, except that study medication will be withheld the AM of fasting.

#### **Visit 7 (Repeat Exercise Visit)**

This visit will follow the same procedures as the Baseline Exercise Visit (Visit 3). Please refer to the description above for procedure details, except that study medication will be withheld the AM of fasting.

**Visit 8 (Repeat Clamp Visit)** This visit will follow the same procedures as the Baseline Clamp Visit (Visit 4). Please refer to the description above for procedure details, except that study medication will be withheld the AM of fasting. Study medication will be discontinued after this visit. The subject will return all opened and unopened pill bottles during this visit. During this visit, subjects will also be given recommendations and education on increasing their amount of exercise due to their identification of being sedentary.

#### **Phone Interview 3**

Subject will be contacted via phone 1 week after Visit 8 (+/- 3 days) to assess adverse events, changes in concomitant medications and medical history, blood sugars.

## 5. Study Laboratory Assays, Exams and Procedures

### 3DPar and Accelerometer

A questionnaire (3DPAR) recalling the physical activity levels of the three previous days will be completed. An accelerometer will then be worn for 7 days following the screening to more accurately estimate each subject's habitual physical activity levels, which may affect insulin sensitivity. The accelerometer will be repeated after visit 6, to assess for changes in physical activity that could confound the study results.

### Screening Blood Draw

Blood will be drawn for Hemoglobin to rule out anemia, HbA1c for determination of chronic glycemia, and serum creatinine and ALT and AST for safety purposes.

### Fasting Blood Draw

Just prior to starting the insulin clamp, blood will be drawn for HbA1c, C-peptide, lipid panel, glucose, glycerol, background enrichment of 6,6-<sup>2</sup>H<sub>2</sub> glucose and <sup>2</sup>H<sub>5</sub>glycerol, insulin, free fatty acids, c-reactive protein, Creatinine, , AST, ALT, DHEAS, Testosterone, Estradiol, Progesterone, sex hormone binding globulin, high molecular weight adiponectin, lipid subfractions (via FPLC in laboratory of Robert Eckel), NT-pro-BNP and apo-B and apo-C3, I-CAM, V-CAM and T-BARs (run in the laboratory of Jane Reusch).

### Body Composition and body fat distribution

Height, weight, waist circumference, and hip circumference will be measured. Body fat distribution will be determined using the waist-to-hip ratio where the waist circumference is measured 1/2 the distance from the xiphoid process to the navel and the hip circumference is measured at the level of the greater trochanter.

Body composition will be measured using the DEXA technique on the pediatric GCRC 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. During the procedure, the subject will be supine on the measurement table, and the arm of the machine will slowly pass over their body.

### Standard Meal

A standard meal will be provided from the CTRC for dinner prior to the hyperinsulinemic euglycemic clamp. The diet will be composed of 55% carbohydrates, 30% fat, and 15% protein and will have no caffeine, no regular soda or syrup, no candy and will be labeled for carbohydrate counting by the CCTSI metabolic kitchen (as in our previous studies) [119, 131]), as diet, activity [171] and circadian rhythms affect IR [119, 131]. The dinner on the evening preceding the insulin clamp will be stored on the inpatient CTRC and eaten on the inpatient floor. Kilocalories will be calculated by Schofield equation: Males:  $(16.25 (\text{weight in kg}) + 137.2 (\text{height in m}) + 515.5) \times \text{Activity Factor}$ ; Females:  $(8.365 (\text{weight in kg}) + 465 (\text{height in m}) + 200) \times \text{Activity Factor}$ . This diet composition is the standard used for the past 10 years by the UCD Center for Human Nutrition prior to insulin clamps as variations in dietary intake affect insulin sensitivity.

### Magnetic Resonance Imaging (MRI)

The MRI will be obtained during visits 2 and 6, at the UCD brain imaging center on the Fitzsimmons campus or at the CHC Radiology Department. Dr. Mark Brown, of UCD radiology, will perform an aorta and calf MRI on a 3.0 T whole-body MRI scanner (GE Medical Systems, Waukesha, WI). Subjects will lie supine while these measurements are obtained, need to hold reasonably still during the scan and cannot weigh >300 lbs.

**Aortic Wall Shear Stress:** This portion will take approximately 30 minutes. The purpose of the cardiac MRI is to obtain phase contrast imaging through several levels of the aorta, including the ascending aorta, the transverse arch, and the descending aorta. MRI images will be taken supine of the chest 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.

**Mitochondrial Phosphorylation Rate:** This portion will take approximately 30 minutes. Assessed by <sup>31</sup>P magnetic resonance spectroscopy saturation transfer performed at 36.31 MHz with the use of a flat, concentric probe made of an inner coil 9 cm in diameter (for <sup>31</sup>P) and a 13-cm outer coil tuned to proton frequency for scout imaging and shimming as previously described [73]. Unidirectional rates of ATP synthesis will be measured with the use of the saturation-transfer method applied to the exchange between inorganic phosphate and ATP. The steady-state magnetization of inorganic phosphate is measured in the presence of a selective irradiation of the <sup>31</sup>P resonance of ATP and compared with the magnetization of inorganic phosphate at equilibrium in a control spectrum (without irradiation of the <sup>31</sup>P resonance of ATP) [73]. The ratio of inorganic phosphate to phosphocreatine in the soleus muscle is also measured by <sup>31</sup>P magnetic resonance spectroscopy as previously described [71, 72]. Subjects will



perform one or two brief (90 second) muscle contractions against resistance separated by rest while the  $^{31}\text{P}$  MRS is performed. Oxygen may also be given through the nose.

### **Euglycemic Insulin Clamp**

Hyperinsulinemic euglycemic clamps are the gold standard measure of insulin sensitivity in youth[172]. Surrogate measures of insulin resistance cannot be used in subjects with diabetes. This is because surrogate markers are based on measuring insulin levels in the blood. In all subjects with T1D, exogenous insulin is injected rather than being made by the pancreas. Therefore, a blood insulin level in these groups reflects the amount of insulin injected, not insulin sensitivity. Thus, performing a hyperinsulinemic clamp is necessary to accurately measure insulin resistance in youth, a central question being investigated in this protocol. Subjects will be admitted to the Pediatric CTRC the evening prior to the clamp study. On the evening of admission, participants will have a finger stick blood sample drawn to measure blood glucose prior to dinner, and urine will be collected to measure ketonuria and, for all female subjects, urine pregnancy testing will be done. Participants will eat the remainder of their 3-day study diet (55% carbohydrates, 30% fat, and 15% protein), for dinner, and will then be fasted overnight except for water or non-caloric beverages without caffeine. Subjects will give their normal short acting insulin (injection or pump bolus) with dinner, but no long-acting insulin will be given.

### **Overnight glucose control**

Two antecubital IV catheters will then be placed, and 2 hours after dinner (approximately 8:00PM), subjects with an insulin pump will have the pump disconnected, and will then begin an overnight insulin infusion to normalize glucose levels. A heating pad may be used to help with ease of IV placement. Blood glucose will be monitored frequently overnight in to assure that it remains in a safe range. Glucose will be allowed to decrease until euglycemia (90-100 mg/dl) is achieved, which is then maintained with an adjustable infusion of 5% dextrose as needed [173], and blood glucose tested every thirty to sixty minutes from 8:00 pm to 8:00 AM. Glucose will be checked more frequently if glucose is out of the target range, with the following schedule (Table 3 below):

AM study medications, and if possible any other home AM medications will be withheld. Glucoses are checked more frequently if they are below 90 mg/dL

### **Fasting Morning Insulin Clamp**

3-stage hyperinsulinemic euglycemic clamp (8, 16, and 80mU/m<sup>2</sup>/min insulin)

Adipose insulin sensitivity: 1st stage (10mU/m<sup>2</sup>/min insulin), 60 minutes. Whole-body lipolysis will be measured under basal conditions and during each stage of the clamp, using a primed (1.6micromol/kg), constant (0.11 micromol/kg/min) infusion of  $^2\text{H}_5$ glycerol[174]. Blood samples will be collected at time 0 (fasting), and at 45, 50, 55 and 60 minutes of the basal period and each insulin stage for determination of insulin, FFA, and glycerol (concentration and isotope enrichment)[175] to determine glycerol rate of appearance[176] and FFA levels at each stage of the clamp.

Hepatic insulin sensitivity: 2nd stage (16mU/m<sup>2</sup>/min insulin), 90 minutes. Hepatic insulin sensitivity will be measured under basal conditions and during each stage of the clamp, using a primed (3mg/kg), constant (0.04mg/kg/min) infusion of 6,6- $^2\text{H}_2$ glucose[116]. Blood samples will be collected at the times specified in section A, for determination of glucose (concentration and isotope enrichment) to determine baseline hepatic glucose output and % suppression by insulin.

