Clinical Research Protocol

National Institute of Diabetes and Digestive and Kidney Diseases

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Title: **Therapeutic targets in African-American youth with type 2 diabetes**
Short Title: Type 2 Diabetes in AA youth
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Estimated Duration of Study: 6 years
Start Date: Fall 2016 **End Date:** Fall 2022
Number and Type of Patients: Accrual Ceiling: 92

	Number	Sex	Age Range	Ethnic Group
Patients:	92	Male/Female	12- 25 years	African-American only
Volunteers	0	-	-	-

Project Uses Ionizing Radiation:
_X__Research indicated: RSC Approval Number: 2581 Expiration Date: May 2019

Project Uses "Durable Power of Attorney": No **Off-site Project:** No
Multi-site enrollment: Yes
Multi-institutional project: Yes; Patient recruitment and data collection will occur at NIH Clinical Center and Pennington Biomedical Research Center (PBRC).
Patient recruitment only will be conducted at Children's National Medical Center.
Pennington Biomedical Research Center (PBRC) site is CLOSED effective 10/05/2021. While a reliance agreement was executed, research was never initiated at this site.

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Précis

Type 2 Diabetes in youth is an emerging public health concern that disproportionately affects minority children. Among minority youth, African-Americans have the highest complication rates, yet the reasons underlying this health disparity are not fully understood. Furthermore, current treatment options are limited, and African-American youth have high treatment failure rates. Metformin therapy is the only oral diabetes drug approved for use in youth with type 2 diabetes. However, metformin works less than 50% of the time in African-American youth and there is marked variability among individuals. Improving outcomes in youth requires understanding the way that drugs such as metformin work in youth and why it does not work in some individuals. New evidence suggests that the ability of metformin to work effectively may be influenced by certain genes or differences in gut bacteria. However, little is known about how genes or gut bacteria may affect youth, especially African-Americans.

To treat this aggressive disease, it is also necessary to simultaneously evaluate new therapeutic options, such as combination therapy of metformin with liraglutide in youth at highest risk for complications. Liraglutide is approved to treat type 2 diabetes in patient 10 years and older as an adjunct to diet and exercise. Liraglutide may be a useful early treatment in youth with type 2 diabetes because it may decrease glucose produced by the liver (an early prominent feature of type 2 diabetes in youth). This study is designed to examine the mechanism of action in the liver of these 2 agents and explore how genetic and gut factors may influence this action.

The primary objective of this pilot study is to compare the ability of two anti-diabetic regimens (metformin and liraglutide versus metformin alone) to lower gluconeogenesis (glucose produced by the liver) in African-American youth with type 2 diabetes. The secondary objectives are to evaluate the effect of these regimens on the following: (1) hepatic glucose production, and insulin sensitivity and (2) insulin and gut hormones concentrations (e.g. incretins). In addition, we will examine the relationship of known differences in genes associated with metformin transport and action with changes in gluconeogenesis and begin to explore the role of gut bacteria to metformin's glucose-lowering effect.

The study design is a parallel-randomized intervention trial of African-American youth with type 2 diabetes who are not on insulin therapy and who are within 5 years of diagnosis. Patients aged 12- 25 years with type 2 diabetes will be enrolled. Participants will be randomized into two intervention arms (16 in each group): metformin and liraglutide versus metformin alone. The study will consist of 5 visits. At Visit 1, a medical history, physical examination and screening labs will be done. Then the eligible participants will undergo a one-week drug-free run-in. At Visit 2 there will be an overnight inpatient stay to perform metabolic testing prior to starting the study drug(s). Participants will start the study drug(s) immediately after Visit 2 and remain on the study drug(s) for 12 weeks. Follow-up monitoring will be performed at 4-week intervals (Visit 3 and 4). The final visit (Visit 5) will occur after 12 weeks.

The ultimate goal of this multi-site project is to begin to address diabetes disparities in African-American youth by understanding the mechanism of action of these diabetes agents to inform precision medicine initiatives. This project brings together the skills and expertise of investigators within the National Institute of Diabetes and Digestive Disorders

and Kidney Diseases (NIDDK), the National Human Genome Research Institute (NHGRI), and the Children's National Medical Center (CNMC). Patient recruitment and data collection will occur at NIH Clinical Center. Eligible patients may be identified through CNMC but no enrollment, informed consent or study visits will occur at CNMC.

Introduction and Significance

Type 2 diabetes is a leading cause of non-communicable disease worldwide and afflicts over 29 million individuals in the United States. Rates of type 2 diabetes in youth are expected to precipitously rise over the next 20 years and African-American youth have a 2 fold increased risk for type 2 diabetes and a 4 fold increased risk for complications compared to white youth[1-3]. Race/ ethnic differences in biological characteristics and/ or pharmacogenomics may contribute to variations in risk for diabetes and/ or medication response. While declining insulin secretion and increasing insulin resistance are the pathophysiological hallmarks of type 2 diabetes, African-American youth may also have additional predisposing factors. Specifically, lower glucagon-like peptide 1 (GLP-1) concentrations in African-American than white youth may contribute to a rapid deterioration in insulin secretion and progression to diabetes[4,5]. Currently, metformin therapy is the only oral agent approved for treating type 2 diabetes in youth[6]. However, African-American youth with type 2 diabetes have the highest metformin failure rates, and more effective treatment options are urgently needed to reduce the physical, psychological and economic burden of this disease[7].

Optimizing initial therapy could dramatically improve outcomes because type 2 diabetes in youth is associated with increased risk for morbidity and mortality within 5-10 years of diagnosis[8]. Therapeutic regimens should target the key pathophysiologic mechanisms of type 2 diabetes in youth (β-cell failure[9] and increased gluconeogenesis[10]). At present, metformin is the first line agent recommended, but metformin alone may not be sufficient to address the severe and accelerated metabolic decompensation observed in youth [6]. We propose that combination therapy with metformin and liraglutide (a glucagon like-1 receptor agonist) would target two key pathophysiologic targets in youth: higher gluconeogenesis and impaired insulin secretion. This pilot study will compare the ability of combination therapy with metformin and liraglutide with metformin alone to lower gluconeogenesis in African-American youth with type 2 diabetes**.** In an effort to better understand the pleiotropic effects of metformin and liraglutide, we will simultaneously evaluate measures of glucose metabolism (hepatic and peripheral insulin sensitivity, insulin and incretin concentrations), lipid metabolism (whole body lipolysis and fatty acid turnover) and gut microbial community. This clinical study will provide the mechanistic framework and foundation for the exploratory pharmacogenomic evaluation. The exploratory pharmacogenomic study will determine whether metformin-induced changes in gluconeogenesis are associated with known pharmacogenomics markers in youth. We anticipate that this integrative approach to therapeutic development will help to define current and future drug targets and focus future pharmacogenomics studies, which are usually expensive, time-consuming and under-represent minority groups[11].

Specific Aims

Aim 1: To quantify and compare rates of gluconeogenesis in African-American youth with type 2 diabetes after short-term combination therapy (metformin and liraglutide) vs. metformin therapy alone, after an overnight fast and during meal absorption.

Hypothesis: *Youth treated with combination therapy will have lower rates of gluconeogenesis after an overnight fast and during meal absorption compared to youth treated with metformin alone.*

Aim 2: To measure the change in GLP-1 and insulin concentrations in African-American youth with type 2 diabetes after short-term combination therapy (metformin and liraglutide) vs. metformin therapy alone.

Hypothesis: *During meal absorption, combination therapy with liraglutide and metformin will be associated with higher GLP-1 and insulin concentrations than metformin alone.*

Exploratory Aim 1: To identify in African-American youth with type 2 diabetes, known pharmacogenomic variants previously associated with metformin response in adults and determine the relationship of these variants with change in gluconeogenesis and metformin concentrations.
Hypothesis: *The 9 known variants of pharmacogenomics response identified in adults (Table 1) will be detectable in African-American youth. A higher genetic risk score (worse risk) of known metformin pharmacogenomic loci will be associated with (a) lower serum and urine metformin concentrations and (b) a smaller delta change in rates of gluconeogenesis.*

Exploratory Aim 2: To compare the composition and distribution of gut microbiota before and after metformin.
Hypothesis: *The composition and distribution of gut microbiota will change after metformin intervention.*

Exploratory Aim 2: *Construct* a pharmacokinetic-pharmacodynamic model based on plasma and RBC metformin concentrations, and treatment outcome response (including change in glucose production, gluconeogenesis, incretin concentrations).
Hypothesis: Metformin concentrations will correlate with treatment outcomes over the 3-month study period.

Background

Metformin

Metformin treatment alone is suboptimal in 50% of all youth and ~ 65% of African-American youth with type 2 diabetes[12,13]. This low efficacy in children was unexpected, not only because of greater metformin efficacy in adults[14], but also because during the 8 week run-in phase of the TODAY study (Treatment Options for type 2 Diabetes in Adolescents and Youth), more than 85% of youth, regardless of race, were able to maintain glycemic control on metformin therapy[15]. Consequently, the reasons underlying this secondary treatment failure with metformin in children remain to be elucidated but do not appear to be related to either stressor scores or poor compliance[16]. We postulate that metformin's durable treatment efficacy is suboptimal in youth, especially African-Americans, because it has only a modest effect on lowering gluconeogenesis (a prominent feature of type 2 diabetes in youth).