Muscle insulin sensitivity: 3<sup>rd</sup> stage (80mU/m<sup>2</sup>/min insulin)[177] 120 minutes. Muscle insulin sensitivity will be determined using glucose infusion rate (GIR) measured with 6,6- $^2\text{H}_2$ glucose, normalized to fat free mass measured by DEXA[120, 178].

Using a metabolic cart and hood placed over the subject's head, resting  $\text{VO}_2$  (ml/kg/min) and  $\text{VCO}_2$  (ml/kg/min) measurements will be collected the prior to the start of the insulin clamp, as well as, during the last 30 min of each stage of the clamp. This will help distinguish between rates of oxidative and non-oxidative glucose disposal. Subjects will rest quietly during this period and breathe normally.

Each time blood is drawn during the clamp, blood will be drawn to a clear line in the syringe to ensure there will be no IV fluid dilution, and will be re-infused into the subjects after obtaining the blood sample to minimize blood loss. The rate of glucose infusion during the 0-270 min insulin infusion period is adjusted based on blood specimens drawn every 5 minutes. Glucose will be measured after centrifugation at the bedside using a Stat Strip glucometer (nova Biomedical) based on the glucose oxidase technique.

Baseline blood samples will be obtained. A primed (4.5 mg/kg), constant (0.03 mg/kg/min) infusion of 6,6-<sup>2</sup>H<sub>2</sub> glucose (Isotec, Miamisville, IA) paired with a primed (1.6 micromol/kg), constant (0.11 micromol/kg/min) infusion of <sup>2</sup>H<sub>5</sub>glycerol[174] (Isotec, Miamisville, IA) will then begin and continue throughout the study. Subjects will rest quietly for 2 hours before the insulin clamp begins to measure resting glucose and glycerol turnover. Blood samples will be drawn at 90, 100, 110, 120 minutes of rest to measure resting glucose and glycerol concentrations as well as stable isotope enrichments.

From 08:00AM- 09:00AM, insulin is then infused constantly at 8 mu/m<sup>2</sup>/min; from 09:00AM-10:30AM, 16 mu/m<sup>2</sup>/min; and from 10:30AM-12:30PM 80 mu/m<sup>2</sup>/min. Since insulin can lower serum potassium concentration, a single oral dose of potassium chloride (20 meq) will be given after the insulin infusion. 20% dextrose (spiked with 6,6-<sup>2</sup>H<sub>2</sub> glucose to maintain stable enrichment of plasma glucose) is infused concurrently to maintain blood glucose at approximately 95mg/dl[177, 179]. The rate of glucose infusion during the 0-270 min insulin infusion period is adjusted based on blood specimens drawn every 5 minutes. Glucose will be measured after centrifugation at the bedside using a glucometer based on the glucose oxidase technique.

Blood draws will be taken at baseline, -30, -20, -10, 0, 45, 50, 55, 60, 120, 130, 140, 150, 240, 250, 260, and 270 minutes of the clamp to measure hormone and substrate concentrations and stable isotope enrichments. -30, -20, -10, 0, 60, 70, 80, 90, 150, 160, 170, 180, 240, 250, 260 and 270 minute samples include glucose, 6,6-<sup>2</sup>H<sub>2</sub> glucose, glycerol, <sup>2</sup>H<sub>5</sub>glycerol. Insulin and FFA are drawn at baseline, -20, -10, 0, 50, 55, 60, 130, 140, 150, 250, 260 and 270 minutes.

The insulin infusion period lasts 270 minutes, during which time the subject will rest in bed in the pediatric CTSC[180]. During the entire 270 minute insulin infusion period, a pediatric CTSC nurse will remain at the bedside, and a Study Physician or Pediatric Nurse Practitioner or Pediatric Physician Assistant will also remain at the bedside to minimize any risks associated with the insulin clamp. The IV site will be continuously monitored to minimize risk of IV infiltration.

Sterile and pyrogen-free <sup>2</sup>H<sub>5</sub>glycerol and 6,6-<sup>2</sup>H<sub>2</sub> glucose will be obtained in powder form from the manufacturer. The CTSC pharmacist will reconstitute the isotopes with sterile technique, filter (0.22 micron) for additional sterilization, aliquot, and freeze at -20 C in the CTSC investigational pharmacy freezer (range -10 to -20c) until use. One aliquot is then sent to the CTSC laboratory for confirmatory quantitative pyrogenicity testing while the second aliquot is sent to Children's Hospital Colorado Clinical laboratory where anaerobic and aerobic culture are performed to confirm sterility, per Children's Hospital Colorado's standard operating procedures. Only after the aliquots are determined to be acceptable by all of the above processes will the labeled glucose solution be released by the CTSC pharmacy for use. Once reconstituted, the labeled glucose and glycerol solutions can be stored in the -20 freezer for up to 6 months, after which time it will be discarded. The labeled glucose and glycerol solutions will remain in the CTSC investigational pharmacy freezer until use. The CTSC investigational pharmacy freezer has secure storage with limited access by CTSC investigational pharmacy personnel only, and is routinely monitored to ensure appropriate temperature control and compliance with expiration dates. The glycerol and glucose solutions will only be used for the current protocol as described in this protocol.

The analysis of <sup>2</sup>H<sub>5</sub>glycerol and 6,6-<sup>2</sup>H<sub>2</sub> will be performed by Mass Spectrometry Core Laboratory using a modification of the negative ion chemical ionization gas chromatography mass spectrometry as previously described [175].

The glycerol rate of appearance (GlycRa) over the last 30 minutes of each stage of the insulin clamp described above will be calculated using the non-steady-state equation of Steele[181] :

$Ra = F - pV((C_2 + C_1)/2)((E_2 - E_1)(t_2 - t_1))/((E_2 + E_1)/2)$  where F is the rate of infusion for <sup>2</sup>H<sub>5</sub>glycerol (0.10 μmol/kg/min), pV is the volume of distribution (0.027 L/kg), C is the plasma glycerol concentration (μmol), E is the plasma isotope enrichment, and t is time (min).

Peripheral glucose disposal will be measured by the glucose infusion rate (GIR) at steady state. The less glucose infused, the greater the insulin resistance, expressed by the value "M", or glucose infusion rate in mg/(kg•min) [177], and expressed corrected for fat-free mass as determined by DEXA (mg/kg fat-free mass/min).

Muscle insulin sensitivity will be determined using glucose rate of disappearance (Rd) measured with 6,6-<sup>2</sup>H<sub>2</sub>glucose, normalized to fat free mass measured by DEXA[120, 178]. Total body GDR will be determined by adding the rate of residual endogenous glucose production to the rate of glucose disappearance. The metabolic clearance rate (MCR) will be calculated to normalize the glucose turnover to the plasma glucose concentration. The rate of appearance (Ra), rate of disappearance (Rd), and metabolic clearance rate (MCR) of isotopically

labeled glucose during the resting period before the clamp will be calculated using equations defined by Steele[176, 181], modified for stable isotopes:

$$R_a (mg \cdot kg^{-1} \cdot min^{-1}) = (F - V((C_1+C_2)/2)((IE_2-IE_1)/(t_2-t_1))) / ((IE_2+IE_1)/2)$$

$$R_d (mg \cdot kg^{-1} \cdot min^{-1}) = R_a - V((C_2-C_1)/(t_2-t_1))$$

$$MCR (ml \cdot kg^{-1} \cdot min^{-1}) = R_d / ((C_1+C_2)/2)$$

F represents isotope infusion rate, IE<sub>1</sub> and IE<sub>2</sub> are isotopic enrichments at sampling time points 1 (t<sub>1</sub>) and 2 (t<sub>2</sub>), respectively. C<sub>1</sub> and C<sub>2</sub> are metabolite concentrations at t<sub>1</sub> and t<sub>2</sub>; V is the estimated volume distribution of glucose (180 ml/kg). All isotopic enrichments will be corrected for background enrichments from blood samples taken before isotope infusion. To account for the “spiked” glucose in the D20 infusion during the clamp, the following equations will be used as defined by Finegood[182]:

$$R_d = [ \frac{F}{(E_2+E_1)/2} - VC_1 \frac{(E_2-E_1)/(t_2-t_1)}{(E_2+E_1)/2} ] - V \frac{(C_2-C_1)}{(E_2+E_1)/2} (t_2-t_1)$$

$$R_a = [ \frac{F}{(E_2+E_1)/2} - VC_1 \frac{(E_2-E_1)/(t_2-t_1)}{(E_2+E_1)/2} + (185) ] - Ginf(t_1) \frac{(E_2+E_1)/2}{(E_2+E_1)/2}$$

$$MCR = R_d / [(C_2+C_1)/2]$$

F = infusion rate of tracer(mg/min), V= estimated volume of distribution of glucose(180 ml/kg), Eg = enrichment of the glucose infusate, Ginf(t<sub>1</sub>) = rate of infusion of exogenous glucose at time t<sub>1</sub>, t<sub>1</sub> = time 1 of sampling, t<sub>2</sub>= time 2 of sampling, C<sub>1</sub> = (tracee) at t<sub>1</sub>, C<sub>2</sub> = (tracee) at t<sub>2</sub>, E<sub>1</sub>= plasma enrichment at t<sub>1</sub>, E<sub>2</sub>= enrichment at t<sub>2</sub>. Ra and Rd are expressed as mg/kg/min. MCR is ml/min.

## FPLC

Lipid subfraction analysis by fast protein liquid chromatography (FPLC) will be done in the UCD laboratory of Dr. Robert Eckel, MD who is experienced in these techniques[16]. Chylomicrons and VLDL are collected close to the void volume of the columns, LDL and HDL elute in separate, well-defined peaks 10-20 ml later, and serum albumin and other non-lipoprotein components elute thereafter. Individual lipoprotein peaks are detected by UV absorbance and collected in 0.5 ml fractions for further analysis of lipid and apolipoprotein composition[16]. In addition, the VLDL, IDL and LDL fractions will be pooled for measurement of TG, cholesterol and apo B in each pool (in addition to in whole plasma TG, cholesterol and apo B), to help interpret baseline and insulin effects on particle number vs. particle composition. Apo-C3 will be collected at baseline; then, frozen and run in the laboratory of Robert Eckel along with the post-clamp lipids.

Upon subject consent, an additional 16 cc of blood will be drawn at baseline and the conclusion of the clamp and saved for potential future research for diabetes-related complications and/or heart disease. Blood will be stored for 10 years past the study's funding, and will be destroyed if requested by the subject in writing.

After the insulin clamp is completed, all infusions will be stopped except for an infusion of dextrose to prevent hypoglycemia until the subject has finished consuming their post-study meal. Subjects will remain on the CTRC for at least 1 hour after consuming their post-study meal with a final glucose concentration check on a portable glucose meter via a blood draw from a catheter. If glucose concentration is 80 mg/dl or greater, the catheter will be removed and the subject allowed to leave with a snack. If glucose concentration is less than 80 mg/dl the subjects will be encouraged to consume 45 grams of carbohydrate, and glucose will be rechecked within 30 minutes until glucose concentration of >80 mg/dl is achieved. The IV will not be removed until stable glucose levels are achieved, to assure safety. Subjects with an insulin pump will then have their insulin pump reconnected. Subjects on long acting insulin will be given an adjusted dose, depending on the normal administration time. All subjects will then be discharged from the hospital with a snack, and to resume their normal home routine. Metformin or placebo will be started with dinner following the first clamp.

Participants will be called by phone to check on how they are feeling the day following the metabolic study. Subjects will be asked to check their blood sugar at least 4 times a day for the 24 hours following the clamp, to make sure medication adjustments do not affect their blood sugars markedly. Participants will be reminded to call the study coordinator or principal investigator if they experience any problems or have any questions or concerns regarding their participation in the study.

### **Accelerometer**

Each subject will wear an accelerometer at baseline and prior to final assessments (MTI Actigraph by Actigraph) to measure habitual level of physical activity, which affects insulin sensitivity. Accelerometers are effective tools for the objective measurement of physical activity [183] because they have the ability to continuously record physical activity data and such data can be used to estimate METs of activity. They provide more detailed information than pedometers, which only measure walking steps, and help get around the recall bias of questionnaires. We are currently using the MTI Actigraph in adolescents in our other diabetes studies; therefore, we are familiar with their use in this population and have the necessary computer software and interpretation skills. Data collected from the accelerometers will be analyzed prior to the insulin clamp to verify that the subjects are sedentary.

### **Urine Metallomatrix Proteins**

At each clamp visit, 5 mls of urine will be frozen from each subject at -20C and used to assay protein levels and activity of the MMPs 2, 9, and 13.

### **Measurements made during bicycle ergometer testing**

During all bicycle tests,  $\text{VO}_2$  (ml/kg/min) and  $\text{VCO}_2$  (ml/kg/min) will be measured, breath-by-breath, at rest and during exercise. Arm blood pressure (by auscultation) and heart rate (by 12-lead ECG) will be obtained every minute during exercise. Cardiac status will be monitored throughout each test by 12-lead ECG. The respiratory exchange ratio (RER) will be calculated as  $\text{VCO}_2$  (ml/kg/min)/ $\text{VO}_2$  (ml/kg/min). A snack will be provided to subjects following completion of the  $\text{VO}_2$ max test.

### **Cardiac Echocardiographic Measurements**

Standard two dimensional and Doppler echocardiography will be performed by an echocardiographer under the supervision of cardiologist Jennifer Dorosz, MD at the University of Colorado Health Sciences Center Heart Center, using [184] to ascertain the presence of left ventricular systolic dysfunction, regional wall motion abnormalities (suggestive of coronary artery disease), pericardial disease or significant valvular pathology. Chamber sizes, LV end-systolic and diastolic chamber dimensions and wall thickness, fractional shortening and the area-length method for measurement of cardiac volume in order to measure ejection fraction will be quantitated by standard techniques for all individuals.

Diastolic function will be assessed by standard echocardiographic parameters (E and A wave velocities, E:A ratio, E wave deceleration time, pulmonary vein Doppler) as well as tissue Doppler of both the septal and lateral mitral annuli (E' and A' velocities, E':A' ratio, E:E' ratio), flow propagation determined by color M-mode, and longitudinal and circumferential strain with speckle tracking. Dr. Jennifer Dorosz, MD and the echocardiographer, who have expertise in echocardiography and these techniques, will be blinded to the diagnostic status of the participant and will supervise and/or perform acquisition of all of the echocardiographic data as well as perform all of the measurements and interpretation. Echocardiograms will be obtained with a commercially available ultrasound system (VingMed Vivid FiVe, GE, Milwaukee, WI) and stored on magneto-optical discs for offline measurement and interpretation using EchoPac (GE, Milwaukee, WI). Subjects will be examined in the left lateral decubitus position using standard parasternal, short-axis, and apical views. All recordings and measurements will be obtained by the same observer according to the recommendations of the American Society of Echocardiography and will always be performed at the same time of day for each subject to avoid the possible influence of circadian rhythm on left ventricular diastolic function (Voutilinen). All cardiac valves will be examined to rule out significant valvular disease. Left ventricular mass (LVM) will be calculated using the following formula:  $\text{LVM (g)} = 0.8 \times 1.04 [(\text{LVEDD} + \text{IVST} + \text{PWT})^3 - (\text{LVEDD})^3] + 0.6$ , where LVEDD is left ventricle end diastolic internal diameter, IVST is interventricular septal thickness, and PWT is left ventricular posterior wall thickness. Cardiac imaging will be performed fasting in the AM at rest following the 3 day study diet, and also during and immediately following exercise on the bicycle ergometer.

### **Brachial Artery Diameter**

Endothelial function will be assessed at the Heart Center of the University of Colorado Health Sciences Center. The brachial artery method will be utilized following the protocol described by Celermajer et al [185, 186], that measures dilation of the brachial artery by ultrasound. The brachial artery diameter will be measured using B-mode ultrasound images, with the use of a GE VingMed ultrasound system and a 10.0-mHz linear-array transducer. Scans will be obtained with the subject at rest and following reactive hyperemia. The subjects will lie quietly for 10 minutes before the first scan. The brachial artery will be scanned in longitudinal section 2-15 cm above the elbow. Depth and gain settings will be set to optimize images of the interface between the lumen and arterial wall. When a satisfactory transducer position is found, the skin will be marked and the arm stationed in the same position throughout the study. Increased flow will then be induced by inflation of a pneumatic tourniquet

placed around the forearm (proximal to the scanned part of the artery) to a pressure of 200 mmHg for 5 minutes, followed by release. Hyperemic response will be measured one minute after cuff deflation. Thereafter, 10 minutes will be allowed for recovery of the vessel, after which an additional resting scan will be performed. The diameter of the vessel will be measured in every case by two independent observers blinded to the status of the participant, stage of the experiment and stage of the study. Flow-mediated dilatation will be calculated by each observer and recorded. The average of the two observations will be used in analysis. The arterial diameter will be measured at a fixed distance from the anatomical marker (such as a fascial plane or a vein seen in cross section) with the use of ultrasonic calipers. Measurements will be taken from the anterior to the posterior “m” line at end-diastole, coincidentally with the R wave on a continuously recorded electrocardiogram. For the reactive hyperemia scan, measurements of diameter will be taken 60 seconds after deflation of the cuff. A minimum of three cardiac cycles will be analyzed for each scan, and the measurements for each observer recorded. The vessel diameter in scans obtained after reactive hyperemia will be expressed as a percentage of the average diameter of the artery in the two resting (or control) scans (considered as 100 percent).