Targeting gluconeogenesis with metformin

To address the therapeutic conundrum of apparent lower metformin efficacy in youth, it is useful to briefly review the literature on the pharmacodynamics of metformin. Although it is widely accepted that metformin's primary mechanism of action is to reduce hepatic glucose production, many questions still remain regarding metformin's target and downstream signaling pathways[17,18]. *In vitro* assays of metformin action do not accurately reflect the gluconeogenic pathways or whether the drug ultimately influences net glucose turnover [19-22]. *In vivo* tracer studies provide more useful information on how metformin affects glucose turnover, but the few studies on this topic disagree on whether metformin's primary physiological target is to inhibit gluconeogenesis (a primary contributor to increased glucose production in youth) or glycogenolysis[23-27]. Moreover, metformin's ability to inhibit gluconeogenesis may be an indirect consequence of reducing hepatic fat and energy stores[28,29]. Since African-Americans have lower hepatic steatosis at the same body mass index when compared to white youth[30], the lower hepatic fat stores could impede a durable metformin effect. However, there are no studies which have examined the effect of metformin on free fatty acid flux in relation to its glucose lowering abilities.

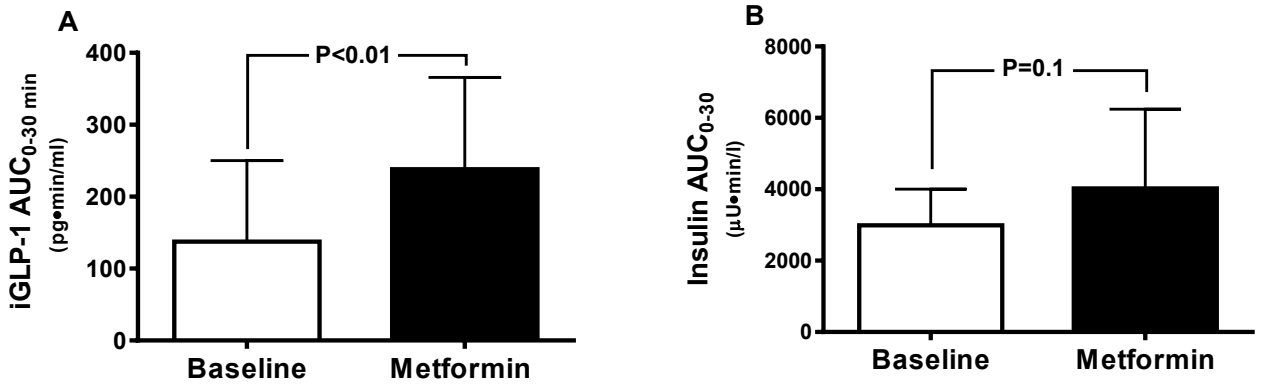
We have previously demonstrated in drug naïve youth with newly diagnosed type 2 diabetes that increased rates of gluconeogenesis are an important contributor to mild fasting hyperglycemia (glucose ~ 7mmol/l)[10]. This finding contrasts with prior adult studies, in which high rates of gluconeogenesis were only observed in individuals with severe hyperglycemia (glucose >8.5 mmol/l) and long-standing disease[31]. This early and severe dysregulation in gluconeogenesis is consistent with the aggressive nature of type 2 diabetes in youth[32] and our preliminary results suggest that metformin may not be a potent first-line agent for this disease. **Preliminary Results:** In a pilot study of 5 multi-ethnic youth with newly diagnosed type 2 diabetes conducted at Baylor College of Medicine, we demonstrated that 3 months of metformin reduced HbA1c (6.8±0.1 vs. 6.0±0.2%, P<0.01) without a change in gluconeogenesis (1.78 ± 0.41 vs. 1.72 ± 0.31 mg●kg_{lbm}●min, P=ns)[33]. The participant characteristics for the pilot study conducted at Baylor were: 5 adolescent girls, age 13.7±1.0 years; 3 African-American and 2 Hispanic. All individuals were maintained on metformin 1g twice daily x 12 weeks without side effects. The participants were instructed on basic education for diet and lifestyle at the start of the study, but there were no ongoing organized or supervised lifestyle regimens. Although not powered to detect weight loss, there was no significant detectable weight loss post metformin treatment. If attenuation of gluconeogenesis is not a primary driver of metformin's initial anti-hyperglycemic effect, metformin alone may be suboptimal in youth with type 2 diabetes. These preliminary results support a multi-mechanistic role of metformin and add credence to the mounting evidence that metformin may have pleiotropic effects.

Targeting incretin secretion with metformin

We hypothesize that modulation of the incretin axis may also be an early mediator in metformin therapy, which could be an important mechanism of action for African-American youth who have lower GLP-1 concentrations[4]. Novel research identifies the gut as an important mediator of the mechanism of metformin action[34], with the most recent publication evaluating a delayed release metformin formulation designed to target the distal ileum and reduce metformin serum absorption. In comparison to immediate release preparations, delayed release metformin optimally lowered overall glucose concentrations, despite lower serum metformin concentrations[35]. Although the exact target of delayed release metformin is not yet clear, enhancing the incretin response could be a potential mediator[36].

Preliminary data: In our pilot study of 5 youth with type 2 diabetes treated with 3 months of metformin, improved overall glycemia occurred independent of changes in gluconeogenesis (see above) or serum metformin concentrations. The improved glycemia was associated with significantly greater early intact GLP-1 area under the curve (AUC) concentrations and a trend for increased insulin during an oral glucose tolerance test (OGTT) (Figure 1)[33].

Figure 1. (A) Early intact GLP-1 and (B) insulin area under the curve concentrations after an OGTT



Notably, although metformin therapy may improve GLP-1 concentrations, the magnitude of the GLP-1 increase by metformin alone would be relatively small and may not sufficiently improve insulin secretion to compensate for the rapid deterioration in β-cell function seen in youth with type 2 diabetes.

Understanding the role of gut microbiome and metformin in African-American youth

Modulation of gut microbiota by metformin may also be an important mechanism of action, especially because intravenous administration of metformin has no direct glycemic effect[37]. The mounting evidence in murine models[38] and humans[34,39] indicate that metformin induced changes in gut microbiota genetic signatures are related to overall drug efficacy. In an elegant study in 14 adults with type 2 diabetes on and off metformin treatment, Napolitano et al. showed that metformin's glucose lowering effect was related to changes in gut microbiota and the entero-endocrine hormone secretion[34]. As new evidence emerges, there is a conspicuous absence of data examining metformin and its effect on the microbiome in populations of African descent and youth. To the best of our knowledge, there is no evidence regarding differences in microbiome by race/ ethnicity and this study will be the first to evaluate the relationship (or lack thereof) of gut microbiota, change in gluconeogenesis and incretin secretion after metformin therapy in African American youth. We hope that this information will provide data to design a more comprehensive evaluation of the gut as a target of metformin action.

Metformin Pharmacogenomics

Inter-individual variability in metformin response

There is marked variability in metformin clinical response in adults (35% non-responders); this may be more prominent in children (50% non-responders), especially African-American youth (~65% non-responders)[6,40]. In the context of these observations, adult pharmacogenomic studies have identified variations in several genes that may be associated with differences in metformin response. Since metformin is not metabolized within the body[41], much of this research has focused on genetic polymorphisms associated with metformin transport, with the strongest evidence for an association with the solute carriers *SLC22A1 (OCT1), SLC22A2 (OCT2), and SLC22A3 (OCT3)* involved in metformin uptake into the bloodstream, and *SLC47A1* (*MATE1*), and *SLC47A2* (*MATE2*) associated with metformin excretion (Table 1). In addition to these candidate genes, a hypothesis-free analysis reported an association of the *ATM* (ataxia telangiectasia mutated) gene with metformin response[42], which was subsequently replicated[43]. The underlying biological mechanism for this association has not been established[42,44] but alteration of AMP kinase activation has been reported with activation or inhibition of ATM[45-47]. Moreover genetic sequencing for integrating physiologic metformin response with a pharmacogenomics approach should also include variants that may be African ancestry-specific or in higher frequency among African ancestry individuals because of the evidence for a greater degree of metformin non-response among African-American youth.

To date, the clinical utility of these genetic polymorphisms of metformin response has not been established[11]. It is also not known whether these genetic variations are associated with metformin's presumed primary mechanism of action, reducing gluconeogenesis. In this pilot study we will provide novel information on the association between change in gluconeogenesis and genetic polymorphisms of metformin response. It will be of particular interest to investigate individuals who do not respond to treatment for genetic variants that might be contributing to that lack of response. This will be the first study in African-American youth to categorize known genetic variants of metformin transport that are common in African ancestry individuals.

Metformin pharmacogenomic targets

There are 3 reasons for integrating physiologic metformin response with a pharmacogenomic approach. First, the frequency of reported variants (Table 1) is sufficiently high that we expect to have carriers of the effect alleles in the studied individuals. For example, we anticipate 13-15 of 32 participants will carry the C allele for rs622342, which has

been associated with reduced HbA1c response to metformin[48]. Second, since the course of type 2 diabetes and metformin response may differ between youth and adults, it is important to understand if the relationship of these variants and clinical outcomes is the same as has been observed in adults. Third, variation in these genes among African-Americans has not been comprehensively described in relation to metformin response. Previous studies of metformin pharmacogenomics that included African-Americans have focused on select variants or on common variation[49-51]. For example, Choi *et al.* began their study by sequencing ethnically diverse individuals, but this study was limited to *SLC47A2 (MATE2)* [49-51]. Given the evidence for a greater degree of metformin non-response among African-American youth, it is imperative to include variants that may be African ancestry-specific or in higher frequency among African ancestry individuals; our sequencing strategy will allow us to detect these variants (Table 1).

Table 1: Single Nucleotide Polymorphisms related to Metformin Response

SNP	Gene	Minor/ Major Alleles	AFR MAF[a]	Chr:bp[b]	Reported Association(s)[c]
rs622342	*SLC22A1 (OCT1)*	C/A	0.27	6:160151834	Increased HbA1c[48]
rs628031	*SLC22A1 (OCT1)*	A/G	0.25	6:160139813	Metformin Intolerance[52]
rs461473	*SLC22A1 (OCT1)*	G/A	0.05	6:160543562	Increased HbA1c[53]
rs662301	*SLC22A2 (OCT2)*	T/C	0.01	6:160696919	Reduced T2D incidence[50]
rs316019	*SLC22A2 (OCT2)*	C/A	0.16	6:160670282	Reduced HbA1c[54]
rs8065082	*SLC47A1 (MATE1)*	T/C	0.37	17:19561878	Reduced T2D incidence[50]
rs2252281	*SLC47A1 (MATE1)*	C/T	0.39	17:19533874	Reduced HbA1c in T2D, reduced glucose in non-T2D [51]
rs12943590	*SLC47A2 (MATE2)*	A/G	0.19	17:19716685	Increased metformin clearance [51], higher HbA1c[49]
rs11212617	*ATM*	A/C	0.24	11:108412434	Less HbA1c control[42,43]

SNP: single nucleotide polymorphism; AFR: African Ancestry; BP: Base Pair; Chr: Chromosome; MAF: Minor Allele Frequency; HbA1c: hemoglobin A1c; T2D: type 2 diabetes. [a]MAF among the African Ancestry samples in the 1000 Genomes data; [b]Human Genome build 37 chromosome and base pair position; [c]Reported association for the individuals with the minor vs. major allele after metformin administration.

We hypothesize that the above 9 variants of pharmacogenomics response will be present in these youth. These variants have been associated with overall glycemic measures (e.g. HbA1c, incidence of type 2 diabetes) and markers of medication tolerance. However, there are no studies examining the association of these variants to the key physiologic target of metformin, the liver. Therefore, despite not having a large sample size, this pilot study will provide new information on the relationship of these variants with changes in gluconeogenesis, and enable us to begin to explore whether known pharmacogenomics variants of metformin response may be useful for predicting changes at the tissue specific level. It is anticipated that the expansion of this study (or other related studies) will be undertaken in due course, depending on the results of the pilot protocol currently being proposed.

Metformin Response and Pharmacokinetics

To elucidate the discrepancy in metformin clinical response in youth and children, a detailed analysis of pharmacokinetic-pharmacodynamic response is needed in youth with type 2 diabetes who are receiving therapeutic metformin doses. Currently, metformin plasma concentrations are not used for routine clinical management and the available literature is sparse and inconsistent. In a recent systematic review, Kajbaf et. Al [55] identified 120 publications with pharmacokinetic and therapeutic metformin values. The majority of the articles cited a therapeutic range between 0.1-4mg/dl although cited individual values varied from 0.129 to 90 mg/dl. The 40-fold variation in these reported therapeutic range is

problematic because it provides no additional information to guide clinical management if levels fall within that wide range.

Interestingly, only 3 of the 120 articles measured "therapeutic" metformin concentrations defined as trough concentrations 12 hours after drug administration and during continual therapy. This is in contrast to the multiple articles that cite maximum concentration (Cmax) as the pharmacokinetic parameter of interest. Therefore, establishing a database or algorithm to define more accurately the therapeutic window of metformin, especially in adolescents is urgently needed. To our knowledge there is only one database of metformin plasma concentrations which was collected from 467 adult patients in France[56]. Mean metformin concentrations were 2.7+/-7.3mg/L with range in plasma levels between 0-61mg/L. This range is markedly greater than the currently published toxic levels of 45mg/L assigned by the International Association of Forensic Toxicologists.

We will assess metformin pharmacokinetics in youth with type 2 diabetes who are receiving metformin daily for 3 months. This information will be used to construct a pharmacokinetic-pharmacodynamic model that may be useful for elucidating mechanisms of metformin responsiveness and non-responsiveness in youth and identifying target drug exposures in this population.

Combination therapy of metformin and liraglutide

Combination therapy is likely to be an ideal therapeutic strategy to address the accelerated trajectory towards metabolic decompensation in youth with type 2 diabetes[57]. We propose that a combination regimen of metformin and liraglutide will be complementary in their glucose lowering effect because of their individual ability to lower gluconeogenesis and enhance the incretin response. This proposal will directly test this hypothesis by comparing African-American youth on combination therapy with metformin and liraglutide to youth treated with metformin alone under basal fasting conditions and during meal absorption.

The secondary aim of this study is to evaluate the between group differences in GLP-1 and GIP area under the curve concentrations. We hypothesize that combination therapy will result in higher GLP-1 and GIP AUC concentrations compared to metformin alone. This hypothesis was based on 2 studies which used a long-acting GLP-1 analog, liraglutide. A preclinical study of liraglutide in mice resulted in 1.4 higher GLP-1 concentrations[58]. In a more recent randomized double blind placebo-controlled cross-over study of liraglutide in 20 individuals with type 2 diabetes, liraglutide significantly increased GLP-1 and GIP concentrations after 17 days[59]. We have also identified 2 additional studies using a short-acting GLP-1 analog (exenatide) which had the opposite effect on GLP-1 response. In a pilot study of patients with Prader Willi Syndrome, exenatide was associated with a reduced GLP-1 response[60]. Similarly, Rother et al. showed exenatide use was associated with lower post-meal GLP-1 concentrations in long-standing patients with type 1 diabetes[61] . Therefore, the effect of GLP-1 analogs on gut hormones (GLP-1 and GIP) is unclear but could be related to type of drug (long vs. short acting), patient characteristics or unknown factors. Our study will help to clarify the effect of a long-acting GLP-1 analog, liraglutide, on incretin hormone secretion in youth with type 2 diabetes.

Liraglutide

Liraglutide is an acylated human GLP-1 receptor agonist that has 97% amino acid sequence homology to endogenous GLP-1 (7-37). Liraglutide directly activates the GLP-1 receptor, leading to glucose-dependent insulin release and glucagon suppression, resulting in improved postprandial glucose homeostasis. A long acting GLP-1 agonist, such as liraglutide, would be a suitable add-on agent to metformin because of its pharmacodynamics, potency and safety[62]. Liraglutide's pharmacokinetic and safety profile in children appears to be similar to that in adults, and it was associated with improved glycemia in a small randomized double-blind placebo-controlled trial of 19 youth[62]. Victoza® (liraglutide) therapy is approved by the Federal Drug Administration (FDA) as an adjunct to diet and exercise to improve glycemic control in patients 10 years and older with type 2 diabetes mellitus (approval on June 17, 2019). The FDA approval was

based on data submitted from the ELLIPSE clinical trial, which was published in the New England Journal of Medicine in April 2019[63]. This trial showed superior efficacy of liraglutide with metformin compared to placebo + metformin for lowering HbA1c. Liraglutide improves overall glucose homeostasis by enhancing glucose-dependent insulin secretion, reducing glucagon secretion and delaying gastric emptying[64]. In addition, liraglutide also lowers fasting glucose concentrations[64,65] but the exact mechanism of action has not been extensively investigated. It is presumed that liraglutide lowers fasting glucose primarily by increasing the endogenous insulin: glucagon ratio that subsequently inhibits hepatic gluconeogenesis and glycogenolysis[66]. However, GLP-1 agonists may also directly lower hepatic glucose production: an extra-pancreatic effect demonstrated in murine models[67], as well as in elegant experiments using prolonged GLP-1 infusion during pancreatic clamps in healthy individuals[68]. If liraglutide decreases glucose production specifically by reducing gluconeogenesis, it would also be important for targeting this early pathological feature of type 2 diabetes in youth.

Study Objectives

The primary objective of this study is to compare the change in gluconeogenesis between the two intervention arms (combination therapy of metformin and liraglutide and metformin only) in African-American youth.

Primary outcome: The change in gluconeogenesis from baseline (prior to study drugs) to 12 weeks.

Secondary outcomes: The change from baseline to 12 weeks in:

1. Glucose production
2. GLP-1 and GIP area under the curve concentrations (AUC) during OGTT and meal absorption
3. Insulin sensitivity
    a. Hepatic insulin sensitivity
    b. Whole body insulin sensitivity
4. Insulin area under the curve concentrations during an OGTT and meal absorption
5. Glycerol and palmitate turnover as an indicator of lipid metabolism.

Exploratory outcomes:

<u>Pharmacogenomic variants of metformin response:</u>

1. Identify the 9 known variants of metformin response (Table 1) in African-American youth with type 2 diabetes
2. Generate a composite genetic risk score based on these 9 variants and determine the relationship of this composite risk score with:
    a. Serum and urine metformin concentrations
    b. Change in gluconeogenesis from baseline to 12 weeks.
3. Screen for unknown genomic variants in metformin response in specific genes of interest.

<u>Gut microbiome and metformin</u>

a) The change in the gut microbial composition and distribution in African-American youth with type 2 diabetes after 12 weeks of metformin.

<u>Metformin response and pharmacokinetic-pharmacodynamic model</u>

b) Construct a pharmacokinetic-pharmacodynamic model based on plasma and RBC metformin concentrations, and treatment outcome response (including change in glucose production, gluconeogenesis, incretin concentrations).

Study Design and Methods

Study Design

This is a pilot randomized parallel design study of African-American youth with type 2 diabetes who are not on insulin therapy, with two intervention arms (combination therapy with metformin and liraglutide, and metformin alone). Participants enter 5-7 day drug-free run-in period prior to initial metabolic evaluation. Participants will be evaluated before and after 12 weeks of study drug(s).

Randomization

The NIH Clinical Center (CC) Pharmacy will perform block randomization stratified by sex. Randomization will be stratified by sex with a block size of 6. The randomization schedules will be constructed by an independent statistician and shared with the NIH CC Pharmacy. The PI and study staff will not view the randomization schedule *a priori*. Subjects will be randomized as they enter the protocol as follows: The PI or study staff will notify the NIH CC pharmacy of the eligible enrolled participant and the pharmacy will assign the intervention arm according to the predetermined randomization schedule. All participant data will be entered into a NIDDK RedCap® database and the participant will be randomized consecutively using the single randomization list maintained by the NIH CC Pharmacy.