### **Dynapulse Measurements**

The Dynapulse Pathway system (PulseMetric, Inc., San Diego, CA) is a noninvasive portable system to measure brachial artery distensibility, utilizing a standard sphygmomanometer cuff inflated in the same fashion as a sphygmomanometer to obtain blood pressure. The instrument derives brachial artery distensibility using the technique of pulse waveform analysis of arterial pressure signals obtained from the sphygmomanometer.

### **Endo-PAT Measurements**

RH-PAT testing 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. 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 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.

### **Carotid Intima-Media Thickness (CIMT)**

The ultrasound transducer is placed on one side of the subject's neck, a single image is obtained and frozen, and software is used to automatically detect the intima media thickness. CIMT will be used to assess differences in atherosclerotic burden between lean, obese and diabetic subjects, and to assist in interpreting differences in FMD between the groups.

### **Exercise Prescription**

The final visit will conclude with education regarding the importance of physical activity, diet and lifestyle modification to mediate the risks associated with sedentary lifestyle and an exercise prescription designed to increase physical activity. The exercise information and prescription are the standard of care used in our Children's Hospital Colorado Pediatric Metabolic Syndrome Clinic, designed by the Children's Hospital Colorado Pediatric Exercise Physiologist. This Exercise Physiologist will also be available to the study for consultation as needed. Families will be provided with standard information about follow up care with their primary care provider and contact information for Children's Hospital Colorado Diabetes and Child Health Clinics if needed regarding any abnormal study findings. In addition, the subject/family will be provided with copies of their study lab results, DEXA scan, physical activity monitoring and exercise test. Study staff will also call the family after the conclusion of the study to check-in on the recommended follow up care and answer any questions about test results.

## **V. Description, Risks and Justification of Procedures and Data Collection**

### **A. Study Duration**

#### **1. Planned Duration of the Entire Study**

The anticipated duration for the study is 5 years.

#### **2. Duration of Participation for Each Subject**

Subject participation consists of 9 visits for study:

**Visit 1 (Screen Visit):** This visit will last approximately 3.5 hours, during which time patients will have demographics and medical history and allergies confirmed, blood drawn and a urine sample taken for laboratory measures, and anthropometrics completed, , and be given an accelerometer to wear for the following 7 days, be instructed on medication administration and glucose monitoring, and subsequent visits will be scheduled.

**Visits 2 and 6 (MRI Visit):** Patients will come to the Brain Imaging Center or CHC Radiology for an MRI. Total duration of visit will be approximately 1 hour.

**Visits 3 and 7 (Exercise Visit):** Patients will come to the adult CTIC in the 12<sup>th</sup> floor adult CTIC inpatient area building for a brachial artery ultrasound, carotid ultrasound and Dynapulse measurements, resting echocardiogram, VO<sub>2</sub>max tests and post-exercise echocardiogram as described. Total duration of visit will be approximately 2.5 hours.

**Visits 4 and 8 (Clamp Visit):** Patients will be admitted to the Pediatric CTIC overnight for stabilization of blood glucose as described. The following morning, clamp studies will be completed. Total duration of visit will be approximately 19 hours.

**Visit 5:** Interim visit will allow assessment of compliance, GI side effects, adverse events, changes in concomitant medications and medical history, height, weight, BP, WC, HC, HbA1c, safety labs and blood sugars, and medication dispensed. This visit will last approximately 1 hour.

**Phone Calls:** A minimum of 2 phone calls will be made to discuss blood sugars and any issues with medications. These calls will last approximately 10 minutes. Other calls may be made if subjects experience illness or other problems with blood sugars or medications.

#### 4. Sources of Research Materials

**To be accessed prior to study:** The patient's medical record will be reviewed for diabetes diagnosis, medications, allergies and other diagnoses that may disqualify patient from participation.

Table 4: Data to be collected during study	
Blood and Urine Samples	VO <sub>2</sub> max Exercise Testing
Questionnaires	Echocardiography
DEXA	Endo-PAT
Anthropometric measurements	Dynapulse measurements
MRI	Electrocardiography
Accelerometer output x 7 days	Brachial Artery Flow Mediated Vasodilation
	Carotid Artery Intima-Media Thickness

#### 5. Subject Recruitment Plan

Subjects for this study will be recruited from the Barbara Davis Center for Childhood Diabetes with whom the PI and Co-I's have a treatment relationship and from study advertisements. Protected health information will only be accessible after the subject has signed a HIPPA recruitment authorization form, provided by the primary care provider, and a research authorization form, provided on entry to the study. Protected health information will only be accessible by study investigators. The initial patient contact will be made by personnel who have a treatment relationship with the subject.

#### 6. Informed Consent Plan

Appropriately qualified and informed personnel who have completed the COMIRB and HIPPA course requirements will fully explain the study protocol and consent form to the subject and guardian verbally in the language they understand. The explanation will be conducted in a quiet environment with adequate time given for the subject and guardian to review the study procedure before the commencement of the study. Asking the subject to explain the study in their own words will assess the subject's understanding. If non-English speaking subjects are enrolled in the study, the investigators will adhere to Section 10C of the COMIRB Instructions for Clinical Investigators regarding the consent of these subjects. The qualified personnel mentioned above will then obtain written consent from the guardian and assent from the subject, co-signed on the consent form, or in subjects who are 18 years or older, direct consent. The PI will make a good faith effort to obtain both parent signatures. The subject and guardian will be provided a copy of the consent form for better understanding and record purposes.

## 7. Special Consent/Assent Plan

Consent will be obtained from all participants in the study. Following explanation, all subjects below 18 years old will co-sign the consent form in addition to the parents signing the consent form. All subjects age 18 or older will sign the standard consent form.

## 8. Subject Compensation, Incentives and Rewards

Subjects will be paid \$425 in gift cards upon completion of all procedures in this study (Table 5 below). If the subject withdraws before the start of the baseline insulin clamp, they will still receive a \$25 gift card for completion of the screening visit. If the subject begins the baseline insulin clamp and needs to stop for any reason, the subject will be given \$100 in gift cards and will be given gift cards for all subsequent completed visits as outlined below. These payments are similar to the payments being made for each visit type in our currently ongoing adolescent studies.

Subjects that bring their completed log book and medication bottles to visits will receive an additional \$5.00 gift card. If co-enrolled, visits 4 and 8 will be performed by and paid for by the T1DX study rather than EMERALD study.

**Table 5 Subject payment schedule**

Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Total
25.00	25.00	50.00	100.00	25.00	25.00	50.00	125.00	425.00

## 9. Potential Risks to Subjects

### Blood Samples

The collection of blood samples may result in temporary discomfort, bruising, bleeding, and on rare occasions, infection.

Nationally, the NIH Clinical Center has a guideline of 9ml/kg in 6-8 weeks for pediatric studies. Certain studies at our institution do draws over 7ml/kg in 6 weeks, or up to 7 ml/kg in a single draw, but include iron supplementation. Otherwise, the routine guidelines in our Pediatric CTSC are 3ml/kg for a single draw and no more than 7ml/kg over an 8 week period. Our baseline and interim visits will include 5 ml or less of blood (1 tsp) (HbA1c, Hb, Cr, AST, ALT). The insulin clamp visit now includes 130.8 ml of blood, which includes 10.8 ml to check blood sugar every 5 minutes by glucometer for the 4.5 hour clamp.). Thus, our insulin clamp visit is within the more conservative pediatric CTSC guidelines of 3ml/kg for a single draw for pediatric studies for subjects 26 kg or greater. This is unlikely to exclude many subjects, as we have already excluded pre-pubertal subjects, and subjects with BMI<5% (due to potential undiagnosed illness), those groups likely to be the lightest in weight. Therefore, we will limit recruitment to subjects 26 kg or greater if all studies are performed. In addition, by study design, subjects <5% for weight are excluded as are subjects with anemia, screened by our baseline Hb, further increasing the safety of the study regarding blood draws. The two clamp visits are separated by 3 months, and therefore the significant blood draws are spaced enough apart to also fall within guidelines.