Study Protocol

Figure 1 illustrates the study design in which there will be 5 study visits: Visit 1 (screening outpatient), Visit 2 (tracer inpatient), Visit 3 and 4 (follow-up outpatient) and Visit 5 (tracer inpatient). Eligible patients may be identified at CNMC but no study visits will occur at CNMC.

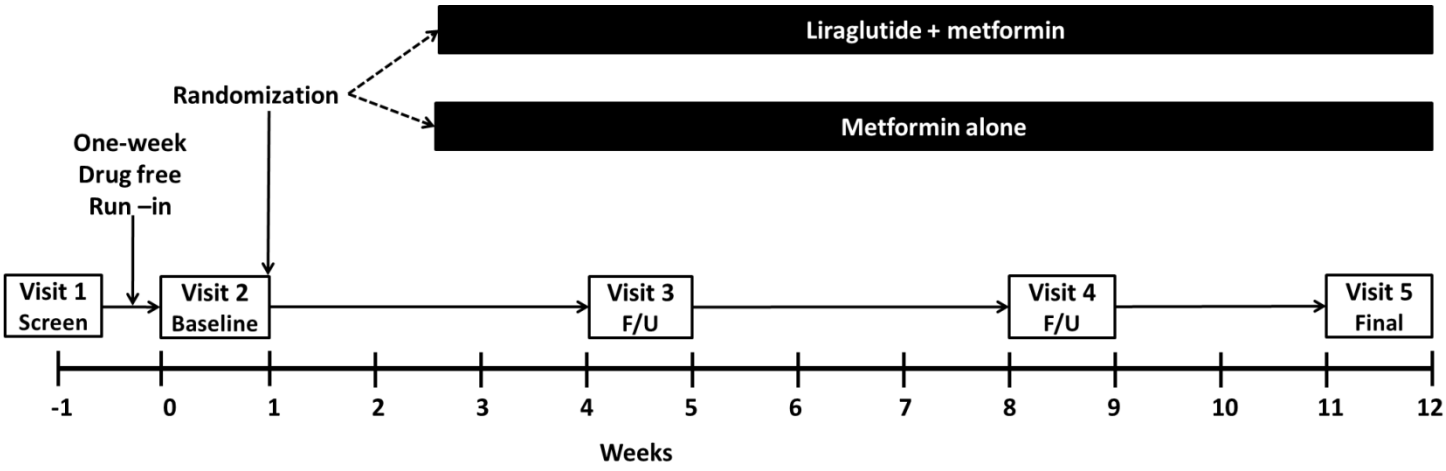


Figure 1 –Study Design: randomized 12-week two-treatment arm clinical trial

Visit 1: Screening Outpatient Visit

This visit is designed to explain the protocol to the participant and their parents, determine participant eligibility, provide study protocol details to the family, and obtain informed consent and assent. Each participant will undergo a history and physical examination, routine blood work (including lipase and amylase) and pregnancy test to determine eligibility. Youth will meet with a member of the nutrition team to discuss your diet and to be educated on keeping 3-day food records before Visit 2 and 5 and will discuss metabolic diet selection during the inpatient stay. In addition, the dietician will perform body measurements including waist, hip and neck circumferences, those measurements will take only few minutes and are not painful.

Education and Blood glucose monitoring during the run-in period

All participants will be seen by a nutritionist and counseled on standard diabetes management, diet and lifestyle management. Participants will be asked to discontinue all anti-diabetic medications for the 5-7 days preceding Visit 2. This drug-free period is necessary to assess the participant's metabolic response off of anti-diabetic medications. During the drug-free period and throughout the study, youth will monitor their blood glucose either by fingerstick blood glucose (FSBG) at least twice daily or they will wear a continuous glucose monitoring (CGM) device. Participants who do not have their own glucometer will be provided with glucose-monitoring devices and supplies and educated on their use. The CGM supplies will be provided by the study team and all participants will receive instruction on how to wear and use the CGM device. All individuals will be closely monitored during the drug-free run-in period with 2-3 times daily blood glucose checks via home glucose monitoring and daily check-in reports by study staff. If severe hyperglycemia, FSBG is >200mg/dl fasting or 300mg/dl pre-dinner, is noted during the run-in period, they will notify the study PI immediately. To monitor participants with elevated FSBG the physician may recommend admission to the NIH metabolic unit prior to Visit 2. Individuals with persistently elevated FSBG and/ or signs and symptoms of metabolic decompensation (e.g. vomiting, dehydration, lethargy, abdominal pain, ketonuria and acidosis) will be withdrawn from the study and restarted on his/ her medication. During the drug-free run-in period, individuals who cannot wear the CGM device or complete fingerstick blood glucose measures or who cannot be reached for ≥3 days during this period, will be withdrawn from the study.

Visit 2: Baseline Tracer Inpatient Visit

Visit 2 will take place ~ 1-2 weeks after the screening visit. All participants will be admitted to the Metabolic Research Unit at the NIH for an overnight tracer visit to measure indices of glucose and lipid metabolism, energy expenditure and obtain samples for genetic analysis prior to initiation of the study drugs. The tracer protocol is illustrated in Figure 2 and outlined below:

a. Admission, oral glucose tolerance test (OGTT) and Dual x-ray absorptiometry (DXA) scan
b. Stable isotope administration and steady state labs
c. Urine collection
d. Indirect calorimetry

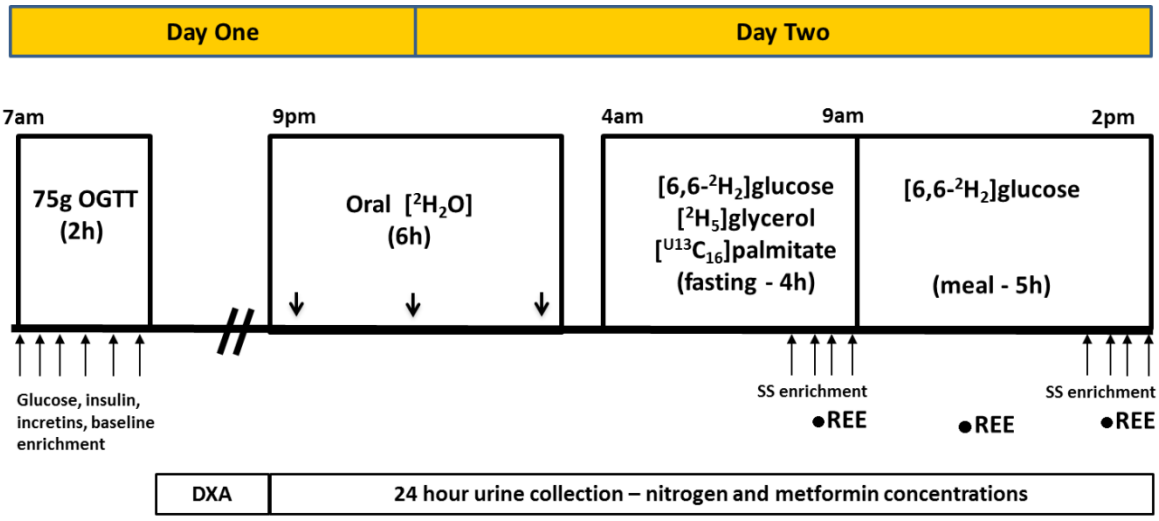


Figure 2 –Study Protocol for Overnight Tracer Visits

a. Admission, OGTT and DXA scan

Youth will be admitted in the morning (~7am) on Day 1 and blood samples for hormones, genetic analysis, inflammatory markers and metabolites (including glucose, insulin, C-peptide, glucagon, and isotopic enrichments of glucose, glycerol and body water) will be collected at baseline. One intravenous catheter will be placed in the arm for blood draws. Blood and urine collection for genetic samples for future analysis will occur on admission for Visit 2 only.

- Oral Glucose Tolerance Test (OGTT)

Youth will undergo a 75gram oral glucose tolerance test with blood samples collection at 0, 15, 30, 60, 90 and 120 minutes for hormones and metabolites (including glucose, insulin, glucagon-like-1 peptide and gastrointestinal inhibitory peptide) and metabolites. To measure blood (plasma and RBC) plasma samples for metformin at baseline, 3ml of blood will be collected at 0-time point.

- DXA Scan

  Dual x-ray absorptiometry scan will be performed during admission Day 1 to evaluate body composition and determine fat mass and lean body mass.

After the OGTT, youth will eat a standard breakfast, a lunch meal (~ 1pm) and a snack (~3pm) to provide ~20%, 30% and 10% of total energy needs respectively. Youth will eat a standard dinner meal at ~ 6pm to provide 40% of total energy needs (50% carbohydrate, 33% fat and 17% protein). Total energy expenditure will be estimated from the from the Mifflin St. Jeor equation [69] with a standardized activity factor. Youth will then be placed NPO at 8pm with ad libitum access to water. Another intravenous catheter will be placed in the evening of Day 1, such that the participant will have two intravenous catheters placed, one in each forearm, for stable isotope infusion and blood draws.

On the first day of admission youth will be asked to review their 3 day food records that they recorded prior to admission with a member of the nutrition team. If youth do not bring these records to this appointment, the nutrition team will perform a 24-hour recall to capture the foods consumed the day prior to admission. This will help the investigators to identify if there are differences in dietary intake prior to admission that may impact the results of the testing. The nutrition staff will also obtain body circumference measures, such as neck, waist and hip circumference. These measurements will be done over minimal clothing with a non-stretch measuring tape.

b. Stable isotope administration and steady state labs

Stable isotope administration will begin the night of the admission; glucose appearance, fractional gluconeogenesis and glycerol turnover will be measured with [6,6$^2H_2$]glucose, deuterated water [^2H_2O], [U$^{13}C_{16}$]potassium palmitate and [$^2H_5$]glycerol respectively. Lean body mass (LBM) measurements from dual x-ray absorptiometry scans will be used to calculate the isotope administration rates. Deuterated water (3 gram●kg_{LBM}) will be administered in 3 divided doses at 9pm, 12am, and 3am. Primed (60×the minute infusion rate) and continuous infusions of [6,6$^2H_2$]glucose and [$^2H_5$]glycerol will start at 4am (t= -240m) and continue until 9am (t= 0m). At 730am, an unprimed [U-$^{13}C_{16}$] palmitate infusion will be started. At 9am (t= 0m), the glycerol and palmitate infusions will be discontinued. The [6,6$^2H_2$]glucose infusion will continue until 2pm (300m) to quantify gluconeogenesis during meal absorption using a continuous feeding protocol with a nutrient drink and [6,6$^2H_2$]glucose intravenous infusion will be used to mimic the steady-state prandial state[70,71]. At 9am (t= 0m), participants will be fed a lactose-free nutrient drink to provide ~50% of estimated total energy needs and macronutrient composition that matches the previous days' meals in equal portions every 30 minutes over the 5h

of feeding. The purpose of this feeding strategy is to evaluate the acute response to refeeding, and not to provide full caloric requirements[71]. Isotope enrichment will be measured over a period of 30 minutes during steady state. Therefore, blood samples for steady-state conditions will be obtained at the following time periods: (1) fasting between 830-9am (t= -30 to 0m) and (2) prandially between 130-200pm (t=270 to 300m). Blood samples for hormones and metabolites (including glucose, insulin, incretins in protease inhibitor treated tubes) will be collected every 30 minutes between 9am and 2pm. Whole blood will be centrifuged and plasma transferred and frozen at -80C until analyses.

c. Urine collection
A timed 24-hour urine collection to measure urinary nitrogen will start at noon on admission and continue until noon on the study day. Total daily urinary nitrogen excretion will be used to determine the total body glucose and lipid oxidation rates as previously described [72].

d. Indirect calorimetry
To calculate substrate oxidation, indirect calorimetry (hood) will be performed for 30-45 minutes between 7-8am, and 1-2pm to measure oxygen consumption and carbon dioxide production rates.

e. Stool Sample
A research stool sample will be collected at home and brought to the visit or collected during the admission. The stool sample will be stored at 4C in a sterile plastic vial and processed within 24 hours of collection.

f. Side-effect Questionnaire
Questionnaires will be completed by the participants at the study visit (Appendix A)

g. Blood glucose monitoring
Blood glucose will be monitored with either fingerstick blood glucose twice daily (fasting and bedtime) or by using an FDA- approved continuous glucose monitoring device (CGM) (for example, the Dexcom® CGM System). The device will be used to monitor glucose in real-time, approximately every 5 minutes. The system consists of a small sensor, transmitter, and hand-held receiver. The small sensor, with a small needle attached, will be inserted subcutaneously. The transmitter, which is attached to the sensor, will send the measured glucose to the receiver. The sensor is changed per manufacturer recommendations. Participants will receive training on removing and replacing the sensor and education on the signs and symptoms of hyperglycemia see Appendix B. All CGM or glucometer supplies will be provided by the study team.

Visit 3 and 4: Follow-up Outpatient Visit
Participants will return to the NIH CC clinic for an outpatient follow-up visit. Visit 3 will occur between 4-5 weeks and Visit 4 will occur between 8-9 weeks. The procedures at Visit 3 and 4 will be identical, and are outlined as follows:

1. Blood glucose records and glucometers will be reviewed.
2. Pill and pen counts performed by the study staff.
3. Blood and urine samples will be collected for glucose and metformin monitoring (including hemoglobin A1c, metformin concentrations).
4. We will review medication adherence and tolerance and emphasize compliance with study visits.
5. A research stool sample will be collected at home and brought to the visit or collected during the admission. The stool sample will be stored at 4C in a sterile plastic vial and processed within 24 hours of receipt.
6. We will collect questionnaire to assess side effects after starting the medication or changing dose. The sample questionnaire is in Appendix A.

Visit 5: Final Tracer Study
Participants will return for an inpatient tracer visit protocol after 12 weeks ± 14 days on study medication. Participants will receive their last dose of study medication at supper in the evening of admission Day 1 during Visit 5.

Visit 5 procedures will be identical to Visit 2 above with the following additional blood sampling for metformin concentrations. To measure blood (plasma and RBC) plasma samples for metformin for participants within the study receiving metformin at doses 1000-2000mg daily. Samples for metformin analysis (3ml of whole blood) will be collected, processed for isolation of plasma and RBC and frozen at -80C until analysis during the OGTT as follows:

a) Day 1 during the OGTT at time 0, 0.5h, 1h, 1.5h, 2h, 3h 4h, 6h, 8h and 8pm (pre-second dose).

b) Day 2 at 8am and at 2pm

After completion of Visit 5, participants will be instructed to resume their home medication regimen.

Study Agents/ Intervention

Two study agents will be used: metformin oral 500mg oral tablet and liraglutide (6mg/ml, 3ml) solution for subcutaneous injection, pre-filled, multi-dose pen that delivers doses of 0.6mg, 1.2mg or 1.8mg. Both metformin and liraglutide will be used within the approved dosing regimens. Neither drug will be altered from the approved dosage formulation. Table 3 illustrates the titration schedule for each study drug.

Table 3: Titration Schedule for Metformin and Liraglutide

Study schedule	Week	Liraglutide (subcutaneous injection)	Metformin (oral tablet)
Run-in	-1 to 0	-	-
After baseline visit	0-1	0.6mg once daily	500mg once daily
	1-2	1.2mg once daily	500mg twice daily
Target study dose	3-12	1.8mg once daily	1000mg twice daily

The participant will start the study drug(s) on Day 2 of Visit 2, after the tracer protocol is completed. The PI will review blood glucose logs and/ or CGM readings weekly and ensure that the study drug(s) titrated to the highest tolerable dose as guided by Table 3 above. For patients on combination therapy, the dose of liraglutide will not be increased if the fasting blood glucose is <75mg/dl on 2 or more consecutive days. If the fasting blood glucose subsequently rises to ≥90mg/dl on 2 or more consecutive days, the dose of liraglutide will increased by 0.6mg once daily every 5-7 days to achieve maximum tolerable dose.

Dose titration for gastrointestinal intolerance
For participants on the combination therapy arm, who experience gastrointestinal side effects, the dose of liraglutide will be decreased first. Liraglutide dosage will be decreased by 0.6mg once daily. If gastrointestinal symptoms persist after 2-3 days, metformin dose will be decreased by 500mg. If symptoms persist, dose adjustments will be made every 2-3 days, alternating between liraglutide (0.6mg) and metformin (500mg) dose reductions. If the highest tolerable metformin dose is < 1000mg daily or liraglutide <0.6mg daily, the subject will be withdrawn from the study.

For participants on metformin alone, metformin will be decreased by 500mg every 2-3 days until symptoms resolve. If the highest tolerable dose of metformin is <1000mg daily, the subject will be withdrawn from the study.

If gastrointestinal symptoms resolve, and at the discretion of the PI, the dose of metformin and/or liraglutide may be increased every 2-3 days to the maximum tolerable dose. Incremental dose increases will be as follows: metformin 500mg every 2-3 days and liraglutide 0.6mg every 2-3 days.

Dispensing and Storing Study Medications

The NIH CC Pharmacy will acquire and store metformin and liraglutide medications for the study. The study medication(s) will be coded and dispensed by the NIH CC Pharmacy. The pharmacy will dispense the study medication(s) according to the predetermined randomization table. The Randomization section contains additional details on the processes we will use to assign randomization. The storage of medications will be according to the package insert for each study drug.

Study medications will be dispensed to the patient by the NIH CC pharmacy. At the end of Visit 2, 3, and 4, patients will receive 1 month supply of medications with 20% overflow to improve compliance and accountability as indicated below.

Medication dispensing schedule:
Visit 2: Metformin (144 tablets) +/- Liraglutide (3 pens)
Visit 3: Metformin (138 tablets) +/- Liraglutide (3 pens)
Visit 4: Metformin (142 tablets) +/- Liraglutide (3 pens)

Compliance and Adherence

When potential participants are considering participation, the importance of compliance with the intervention assignment will be stressed. The ability to adhere to the protocol of the study, and take medication daily, as instructed is one of the inclusion criteria of the study and will be underscored. One month supplies of medication will be dispensed by the NIH CC pharmacy. Medication adherence will be determined by study personnel counting of pills and pens that were dispensed at each medical visit and returned at the next medical visit (Visit, 3, 4 and 5). The percent of pills taken over the 3-month study period will be assessed. We will employ the following strategies:

1. Twenty-percent extra of metformin tablets will be added to improve compliance, as the participants will not know how many extra pills are sent (although they will be told that there are more than the exact amount of pills so that we can be sure they are taking it). Participants will return the remaining doses at the next visit and receive another month supply with 20% extra. The number of unused tablets will be recorded.
2. Adherence to scheduled blood glucose monitoring will be assessed by reviewing the participant's glucose meter or CGM device.
3. The participants will be contacted every 1-2 weeks by phone, email and/or text message to encourage and assess compliance. Text and email-based reminders are an important adherence tool which can be tailored to the needs of the individual youth and family. Text messaging has been shown to improve outcomes in African-American children with other chronic diseases, such as asthma [73] and sickle cell anemia [74] and in adults with type 2 diabetes [75].

Follow-up

Post-Study Treatment

The patient's clinically relevant data, available at the end of the study, will be shared with their diabetes provider with the consent of the participant and/or the parent. At the family's request, it will also be provided to his or her primary care provider. After completion of this study, participants and families will discuss their diabetes control with their providers and it will be up to the clinical diabetes provider and the patient/ family to decide whether they would like to continue liraglutide, after reviewing the potential risks and benefits.