### 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.

**Hyperinsulinemic Euglycemic Clamp:** Hyperinsulinemic euglycemic clamps are the gold standard measure of insulin sensitivity in youth[172]. Surrogate measures of insulin resistance cannot be used in subjects with diabetes. This is because surrogate markers are based on measuring insulin levels in the blood. In all subjects with T1D, exogenous insulin is injected rather than being made by the pancreas. Therefore, a blood insulin level in these groups reflects the amount of insulin injected, not insulin sensitivity. Thus, performing a hyperinsulinemic clamp is necessary to accurately measure insulin resistance in youth, a central question being investigated in this protocol. There is a rare risk of hypoglycemia during the insulin clamp. If blood sugars became extremely low for a prolonged period, this could cause a seizure. To minimize this risk, IV access is obtained prior to insulin administration, and blood sugars will be monitored every 5 minutes throughout the procedure and maintained at 95 mg/dL, to be well above a low level. Two IV's will be in place, in case there is a problem with one IV. If the glucose level is decreasing, glucose will be given to stabilize and return blood sugar levels to normal. IV access will also be left in place until blood glucose values are stable after the study is completed.



During the clamp, there is also a risk of infiltration of the IV solution, which could lead to skin burn or tissue damage. To minimize the risk of any complications with the clamp, during the entire clamp procedure, a pediatric CTRC nurse will remain at the bedside, and a Pediatric Endocrinologist (study Physician) or study Nurse Practitioner or study Physician Assistant will also remain at the bedside. The IV site will be continuously monitored.

Hyperinsulinemic euglycemic clamps with stable isotopes can and have been done safely in diabetic and nondiabetic subjects by the highly experienced investigators in this protocol, in the setting of the highly specialized pediatric CTRC at this institution. We are currently conducting them in adolescents with T1D, T2D obese and lean control youth (PI Nadeau, COMIRB# 06-0665) in the same age group as we now propose. Our current clamp experience at the CTRC by the PI includes approximately 140 clamps overall. Adolescent subjects have tolerated the clamps well and we have had no hypoglycemia and no unanticipated adverse events or serious adverse events to date. In addition, our colleagues at the University of Colorado adult CTRC have performed thousands of hyperinsulinemic euglycemic clamps. Current investigators with adult CTRC clamp protocols include Leigh Perrault, MD (approximately 100 clamps, only adverse event: superficial thrombophlebitis), Terri Hernandez, RN, PhD (approximately 200 clamps, 1 mild superficial phlebitis/mild arm pain), Marc Cornier, MD (approximately 150 clamps, one mild superficial phlebitis), Rachel VanPelt, PhD (approximately 150 clamps, no adverse events), Irene Shauer, MD, PhD 70 clamps (no adverse events), our co-investigator David Maahs, MD (17 clamps, no adverse events and our co-investigator Megan Moriarty (10 clamps, no adverse events). Many of the insulin clamp protocols in the adult CTRC use only an RN at the bedside rather than an endocrinologist or specialized NP/PhD like our protocol and check blood sugars only every 10 minutes rather than every 5 minutes like our protocol. Despite these less stringent requirements, of the approximately 800 clamps performed recently by the investigators above, no significant hypoglycemia or severe adverse events have occurred.

### **Tracer Infusions**

This study involves the use of the stable, non-radioactive glucose and glycerol isotopes. These are substances normally present or produced in the body, and thus pose no more risk than typical glucose infusions. There is a theoretical risk of infection from the stable isotope infusions. However, these isotopes are specially produced to be sterile and pyrogen-free by the manufacturer, and in addition, after being reconstituted by the Children's Hospital Colorado Investigational Drug Pharmacy, they are retested for pyrogens and sterility prior to use at the University of Colorado CTRC laboratory and the University of Colorado Clinical Laboratory, respectively, and discarded if not used in the standard expiration period. This is the standard procedure used by numerous studies at the University of Colorado. The stable glucose and glycerol isotopes have previously been determined to be usable for research at our institution (currently being used in our protocols 06-0665 and 07-0988) and others, and an IND is not required. To date, we have had no complications from the stable isotope infusions. However, as with any infusion, there is the possibility of infection.

Sterile and pyrogen-free  $^2\text{H}_5$ glycerol and 6,6- $^2\text{H}_2$  glucose will be obtained in powder form from the manufacturer. The CTRC pharmacist will reconstitute the glucose with sterile technique, filter (0.22 micron) for additional sterilization, aliquot, and freeze at  $-20^\circ\text{C}$  in the CTRC investigational pharmacy freezer. One aliquot is then sent to the CTRC laboratory for confirmatory quantitative pyrogenicity testing and a second aliquot to the Children's Hospital laboratory for anaerobic and aerobic culture to confirm sterility.

### **Metabolic Cart**

The metabolic cart measures the amount of air that the subject breathes in and out. The machine attaches to the subject's mouth through a tube, or a plastic bubble that is placed over the subject's head. There is the potential for experiencing claustrophobia from having the plastic bubble over the subject's head.

### **Magnetic Resonance Imaging**

The MRI is a non-invasive scan of the aorta and calf muscle. MRI uses a magnet and there is no radiation and no risk involved with the MRI. The scan may be loud, therefore the subject is provided with audio protection and optional television.

### **Accelerometer**

The accelerometer will be worn in a pouch that rests on the subject's hip and is positioned upright against the body to measure movement, similar to a pedometer. There is no risk involved with the accelerometer.

### **Study Diet**

There is a small risk for diabetic subjects 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, blood sugars will be checked before and after the meal.

### **Electrocardiography**

The ECG and near infrared spectroscopy device involve adhesive pads, which may cause minor skin irritation.

### **Exercise Test**

The exercise test involves exercising for a short time while the pulse and blood pressure are watched. It is routinely used to diagnose heart and lung problems. Rarely, people get abnormal heartbeats or chest pain while doing this test. There is also a risk of hypoglycemia during exercise in diabetic subjects, which will be minimized by monitoring blood sugars and having snacks and juice available during the exercise testing. The risk of hypoglycemia following the exercise test will be reduced by the provision of a small meal to subjects after completion of the exercise test. Because sedentary and obese subjects are at increased risk of reduced exercise capacity, the results of exercise testing are of potential benefit to subjects.

### **DEXA Scan**

The DEXA scan will measure the percentage of fat and muscle in the participant's body. This procedure will deliver the following amounts of radiation exposure: 10  $\mu$ Sv total body equivalent dose per measurement. The amount of radiation that the participant will be exposed to during the DEXA test is approximately equal to the amount of radiation they would receive being outdoors for one to two days in a high altitude city, such as Denver. Because T1D and sedentary and obese subjects are at increased risk of reduced bone mineral density, the results of the DEXA are of potential benefit to these subjects. **Dynapulse, endo-PAT and Brachial Artery FMD** There may be slight discomfort when the cuff is inflated.

### **Carotid Artery Ultrasound**

There is no risk or discomfort associated with this procedure.

### **Metformin Intervention**

Adverse effects of metformin include nausea, anorexia and diarrhea, and reduction in blood sugar due to improved insulin sensitivity. The adverse GI effects could potentially interfere with patient compliance. These effects have been shown to be reduced when the dose is titrated up slowly, given with food, and when blood sugars are followed closely as described in the Study Design section. Metformin has been found to rarely cause lactic acidosis (about 0.03 cases per 1,000 person years), and then only when used in persons with renal or hepatic insufficiency or during episodes of hypoxia or circulatory failure. This risk is minimized by stopping the medication with vomiting, diarrhea, before IV contrast or alcohol ingestion, and by the screening safety labs. A very rare risk of anemia is possible, but minimized by a screening and post-intervention check for anemia. We have already obtained an FDA exemption for use of metformin in this study. Metformin is often used to help women get pregnant and continued in the first trimester of pregnancy. However, to maximize safety, pregnancy is an exclusion criterion in the study.

### **Violation of Privacy and Loss of Confidentiality**

These are both risks to which research participants are exposed. The possibility of these risks increases when protected health information is collected. Every effort will be made to decrease this risk by limiting access to protected health information, storing this information in a password protected database, and identifying subjects only by a unique identifier that is kept in a separate location in a locked container, traceable only by study personnel. All of the tests involve the risk of identifying asymptomatic abnormalities. The study may include risks that are unknown at this time.

## **10. Protection Against Risks**

Study diet: Subjects will be asked to monitor blood sugars at least four times a day for the 3 days prior and the 24 hours after the study. Subjects will be called to check on blood sugars. Insulin doses will be adjusted as indicated.

Glucose levels will be monitored closely during both the clamp study and the overnight insulin infusion to avoid hypoglycemia in diabetic subjects. Women will have a urine pregnancy test performed in order to ensure that neither the DEXA nor the clamp study is performed on any woman who is currently pregnant.

Proper sterile technique will be used with blood draws and IV placement to decrease the infection risk. EMLA cream will be used if subject desires to minimize pain of IV. Frequent, rapid blood glucose measurements will be monitored during the euglycemic clamp to ensure safety.

To minimize the risk of any complications with the clamp, during the entire clamp procedure, a pediatric CTRC nurse will remain at the bedside, and a Pediatric Endocrinologist (study Physician) or study Nurse Practitioner or study Physician Assistant will also remain at the bedside. The IV site will be continuously monitored.