Post-Study Obligations

There are no anticipated post-study obligations.

The study drugs (metformin and liraglutide) will not be made available to participants through this study protocol either at the end of the study or if they are withdrawn from the study prior to completion. However, the patient's clinically relevant data will be shared with their diabetes provider with the consent of the participant and the parent in order to help them decide whether they would like to continue the medication(s) outside of this study protocol.

Inclusion and Exclusion Criteria

Inclusion Criteria

Primary randomized protocol

1. Youth must self-identify as African-American and identify both parents as African-American
2. Age 12- 25 years
3. Pubertal or post-pubertal: Girls – Tanner stage IV-V breast; Boys – Testicular volume 11-25cc
4. Diagnosis of type 2 diabetes of ≤5 years duration, as per American Diabetes Association Criteria[76]
5. Hemoglobin A1C <9% at study initiation
6. Negative to mild ketonuria without acidosis (negative or 1+ ketones on urinalysis)
7. Negative test for diabetes-related autoantibodies (glutamic acid decarboxylase 65 and tyrosine phosphatase-related islet antigen 2 (IA-2))
8. Willing and able to take daily medications and check blood glucose levels at least twice per day or wear a continuous glucose monitoring device (CGM).

Exclusion Criteria

1. Pregnancy or breastfeeding
2. Allergy to study medications
3. Allergy to milk protein
4. Chronic insulin therapy
5. Treatment with other medication which are known to affect the parameters under study **(**for example sodium-glucose transporter 2 (SGLT-2) inhibitors, dipeptidyl peptidase-4 (DPP-IV) inhibitors, non-selective beta blockers).
6. Metabolic derangement such as metabolic acidosis, severe hyperglycemia (fasting blood glucose ≥ 200mg/dL), and/or liver enzymes > three times the upper limit of normal.
7. Personal or family history of medullary thyroid cancer or Multiple Endocrine Neoplasia syndrome type 2
8. Any other condition that, in the opinion of the investigators, will increase risk to the subject, or impede the accurate collection of study-related data.
9. Body weight ≥ 450lbs
10. Body weight ≤58kg
11. Serum triglyceride concentrations ≥500mg/dl
12. Hemoglobin concentration <10g/dL

Rationale for Inclusion Criteria

African-American Youth

Individuals will be considered to be African-American if they self-identify as African-American and describe both parents as being African-American. In brief, African-American youth are disproportionately affected by type 2 diabetes and have lower treatment efficacy and higher morbidity rates compared to other ethnic groups. This project is designed to

investigate the underlying biological determinants (including genetic) in African-American youth with type 2 diabetes and provide information that will help to reduce the burden of disease in this vulnerable population.

Age Range 12- 25 years and pubertal status

The age range of 12- 25years was chosen to maximize recruitment and includes a wide range of youth afflicted with type 2 diabetes. However, using a wide age range increases the potential to recruit youth across the pubertal spectrum and puberty is associated with changes in insulin resistance. Therefore, to minimize clinical variability, we will only recruit pubertal or post-pubertal youth (defined as Tanner stage IV-V for girls and testicular size 11-25cc for boys). We believe this approach is reasonable because we will have a well-defined cohort (by pubertal status) and a balanced distribution in intervention arm by sex (randomization stratified by sex). In addition, the primary outcome for this study (change in gluconeogenesis) varies minimally with age or pubertal status among pre-pubertal and pubertal youth[10,77].
Pubertal status will be defined based on breast examination (Tanner stage IV-V) in girls and testicular examination (11-25cc) in boys. Pubic hair and bone age will not be used to characterize pubertal stage.

Diabetes diagnosis criteria and HbA1c <9%

Type 2 diabetes in youth will be diagnosed according to ADA criteria[76] and eligibility criteria were based on the Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) study design[6]. Our overall aim is to help identify early therapeutic targets in youth with type 2 diabetes. In order to achieve this goal, we want to evaluate drugs that could be used early in the course of diabetes, before the onset of severe hyperglycemia, and ones that will work effectively at physiologic targets to help stem the rapid progression of type 2 diabetes in youth. Therefore, we are recruiting youth who are relatively early in the disease course as defined as having a diagnosis of diabetes ≤5 years duration with mild to moderate hyperglycemia as defined by HbA1c <9%. Youth with HbA1c ≥9% often have severe hyperglycemia and glucotoxicity which is associated with increased risk for dehydration and ketoacidosis and these conditions would interfere with measurements of insulin sensitivity and glucose production.

Rationale for Exclusion Criteria

Pregnancy and Breastfeeding

Pregnancy and breastfeeding are associated with physiologic increases in insulin resistance, which would confound data interpretation. In addition, the DXA scan cannot safely be performed in pregnant women. Female participants of childbearing age who meet other eligibility requirements and wish to participate in the study will be informed of the potential risks to a pregnancy conceived while on any study medication. Participants will also be informed of the potential risks of hyperglycemia to a pregnancy including fetal malformations, pre-term delivery and increase maternal complications. Participants who consent to participate will be asked to practice reliable birth control including systemic hormones and/or barrier methods. Patients who are pregnant and/or sexually active and not using adequate birth control will be excluded from enrollment in the trial. Pregnancy tests will be obtained from all female participants before inpatient visits and right before DXA scans.

Chronic insulin therapy

Participants who are currently taking insulin therapy or who are within 3 months of insulin treatment will be excluded from the study. Insulin therapy will directly affect hepatic glucose production and insulin sensitivity.

Any condition which may increase risk to the subject or impede accurate data collection

Examples include participants with a history of active thyroid disease, liver disease, pancreatitis, nephrotic syndrome or lupus. Liraglutide may be associated with an increased risk for pancreatitis and C-cell carcinoma. Therefore, any individual with a history of acute or chronic pancreatitis, or increased risk for pancreatitis as indicated by elevated serum triglycerides≥500mg/dl at baseline, will be excluded. Hypertriglyceridemia is a well-known common cause of

pancreatitis. In general, the risk for pancreatitis occurs in patients with severe hypertriglyceridemia >1000mg/dl[78]. However, the exact lower threshold of risk is not well defined. Although African-Americans have lower triglyceride concentrations compared to other US ethnic groups, there are no studies that would suggest that African-Americans have a higher population risk for pancreatitis at lower triglyceride concentrations. Therefore, in accordance with National Cholesterol Education Program ATPIII guidelines, we propose using the current recommended triglyceride threshold of ≥500mg/dl for denoting increased risk for pancreatitis[78]. In addition, any individuals with a personal or family history of medullary thyroid cancer or Multiple Endocrine Neoplasia syndrome type 2 will be excluded.

Weight ≥ 450lbs
Individuals with weight ≥ 450lbs will be excluded because the maximum weight for the dual energy absorptiometry (DXA) scan is 450lbs.

Clinical and Laboratory Methods

Glucose Turnover

During the stable isotope tracer protocol, hepatic glucose production will be partitioned to evaluate rates of gluconeogenesis and glycogenolysis overnight and during a meal. Rates of glucose and glycerol turnover will be calculated as described below.

Rates of glucose appearance ($Ra_{glucose}$) will be calculated under near-steady-state conditions using the average enrichment of [6, 6-$_2H_2$]glucose into the systemic circulation using conventional isotope dilution calculations [77].

$$Raglucose = \frac{Infusate\ enrichment}{Plasma\ enrichment} \times \ Rate\ of\ infusion\ of\ labeled\ glucose$$

Under fasting and steady state conditions, rate of glucose appearance reflects hepatic glucose production.

Glucose production rate is the sum of the rates of gluconeogenesis and glycogenolysis [20]:

$$Glucose\ production\ rate = Ra\ total - \text{rate of infusion of labeled glucose}$$

Fractional gluconeogenesis will be determined using ^2H_2O and the average enrichment of ^2H enrichments of carbon 1,3,4,5,6 of glucose as previously described [79].

$$Fractional\ gluconeogenesis = \frac{\left[\frac{(M+1)2_H(m/z169)}{6}\right]}{2_H2O}$$

where $(M+1)^2H(m/z169)$ is the M+1 enrichment of ^2H glucose measured using the mass-to-charge ratio (*m/z*) 170/169 fragment of glucose, 6 is the number of ^2H labeling sites on the *m/z* 169 fragment of glucose, and ^2H_2O is the enrichment in body water.

Absolute rates of gluconeogenesis and glycogenolysis will be calculated as follows:

$$Rate\ of\ gluconeogenesis\ = Ra\ total \times\ fractional\ gluconeogenesis$$

$$Rate\ of\ glycogenolysis = glucose\ production\ rate - rate\ of\ gluconeogenesis$$

During meal ingestion, the Ra of dietary glucose into the plasma pool (Ra meal) will be estimated from the carbohydrate content in the liquid meal. We assumed that ~85% of orally ingested glucose enters the systemic circulation in the normal volunteers [80].

The rate of glycogenolysis during meal absorption ($Glycogenolysis_{feed}$) will be estimated as:

$$Glycogenolysisfeed = Ra\ total - (Gluconeogenesis + Ra\ meal + infusion\ rate\ of\ labeled\ glucose)$$

Glycerol and Palmitate Turnover

Rates of appearance (*Ra*) will be calculated under near-steady-state conditions using the average enrichment of [$^2H_5$]glycerol or [U-$^{13}C_{16}$] palmitate into the systemic circulation using conventional isotope dilution calculations [77].

$$Ra = \frac{Infusate\ enrichment}{Plasma\ enrichment} \times\ Rate\ of\ infusion\ of\ labeled\ glycerol\ or\ palmitate$$

Insulin Resistance

Hepatic insulin sensitivity we will be calculated using the hepatic insulin sensitivity index by measuring hepatic glucose production in relation to the fasting insulin concentration [81]. Since acute changes in insulin concentration decrease glycogenolysis but gluconeogenesis remains constant, glycogenolysis is the component of hepatic glucose production that is most sensitively regulated by insulin [70,82]. Indices of hepatic sensitivity that include gluconeogenesis may underestimate the effect of hepatic insulin action and could blunt the assessment of insulin sensitivity. Therefore, we will also calculate a hepatic insulin sensitivity index using glycogenolysis and fasting insulin concentration[83].
Whole body insulin sensitivity will be calculated with the Matsuda index during the oral glucose tolerance test [81].

Pharmacogenomic Analysis of Metformin

Blood collection for genetic samples for future analysis will occur on admission for Visit 2. We will take two approaches to address this exploratory aim. First, we will assay all participants using a genome-wide genotyping chip. The chip used will be selected based on the best option for this purpose at the time of assay, though one likely option is Illumina's Multi-Ethnic Genotyping Array (MegaChip), which included population genetic studies of African-Americans in its design and allows for some customizable content. The Chip data will give us genome-wide genotyping of common variants that will include (by design or customization) variants previously associated with metformin response (Table 1). In addition, chip data will be used to adjust the analyses for genome-wide proportion of African ancestry [84], an important covariate in the data analysis of admixed African-Americans. Variants were selected for analysis based on evidence for an interaction between the variant and glucose-related metformin response. These variants were further limited to those with evidence from more than one study or in genes with strong biological plausibility. Additionally, we will sequence the entire gene and promoter region of genes with the strongest evidence for an association with metformin response: *SLC22A1*, *SLC22A2*, *SLC22A3*, *SLC47A1*, *SLC47A2*, and *ATM*. As most of the study of these pharmacogenomic loci has been conducted in non-African ancestry samples, genetic variation relevant for African-Americans youth may have been missed.
Secondary genetic findings: A "secondary genetic finding" describes a finding that is not related to the primary reason that the study is being done. While identifying secondary genetic findings is usually the realm of genome- or exome-wide sequencing studies, common genome-wide genotyping chips, such as the MegaChip, have added a set of rare variants that are considered by the American College of Medical Genetics and Genomics (ACMG) to be actionable. An individual's knowledge of carrying these rare variants can have significant implications for medical decisions in terms of treatment or monitoring in individuals and their families. It is expected that whole exome sequencing would identify a clinically actionable variant among 1.2 - 3.4% of individuals sequenced [85]. With the sample size of the current study and considering that chip array is substantially less comprehensive than whole exome sequencing, this analysis is likely to identify one or no participants with actionable variants. Given the potential medical value of this information, however, we will evaluate the ACMG variants on the chip and will be prepared to return these findings to participants using the following procedures. First, the ACMG variants will be extracted from the chip data, and the genotypes for all

participants at these loci will be evaluated. If an actionable variant is identified at one of the ACMG loci, a new blood sample will be requested, as these results are intended for research purposes only. At this point, participants will be informed that they may carry a genetic variant that could have important consequences for their health, but that these findings will need to be confirmed. The blood sample will be sent to a commercial laboratory to obtain Clinical Laboratory Improvement Amendments (CLIA)-certified results. If the finding is confirmed, a genetic consult will be requested at the NIH Clinical Center, and a genetic counselor will meet with the participant and/ or the participant's parent. The research team will offer to pay for the cost of confirmatory findings.

Microbiome Analysis

Fecal samples will be collected in sterile cryovials and stored at 4C for less than 24 hours. The sample will be transferred to -80C and stored until further analysis. This analysis will be performed in collaboration with Dr. Sushil Rane and Dr. Hariom Yadav. After thawing of fecal sample, fecal DNA will be isolated for microbial compositional analysis using PowerSoil® DNA Isolation Kit. Amplicon sequencing using next generation technology (bTEFAP®) will be used to for microbial sequencing using Illumina MiSeq and HiSeq platforms technologies. In brief, the 16S universal Eubacterial primers 27F AGAGTTTGATYMTGGCTCAG and 519R GTNTTACNGCGGCKGCTG will be utilized to evaluate the microbial ecology of each sample on the Illumina MiSeq with methods via the bTEFAP® DNA analysis service. A single-step 30 cycle PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) will be used under the following conditions: 94C for 3 minutes, followed by 28 cycles of 94C for 30 seconds; 53C for 40 seconds and 72C for 1 minute; after which a final elongation step at 72C for 5 minutes will be performed. Following PCR, all amplicon products from different samples will be mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Samples will be sequenced utilizing the Illumina MiSeq chemistry following manufacturer's protocols.

The Q25 sequence data derived from the sequencing process will be processed using a pipeline (MR DNA, Shallowater, TX). Sequences will be depleted of barcodes and primers then short sequences < 200bp are removed, sequences with ambiguous base calls removed, and sequences with homopolymer runs exceeding 6bp removed. Sequences are then denoised and chimeras removed. Operational taxonomic units (OTU) will be defined after removal of singleton sequences, clustering at 3% divergence (97% similarity)[86-89]. OTUs will be taxonomically classified using BLASTn against a curated GreenGenes/RDP/NCBI derived database[90] and will be compiled into each taxonomic level into both "counts" and "percentage" files. Count files contain the actual number of sequences while the percent files contain the relative (proportion) percentage of sequences within each sample that map to the designated taxonomic classification.

Metformin Pharmacokinetics

Samples will be processed for isolation of plasma and RBC and frozen at -80C until analysis. Both plasma and RBCs will be processed in duplicate cryotubes (800 – 1000 µl per cryotube for each time-point collected). Cryotubes will be labeled with participant ID and sample time collection and the timing of metformin administration documented. Metformin is stable under refrigerated conditions for up to 24 hours after collection- therefore samples may be kept in the fridge for pickup and processing the next day (the later PK time points- 8 and 12 hours). Analysis for metformin concentration will be via a validated LC-MS/MS assay.

Collection and Storage of Human Specimens or Data

Research Use, Storage and Disposition of Human Subjects' Samples

For future reference and potential use, we will store all samples (blood or fluids) in our locked freezers for an unlimited period of time. Samples will be labeled with coded identifiers linked to patient identity only via a secured database.

Research records and data with personal identifiers will be stored in our locked offices, the medical record department, and the electronic study database. This material will additionally be protected by medical record and computer access procedures. Access to records and data associated with personal information will be restricted to the Principal Investigator, Co-Investigators, study support staff, and database support staff.

Subjects may request that unused samples be removed from our freezers and be destroyed. If no such request is made, we will keep samples until they are completely used or no longer of scientific value, at which time they will be destroyed. We do not plan to destroy personal medical information or stored data. The Principal Investigator will report loss or destruction of data or samples to the IRB.

Genomic Data Sharing Plan

This protocol involves the collection of human and non-human genomic DNA. In the pharmacogenetics study of metformin, genomic DNA from patients will be stored for genome-wide testing and targeted gene sequencing. Fecal samples will also be stored for analysis of microbial DNA. The participants will consent for their data to be shared through publicly accessible databases. De-identified data from this study will be made available by submission to dbGAP or a similar publicly available database, for which user registration is required. Data will be submitted at the time of publication and will include the relevant genomic data and corresponding phenotypic data. Users must agree to the conditions of use governing access to the public release data, including limitation of research to investigations consistent with the participants' consent, restrictions against attempting to identify study participants, destruction of the data after analyses are completed, reporting responsibilities, restrictions on redistribution of the data to third parties and proper acknowledgment of the data resource.

Materials Transfer Information

Stored samples and/or data may be sent to outside collaborating laboratories, or shared with other NIH collaborating investigators, to study questions related to diabetes or its complications (including, for example: glucose metabolism, diabetes, obesity, weight, and lipid metabolism). Samples may be sent to outside commercial laboratories for analysis. Samples and data sent to outside laboratories and collaborators for analysis and/or testing will contain only coded numbers, without personal identifiers. Materials Transfer Agreements will be completed before the exchange of samples and/or data with outside collaborators.

Samples for isotopic enrichment of gluconeogenesis and other metabolites will be centrifuged and plasma stored at -80C. The samples will be run in duplicate at the Baylor College of Medicine, Children's Nutrition Research Center and at the NIDDK, Mass Spectrometry Core Laboratory. Coded samples without personal identifiers will be sent to Dr. Morey Haymond (co-investigator) at the Children's Nutrition Research Center, Baylor College of Medicine. After IRB approval, a Materials Transfer Agreement will be executed between NIDDK and Baylor College of Medicine, Houston, TX. No data or samples will be transferred without fully executed tech transfer agreements.

Statistical and Power Analyses

Sample Size Justification – Primary Study

Sample size estimates are based on the mean changes and standard deviations observed in our pilot study (Table 2). Assuming a two-sided alpha of 0.05 and standard deviations of change from baseline of 0.20 mg●kg_{lbm}●min for gluconeogenesis and 0.50 mg●kg_{lbm}●min for glucose production, a trial with 12 participants in each group will have 80% power to detect a difference between metformin and combination therapy of 0.24 mg●kg_{lbm}●min in gluconeogenesis and in 0.60 mg●kg_{lbm}●min in glucose production. Analyses within each group will have 80% power to detect a change from zero if the true change is ≥0.18 and ≥0.44 mg●kg_{lbm}●min for gluconeogenesis and glucose production respectively. For

the secondary outcome of change in GLP-1 AUC after an OGTT, we will have > 95% power to detect the mean difference of 101 pg●min/ml. **Assuming a 25% attrition rate, we will recruit 16 in each group.**

Table 2: Mean and Estimated Rates of Gluconeogenesis and Glucose Production and Projected Effect Sizes*

	Baseline Mean (SD)	Follow-up Mean (SD)	Difference Mean (SD)	15% Decrease	20% Decrease
Gluconeogenesis (mg●kg_{lbm}●min)	1.78 (0.41)	1.73 (0.31)	0.05 (0.19)	0.27	0.36
Glucose Production (mg●kg_{lbm}●min)	2.93 (0.92)	2.67 (0.54)	0.27 (0.50)	0.44	0.59
Early intact GLP-1 AUC (pg●min/min)	137 (113)	238 (128)	101 (32)	-	-

**Data from pilot study in 5 youth with type 2 diabetes treated with metformin 1000mg twice daily for 3 months.*

This power analysis was performed with two-sided T tests to detect a between group difference of a 15% change in gluconeogenesis which would require 12 individuals per group. Our primary assumption is that there will be a 15% difference in gluconeogenesis between the two study arms. This assumption is underscored by the fact that metformin will not result in a change in gluconeogenesis *in vivo* [24,26,27] but liraglutide will decrease rates of gluconeogenesis by 15%. This sample size is in keeping with previous studies assessing metformin effect using similar techniques to measure of gluconeogenesis [24,25]. Nevertheless, we acknowledge that the delta difference and SD for change between two differently treated groups could be larger. Therefore, this pilot study will provide valuable preliminary information for the magnitude of the difference between intervention groups that would be crucial for a larger randomized placebo controlled trial.