To minimize the risk of hypoglycemia in diabetic subjects during the VO<sub>2</sub>max test, blood glucose levels will be monitored before and after the test. Snacks and juice will also be available for subjects during the test. A light meal will also be provided to subjects follow the exercise test. To minimize GI side effects of metformin or hypoglycemia, the dose is titrated up slowly, given with food, and blood sugars are followed closely as described in the Study Design section. In addition, in the preliminary studies presented the effect of metformin on HbA1c was small, and the meta-analysis cited found no significant effect on HbA1c, therefore major changes in blood sugars are not expected. Other adverse effects of metformin include lactic acidosis. This side effect is extremely rare and is associated with dehydration. The risk of lactic acidosis will be decreased by having the subject stop their metformin temporarily if they are going to have surgery, IV contrast, alcohol ingestion or a significant illness associated with vomiting. Anemia is avoided by a screening and post-intervention Hb, and by blood volumes within recommended guidelines.

Emergency Response Plan: EKG, echocardiogram and blood pressure measures are performed prior to exercise testing to screen for any heart problems making exercise contraindicated. A physician or trained proctor will be present during the test. The CTRC performs graded exercise testing on a regular basis and is equipped with an oxygen supply and defibrillator. In the event that a patient goes into cardiac arrest during the procedure, the research team will call 911.

Blood Volume: We will use a minimum weight cutoff of 26 kg to remain below the most conservative pediatric CTRC blood drawing guidelines. In addition, we pre-screen subjects with a hemoglobin and hematocrit at their baseline visit, and exclude subjects with anemia. This screening also helps to increase the safety of the blood draw. In addition, the blood planned to be frozen and held could also be omitted if needed to reduce blood volume for a particular subject. Finally, our CTRC has a system to track other studies subjects might enroll in, and we ask during our consent process if the subject has been involved in any other studies in the past 6 weeks to avoid excessive blood drawing.

Pregnancy: Pregnancy is an exclusion criterion in the study and is tested for prior to each DEXA and insulin clamp.

Every effort will be made to decrease the risk of loss of confidentiality by limiting access to protected health information, storing this information in a password protected database, and de-identifying study specimens.

## **11. Potential Benefits**

### Evidence of Direct Benefit to subjects

Benefit from metformin intervention: Our preliminary data shows that youth with T1D have IR, cardiac, vascular, muscle mitochondrial and exercise dysfunction. We also provide rationale that metformin may improve each of these abnormalities in the youth with T1D studied in this protocol, as well as likely improve BMI if overweight/obese, lipid profiles and glycemic control. Therefore T1D youth have clear evidence of likely benefit from being in the trial. In addition, all T1D youth, even if on placebo, will benefit from the screening tests for these complications that they are at increased risk for, and from the increased attention to checking blood sugars, the exercise prescription and from increased interaction with diabetes providers and educators.

There is potential benefit of participation by subjects with diabetes because the results of the Hb, HbA1C, and DEXA are made available to the patient/family and their health care provider. Because sedentary and obese adolescents are at increased risk for reduced bone mineral density, reduced exercise capacity, cardiovascular and muscle dysfunction and increased hepatic and visceral fat non-diabetic subjects may potentially benefit from the results of DEXA, cardiovascular, MRI and exercise testing. Subjects with diabetes have a condition which we will learn more about from performing this study.

The primary benefit from the clamp would be the discovery of undiagnosed insulin resistance. As mentioned before, surrogate measures are unable to detect IR adequately, and IR is the strongest predictor of CVD [12, 14, 187-189]. Discovery of IR would enable us to inform the family of an increased risk in the future for the individual for CVD, which may prompt them to seek greater screening tests when the individual is older. The direct benefit of

the isotopic tracers would be the differentiation of whether the liver, and/or the muscle and/or the adipose tissue are involved in the IR.

The echocardiogram can detect previously undiagnosed asymptomatic cardiac lesions such as mitral valve prolapse, bicuspid aortic valve, or small ASD/VSD in addition to any abnormal cardiac function. The blood tests done for screening can detect alterations in blood glucose, pubertal sex hormones, as well as abnormalities in fasting lipids. The risk of all of these endpoints is increased in T1D subjects, especially those who are also obese, and if left untreated can increase long term health risk, thus the benefit of detecting any of these would directly impact both the health and the longevity of the individual subject.

At the conclusion of the study, all subjects in the protocol will also be given counseling on the benefits of exercise as discussed above, and given an exercise prescription (as done as the standard of care in our pediatric metabolic syndrome clinic). The subjects will also be re-contacted once by phone call to follow-up on their study results and exercise recommendations. Sedentary subjects will thus also gain direct benefit from the study through the benefits of specialized counseling and recommendations for increased physical activity that would not otherwise be available to them.

#### Evidence of Benefit to Society

The importance of the knowledge gained from this protocol is very high, since both T1D is a relatively common disease, increasing in prevalence in youth, and increases the risk of death from CVD and shortens lifespan. Diabetes mellitus is the third most prevalent severe chronic disease of childhood. Key research questions now focus on descriptive epidemiology (prevalence and incidence rates), etiology (metabolic, behavioral, genetic and immunologic risk factors), and appropriate treatment approaches. Macrovascular complications such as coronary artery disease continue to be the main cause of death in persons with diabetes, shortening average lifespan by 15 years, and were the topic of a recent NIH working group that evaluated opportunities to pathogenesis of and intervention to prevent CVD [10]. Although data to guide care of CVD in adults with diabetes are limited, even less data exist regarding the antecedents of CVD in youth with diabetes. Yet, these antecedents of adult CVD are present in children [190] with studies such as the Bogalusa Heart Study [191-193] and the Young Finns Study [194] demonstrating tracking of factors such as lipid levels and obesity. As CVD is the leading cause of death in people with diabetes and the antecedents develop in childhood, data to inform clinicians as to appropriate screening and treatment in this high-risk population are of great public health importance.

Our preliminary studies suggest that adolescents with T1D may already display symptoms of cardiovascular dysfunction including reduced exercise capacity, diastolic dysfunction, left ventricular hypertrophy and reduced vascular reactivity as measured by brachial artery ultrasound, endo-PAT plethysmography and Dynapulse. This has serious implications for the treatment of these patients as they age.

Traditional CVD risk factors remain important, but do not explain the excess risk of CVD in T1D [12]. Conventionally, T1D is considered a disease of insulin deficiency, leading to a research focus on improving insulin secretion or delivery to improve glucose control. However, a glucose-centric view may overlook the potential role of IR in T1D and CVD. Since IR is a contributor to accelerated atherosclerosis in the general population, superimposed IR may hasten the development of CVD in T1D [12, 14, 189]. In addition, due to rising rates of obesity in all youth, IR is likely to become increasingly important in the pathophysiology of T1D.

In addition, microalbuminuria and later diabetic kidney disease also likely contribute to increased risk of CVD. Preliminary evidence indicates that metalloproteinases (MMP) 2, 9, and 13 may be helpful noninvasive markers of kidney dysfunction. Elevated activity of these MMPs may be a biomarker specific for diabetic kidney disease in terms of glomerular basement membrane integrity. We hope that these tests may help to segregate out patients who will progress at a faster rate towards renal complications and therefore could be targeted for more intensive therapy.

Analysis of lipid subfractions with fast protein liquid chromatography (FPLC)[16] and apoB will help determine the baseline atherogenicity of lipid profiles in T1D youth, and the effects of improving IR on lipoprotein metabolism. Using apo B in combination with VLDL TG will allow interpretation of the effect of insulin on particle composition vs. particle number. By examining hepatic insulin sensitivity, and body composition proposed in Aim 2, and the vascular measures proposed, the relationship between lipid profiles and these factors can also be examined.

#### **12. Alternative Treatment**

Insulin in T1D is the only alternative treatment and will still be continued.

### **13. Consideration of Specific Subject Categories**

#### **1. Inclusion of Women**

Every effort will be made to include approximately equal numbers of males and females in this protocol. The prevalence of T1D in adolescents is approximately equal in males and females, so we expect equal numbers of males and females being available for the study.

#### **2. Inclusion of Minorities**

Every effort will be made to include a diverse subject distribution.

#### **3. Inclusion of Children**

All subjects will be between ages 12 and 21. Insulin sensitivity needs to be studied in the adolescent age group as no data is currently available in this age group and it is critical to understand the pathophysiology of T1D in its developing stages.

## **VI. Potential Scientific Problems**

### **Subject drop-out**

To account for screen failures and potential 30% dropout, up to 100 subjects may be needed to be recruited to obtain the required 60 subjects. 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 TODAY study participants. Due to our TODAY and other metformin studies, our staff is experienced with long-term adolescent studies, with excellent study retention for as long as six years. Adverse effects of metformin (nausea, anorexia, diarrhea and vomiting), have been shown to be minimized when the dose is titrated up slowly and taken with meals, and in our blinded study of metformin in obese adolescents, adverse effects did not result in decreased dose or significant patient attrition vs. placebo.

### **Hypoglycemia during the insulin clamp**

Hypoglycemia will be limited by monitoring blood sugars every five minutes, choosing a goal glucose well above the hypoglycemic range (95mg/dl), having IV access obtained prior to insulin administration, having two IVs in place in case problems arise with one IV, and leaving IV access in place until blood glucose values are stable after the study is completed. In addition, during the entire insulin infusion period, a pediatric CTSC nurse will remain at the bedside, and a Pediatric Endocrinologist (study Physician) or study Nurse Practitioner or study Physician Assistant will also remain at the bedside to minimize any risks associated with the insulin clamp.

### **Inability to Stabilize Glucose Prior to Euglycemic Clamp**

Stabilization of blood glucose by intravenous infusion of glucose and insulin should not be difficult in patients with reasonably well-controlled diabetes and is standard procedure. If serum glucose has not stabilized between 80-120 mg/dL by the start of the clamp, the clamp will be cancelled and the patient given the option to return for a second attempt within one week.

### **Hepatic Glucose Output**

Hepatic glucose output should be suppressed during the high dose clamp [195], but may not be in pubertal adolescents with higher Hba-1c [39], hence stable isotope tracer techniques will be used to measure hepatic glucose output.

### **Variability in Subject Activity and Diet**

Subject activity and diet that affect IR will be addressed by recruiting sedentary subjects, prescribing a standardized study diet (with carbohydrate content labeled for counting) and limiting strenuous exercise for 3 days prior to the clamp and exercise visits.

### **Insufficient Number of Subjects**

This is unlikely as the Barbara Davis Center for Childhood Diabetes is a very large, tertiary care center for type 1 diabetes, with approximately 3000 type 1 patients. Likewise, we have previous experience recruiting from these demographics for similar procedures.

### **Hypoglycemia During Exercise Testing or Metformin Intervention**

This will be limited by monitoring blood sugars before and after exercise and as needed, and having juice and snacks available in the exercise testing room as needed. In addition, subjects will be provided with a small meal following the VO<sub>2</sub>max test. Metformin may improve IR requiring insulin reductions, but with the expected mean A1c of 8.5% in our adolescent population, and an expected metformin effect of less than 1% change in A1c, significant hypoglycemia should not be common. Frequent blood sugar monitoring and contact with study staff will

help avoid hypoglycemia, and hypoglycemia was not significant in our preliminary studies or the metformin meta-analyses quoted. Metformin is well tolerated in youth and unlikely to present risk. On the contrary, it is likely to present benefit to the youth with T1D.

## **VII. Data Management and Security Plan**

### **Data Entry**

Data will be entered from paper forms. Once forms are completed, verified and corrected for inconsistencies, they will be manually entered at our site using a computerized data management system (Redcaps).

### **Edit Checks**

Computerized data validation routines will be used to enhance data quality and verify the accuracy of data within predefined value ranges. These checks include, but are not limited to: (a) initial screening of data, using logic and range checks built into data entry screens; (b) cross-form functional and consistency checks; and (c) edits assessing the serial integrity of data.

### **Disaster Recovery**

Routine data backup will occur on data in conjunction with the children's hospital secure server and Redcaps.

### **Security and Confidentiality**

All hard copy forms will be de-identified with a study number and filed in a locked cabinet, to which only the investigators will have access. Standard protection against computer hackers is implemented. Recovery from natural disasters (water, fire, or electrical) can occur through the ability to reconstruct both the database management system and the data from nightly backups.

## **VIII. Data and Safety Monitoring Plan**

The principal investigator and a Safety Officer, Christine Chan, MD will monitor the protocol and the safety of the research subjects. The PI will review all laboratory data and report any abnormal values to the patient and guardian and instruct the subject to follow-up with their PCP. The PI will report adverse events, and any decision to suspend or halt the protocol to the Safety Officer, CTRC and COMIRB immediately. She will meet every 6-12 months with the Safety Officer. She will also prepare a written report for the yearly continuing review required by the Safety Officer, COMIRB and the CTRC. There are no other entities that require notification about this protocol.

No protected health information will be collected until the appropriate HIPAA forms are completed. The protected health information that will be collected will include: Name and phone number, demographic information (age, sex, ethnicity, address, etc.), diagnosis (es), history and physical, laboratory or tissue studies, radiology studies, procedure results, survey/questionnaire results, research visit records, and portions of previous Medical Records that are relevant to this study. This information will be accessible only by the study investigators, Federal agencies overseeing human subject research, the Colorado Multiple Institutional Review Board, regulatory officials from the institution where the research is being conducted to monitor safety and compliance with policies.

### **A. Adverse Events (AE)**

The hyperinsulinemic euglycemic clamp is a standard procedure used in a large number of research studies and settings. Adverse events are uncommon when the procedure is done by experienced personnel in an appropriate setting.

#### **1. Adverse Event Definition**

For the purposes of this study, an Adverse Event (AE) is defined as any untoward medical event associated with the use of a drug in humans, whether or not considered drug-related. AEs also include any significantly abnormal physical finding identified on examination and any significantly abnormal laboratory result obtained on the patient between visits or at the time of the visit. Questions answered YES and any new abnormal physical findings are pursued by the study staff in order to determine the seriousness of the event and the need for further evaluation, follow-up, or referral.

#### **a) Adverse Event Reporting**

AEs are reported on a standard form that is completed by the study staff at each regular follow-up visit and phone interview. Adverse events reported or ascertained between clinic visits are captured and reported at the time of the next scheduled visit.

Pre-existing conditions (that is, conditions present prior to randomization) are not considered or recorded as AEs or SAEs unless the condition worsens in intensity or frequency after randomization. Likewise, continuing adverse events are not reported as AEs at subsequent visits unless they increase in severity or frequency between the visits, they result in criteria for an SAE, and/or they resolve between visits.

## **B. Serious Adverse Events (SAE)**

### **1. Serious Adverse Event Definition**

Events are divided into those that are not serious (AE) and those that are serious (SAE). The distinction between an SAE and an AE is a regulatory definition established by the FDA, not a clinical definition. The definition of SAE is not always related to clinical severity of the event. For the purposes of this study an AE is considered a Serious (SAE) when it satisfies any one of the following criteria:

- The event results in an inpatient hospitalization (any overnight stay associated with an admission).
- The event results in the prolongation of a hospital stay.
- The event results in permanent or severe disability.
- The event results in death.
- A pregnancy results in a congenital anomaly.
- The event results from an overdose (either accidental or experimental) of the study medication.
- The event is life-threatening.
- Treatment is required to prevent a serious event.
- The patient experiences a bout of lactic acidosis.
- An episode of severe hypoglycemia requiring assistance occurs.

There have been no SAE's in the research groups experience in the Pediatric CTRC. We do not anticipate encountering SAE's; however, we have identified the following as possible SAE's for the purposes of monitoring:

- Infection related to blood draw or IV placement
- Severe hypoglycemia during the euglycemic clamp or VO<sub>2</sub> max test (blood glucose < 45 mg/dl)
- Severe adverse effects from taking placebo or metformin

### **a) Serious Adverse Event Reporting**

Study patients are instructed to contact the clinic with any serious adverse event meeting the above criteria. Each SAE is recorded on the study form and the PI is informed soon as possible after they occur and preferably within 24 hours of the notification of the clinic staff. This notification should occur even if data are incomplete. Additional data and follow-up information are documented and sent subsequently as an update to the original report. The PI immediately forwards SAE reports to the Safety Officer and COMIRB and any other required institutional monitoring committee.

## **C. Subject Discontinuation Criteria**

If a subject experiences any of the following, the subject will be withdrawn from the study.

1. Inability to complete study procedures
2. Inability of subjects to tolerate a minimum of 500 mg BID of metformin or placebo tablets
3. Development of anemia or renal dysfunction (anemia: hemoglobin < 9; renal dysfunction: creatinine >1.2)
4. Recurrent severe hypoglycemia (severe defined as symptomatic blood glucose < 45 mg/dl requiring assistance or seizure; recurrent as determined by PI)
5. Development of Lactic acidosis (as determined by PI)
6. Subject becomes pregnant during study

## **D. Protocol Stopping Criteria**

If one or more subjects experience any of the SAE's listed above, the PI will consult with the Safety Officer prior to continuing study visits with subjects. The Safety Officer and RSA will consult about the significance of the SAE's and make a recommendation to the PI.

# **VIII. Data Analysis Plan**

## **A. Data Analysis Plan**

Data analyses and power: For all aims, variables will be checked for the distributional assumption of normality and appropriate transformations applied to normalize the data.



Specific Aim 1a: A placebo-controlled design will be employed using a mixed effect model to determine the effect of treatment and check for the effect of sequence on the effects of metformin to placebo on whole body IR (GIR, mg/kg/min, 1<sup>o</sup> outcome). Based on the Sarnblad et al article, who had a mean difference of 0.8 and SD of 0.9, with a sample size of 30 per group and an alpha of 0.05, we will have 92% power to detect a difference of 0.8 in the primary outcome of insulin sensitivity. Additional 10 patients will be recruited per/group for a total N = 80 to account for 30% dropout. 100 will be screened to account for screen failures.

Specific Aim 1b: A placebo-controlled design will be employed using a mixed effect model to determine the effect of treatment on the effects of metformin to placebo on ADP t<sub>1/2</sub> adjusting for baseline mitochondrial function, gender, and Tanner stage. Our preliminary baseline data shows a difference in ADP t<sub>1/2</sub> between T1D and controls (19.1 ± 0.85 vs. 14.6 ± 0.63, respectively), or an effect size = 5.294. Assuming that T1D patients treated with metformin improve ADP t<sub>1/2</sub> compared with placebo, we will have > 80% power to detect an effect size as small as 0.645 and a two-sided  $\alpha$  = 0.0125 adjusted for 4 endpoints.

Specific Aim 2a: A placebo-controlled design will be employed using a mixed effect model to determine the effect of treatment on the effects of metformin to placebo on BrachD, adjusting for baseline BrachD, gender, and Tanner stage. Agarwal et al demonstrated a mean difference between 3 months of metformin and placebo in young women with PCOS using Aix (-6.1; 95% CI of -8.46-3.74)[93]. If we see a similar effect with common SD = 5.066 calculated from the CI, N = 11 per/group is required for 80% with a two-sided  $\alpha$  = 0.0125 to adjust for 4 endpoints, thus with N = 80 we will have > 99% power for this aim.

Specific Aim 2b: A mixed effect model will be used to compare the effects of metformin to placebo on cardiac dysfunction measured by CS. With N = 80 we will have > 80% power to detect an effect size as small as 0.645 and a two-sided  $\alpha$  = 0.0125 adjusted for 4 endpoints.

Secondary Analysis: For all Aims, correlations will also be made between each primary outcome and IR, A1c, T1D duration, and BMI as continuous measures with Pearson correlation coefficients without adjustment for covariates. Multivariate linear regression will determine which outcomes relate independently to IR, A1c, T1D duration, BMI and/or gender after adjusting for covariates. Similar analyses will assess for correlations between changes in IR, A1c and BMI on changes in each primary outcome to the intervention.

Exploratory analyses: Secondary outcomes will be analyzed at baseline and correlations between potential contributors to each outcome will be examined with Pearson or Spearman correlation coefficients, as appropriate. Multivariate linear regression will then determine which potential contributors independently relate to each baseline measure, adjusting for covariates. A mixed effect model will also be used to compare the effects of metformin to placebo on exploratory outcomes.

#### Analysis plan:

The primary analysis will be a treatment group comparison of mean change in whole body IR (GIR, mg/kg/min, 1<sup>o</sup> outcome) obtained at the 3 month visit adjusted for baseline IR, tanner stage, sex, and race/ethnicity in an analysis of covariance (ANCOVA) model.

The primary analysis will follow the intent-to-treat principle. The data of all randomized patients will be included in the analysis regardless of whether the assigned treatment was actually received, according to randomization group.

Multiple imputation using Rubin's method 24 will be used to impute the GIR outcomes that are missing. The primary analysis will be repeated including data from participants with no imputation for missing data. The results will be compared with those using imputation to verify that conclusions are not sensitive to the method for handling missing data.

#### 6.2.2 Secondary Endpoints:

Secondary analyses will include treatment group comparisons of the following:

1. Mean change in hepatic IR
2. Mean change in adipose IR
3. Mean change in ADP t<sub>1/2</sub> from calf MRI
4. Mean change in BrachD from brachial artery ultrasound
5. Mean change in circumferential strain from echo

#### 6.2.3 Exploratory Analysis:

Correlations between changes in HbA1c and BMI and IR will be assessed.

## **IX. Knowledge to be Gained From This Research**

T1D is increasing among youth. The pediatric incidence in Colorado rose from 14.8/100,000 in 1978-1988 to 23.9 in 2002-2004[196], translating to a lifetime of exposure and risk for early death from CVD [196-201]. Approximately 17 years of life are lost for a child diagnosed with T1D at age 10, a barrier that has not improved over the last four decades [202]. Thus, youth onset T1D creates an especially vulnerable population. Intensive glycemic control is undoubtedly critical to decreasing renal, neurological, and retinal complications, and also correlates with reduced CV mortality [9, 203-205]. However, we currently lack safe or realistic intensive glycemic treatment strategies for youth. As evidence, only 25% of youth in our multi-center T1D EXCHANGE Registry were able to reach HA1c goals, making novel approaches to preventing complications critical[122].

Unaddressed IR may contribute to CVD in T1D, as it does in T2D [17-21, 206]. For example, estimated IR, not glycemia, predicted CVD events and mortality in two adult T1D studies[9, 24], and in our collaborators Coronary Artery Calcification in T1D adults (CACTI) study, clamp-derived IR, not glycemia, predicted CAC[25]. Our previous studies also reveal the presence of IR unrelated to glycemia, along with a dangerous constellation of cardiac, vascular, muscle mitochondrial and exercise dysfunction in normal-weight T1D children, despite short diabetes duration and absence of other typical IR-related co-morbidities. Thus, IR appears to be a common, integral component of T1D pathophysiology, which is not explainable by hyperglycemia or obesity alone. However, the currently increasing rate of obesity in T1D youth[122] is also a critical barrier to progress, as obesity super-imposed on T1D is likely to worsen IR. We propose that controlling IR early in the care of T1D patients may decrease CV morbidity and mortality in this population.

The mechanism of IR in T1D is distinct from T2D as the IR phenotype clearly differs. Differentiating each tissue's response to metformin in T1D, and exploring metformin's impact on the CV system will help direct future therapeutic targets, as will clarifying the relative importance of hyperglycemia vs. IR in the cardiac, vascular, exercise and muscle dysfunction in T1D youth. A 2010 meta-analysis concluded that "properly designed randomized controlled clinical trials of metformin" designed to assess "reductions in CVD in T1D should be conducted forthwith"[60]. Our proposal addresses this significant unmet need. Findings supporting directly treating IR in T1D youth may change the clinical approach to T1D care.

T1D shortens lifespan despite modern advances in control of established CV risk factors[207]. Thus, innovative therapeutic approaches are required if we are to address the excess CVD resulting from T1D. Metformin has been attempted previously in T1D youth, but its limited impact on HbA1c tempered enthusiasm. Few studies have examined the non-glycemic benefits of metformin in T1D. Thus, additional benefits of insulin sensitization or clues to causes of IR in T1D may have been overlooked in the literature. The meta-analysis above determined that metformin significantly reduced insulin dose in T1D, but found no trials that included CV outcomes or their surrogates[60]. Therefore, our proposal is highly innovative in assessing metformin's effects on the CV system in T1D. Our proposal also moves beyond the typical paradigm of approaching IR in T1D as strictly a consequence of obesity. Our preliminary data strongly demonstrate two key novel observations: 1. Both T1D and T2D youth have evidence of mitochondrial, vascular, cardiac and exercise dysfunction that correlates with IR. 2. IR is nearly universal in T1D youth but is not predicted by usual metabolic syndrome features, identifying an entirely unique phenotype. Thus, the mechanism of IR is likely also unique in T1D and may respond differently to therapies than it does in T2D. Our data reframe the traditional model of IR as synonymous with a metabolic syndrome phenotype.

We propose that a new approach of treating or preventing IR in T1D patients at an early age, when defects in CV function may still be reversible, will decrease CVD burden in T1D at later stages of life and modify current standards for care. Knowledge gained from this proposal, the first to rigorously examine the response to metformin in T1D, will provide novel information about the role of IR in early CVD development in T1D and focus future interventions. We are uniquely poised to address the nature and consequences of IR in T1D youth. Our novel imaging techniques that can noninvasively assess mitochondrial dysfunction (MitoD) in youth with exercise. Our advanced echocardiography indices and are validating noninvasive vascular biomarkers to identify early progressors that may benefit from more intensive interventions. Our expertise in 3-stage hyperinsulinemic clamps with isotopes in T1D youth is also a unique strength. In parallel with this proposal in T1D youth, we are also performing similar studies of IR, on control, obese and T2D youth, which will enable novel comparisons to the T1D youth studied in this protocol.

## X.

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