Analysis of Primary and Secondary Outcomes

The primary outcome, the change in gluconeogenesis from baseline to 12 weeks, will be analyzed using an analysis of variance that compares the two randomized groups adjusting for baseline levels. Similar analyses will be performed for the secondary outcomes, using transformations or nonparametric tests as appropriate. Secondary regression analyses will adjust for the effect of other variables (such as fat mass, metabolite and hormonal concentrations etc.) on these changes.

Analysis of Exploratory Outcomes

Pharmacogenomic Variants of Metformin Response

Given the limited sample size, the proposed genetic aims are exploratory in nature. First we will use 9 previously-identified genetic variants (Table 1) to construct a genetic risk score, calculated as the sum of the risk alleles that the individual has for any of the variants. Metformin response will be assigned as a 15% change in gluconeogenesis from baseline (minimum clinically relevant effect size) for each individual[26,91]. We will then compare this risk score in the metformin responders vs. non-responders. The genetic risk score based on these 9 variants associated with metformin response in adults will be analyzed as a single predictor, and not by individual variants. Since this approach may miss important variation in these genes, particularly given that the question of metformin response has been less well studied among African-Americans, we will also sequence the genes of interest in these individuals.

We will use regression models to determine if this risk score predicts metformin response in terms of changes in rates of gluconeogenesis and metformin concentrations in serum and urine, after adjustment for genome-wide proportion of African ancestry (additional covariates will be evaluated for inclusion), with $p<0.05$ considered statistically significant. Rare variants identified during targeted gene sequencing will be analyzed in a composite way, collapsed by gene and considered with a rare variant analysis tool (e.g. SKAT [92]). While this analysis may have limited power, the identification

of additional variants in these genes among African-American youth will inform future larger studies. Additionally, we will determine whether adding the sum of the risk alleles as covariates modifies the statistical analyses for the outcomes in Aim 1 and Aim 2. The composite genetic risk score will be used to adjust the primary outcome analysis of change in gluconeogenesis to see if accounting for this variation influences our conclusions. These results will be evaluated using a likelihood ratio test comparing a regression model adjusted and unadjusted for the variant.

Gut Microbiome and Metformin

Statistical analysis will be performed using a variety of computer packages including XLstat, NCSS 2007, "R" and NCSS 2010. Alpha and beta diversity analysis will be conducted as previously described[86-88,93] using Qiime (www.qiime.org). Significance reported for any analysis is defined as $p<0.05$.

Metformin Pharmacokinetics

Statistical analysis and non-linear mixed effect modeling will be performed using the "Phoenix WinNonlin", an industry standard for non-compartmental analysis, pharmacokinetic/ pharmacodynamic modeling.

Multiple-site Studies

Eligible patients may be identified through CNMC but no enrollment, informed consent or study visits will occur at CNMC.

The primary IRB on record will be NIH IRB. A Federalwide Assurance for Human Subject's Protection (FWA) for Children's National Medical Center is FWA00004487 and for PBRC is FWA 00006218.

We will also utilize the Translational Research in Pediatrics Program (TRIPP), an established contractual agreement between CNMC and the National Institutes of Health Clinical Center (NIH CC). A Reliance agreement has been executed between NIH and CNMC.

Human Subject Protection

Informed Consent/Assent Procedures

Informed consent/ assent will be obtained as consistent with the requirements of SOP 301 and 402. Written consent/assent will be obtained from each subject after detailed explanations of the planned procedures by the principal or an associated investigator. . Prospective subjects will be given the research information in layman's term and in language understandable to the subjects. The consent process will take place prior to any study procedures. Subjects have the right to withdraw participation from this protocol at any time.

When a document that is in electronic format is used for the documentation of consent, this study will use the iMED platform, which is 21 CFR, Part 11 compliant, to obtain the required signatures. The iMED consenting process will happen at NIH clinical center. All patient identification will be confirmed with hospital issued wrist band. During the consent process, participants and investigators will view individual copies of the approved consent document on screens at the NIH Clinical Center. Both the investigator and the participant will sign the document with a hand signature using a finger, stylus, or mouse.

Language for Minors

Written informed consent and assent will be obtained from the minor and his/her parent/guardian or (LAR) prior to any screening visits, study procedures or interventions. The Principal Investigator or other designated qualified protocol investigators will explain the study in language understandable to the parent/guardian or (LAR)). The investigator will

also explain the study to the minor who is of a younger age and level of understanding. Sufficient time and opportunity will be given for discussion of the research as well as to answer any questions they may have, taking care to minimize or eliminate the perception of coercion or undue influence. In accordance with 45 CFR 46.408, [the parent/guardian or (LAR)], will sign the current IRB approved informed consent. The minor will provide assent as appropriate for the child's age and level of understanding, and sign the assent document if possible. The investigator will sign the assent as well. A copy of the consent and assent will be given to the minor and [his/her [parent/guardian or (LAR)] for future reference. The signed documents will be sent to the Medical Records Department for placement in the subject's permanent CC medical record. The consent process will additionally be documented in the electronic medical record (CRIS).

For minors who reach the age of consent while enrolled in our protocol, the parent and the minor will be forewarned at the time of enrollment, that there will be a need to consent the minor on reaching the age of consent as consistent with the requirements of SOP-301 and 402—Requirements for Informed Consent. This is because the prior parental permission and child assent are not equivalent to legally effective informed consent for the now-adult subject (consistent with NIH HRPP SOP 402). All individuals will be given the opportunity to ask questions. In these situations, reconsenting will be performed at the NIH CC.

Rationale for Subject Selection

Type 2 diabetes in youth is a rapidly progressive and aggressive disease yet current treatment options are limited and not effective. Furthermore, African-American youth are disproportionately affected by 2-fold increased risk for the disease and a 4-fold increased risk for complications compared to white youth. Currently, clinical therapy with metformin in suboptimal and African-American youth have the highest failure rates. To improve outcomes, we need to understand the pathogenesis of the disease as well (as the pharmacodynamics and pharmacogenomics) in these high risk youth. Therefore, this study was designed to specifically examine these parameters in African-American youth to improve outcomes and inform precision medicine initiatives. Volunteers eligible for this study include mature adolescents and young adults with type 2 diabetes. Young adults will be included in the protocol to enhance recruitment and because the disease progression is similar to adolescents with type 2 diabetes. We will not recruit pre-pubertal or peri-pubertal children with diabetes because this increases the risk that they have type 1 diabetes (a condition not relevant to the aims of this study).

Rationale for Exclusion of Vulnerable Populations

This study will involve recruitment of children age 12-17 years as described above. The study will not involve recruitment of other vulnerable populations including pregnant women, prisoners, adults who are or maybe unable to give consent because they do not meet the inclusion criteria for this study. We will protect participants as described in SOP 402.

Recruitment Strategies

Local subject recruitment documents, including listserv, flyers, social media posts, press releases, correspondence to local subjects will be submitted for approval to the relying IRB.

Tools used for the recruitment of subjects will include the posting of the study description on the Clinical Center Public Service Announcement (NIH CC website, the NIH Record, the Clinical Center News , Clinical Center Facebook, Clinical Center Twitter, OPR LISTSERV, ResearchMatch), and CNMC; radio media, referrals from other protocols, outside physician referrals and self-referral, newspaper advertisements and video ad. Additionally, fliers may be distributed throughout the NIH campus, the CNMC, George Washington University, the University of Maryland and other pediatrician or doctors' office sites in the Washington DC Metropolitan and Baton Rouge areas.

Strategies to address the challenges of clinical trials in youth with type 2 diabetes

There are two major challenges to pediatric research in type 2 diabetes in youth: (1) difficulty with recruiting eligible participants and (2) poor retention rates[94]. Multiple social (decreased recognition of importance of research within this field) and physical barriers (inconvenient study site locations and lack of travel reimbursement) contribute to these challenges. Therefore, to maximize recruitment opportunities our research approach includes:

1) Partnering with CNMC, the largest tertiary care pediatric diabetes center in the Mid-Atlantic region, and leveraging that relationship to build relationships with physicians and families within the community – with a view to promoting health and research literacy.
2) Collaboration between myself and Dr. Fran Cogen, the Director of Diabetes Clinic at CNMC where we care for many youth with type 2 diabetes (see below).
3) Utilizing the Transitional Research in Pediatrics (TRIPP) agreement, an agreement established in 2013 between CNMC and the NIH Clinical Center, which provides additional administrative and institutional support to facilitate transportation and organizational support.

The Pediatric Diabetes and Endocrinology Division at CNMC, Northeast location in Washington DC cares for over 250 youth and young adults with type 2 diabetes per year, the majority of whom are African-American. Table 4 summarizes the demographics of eligible patients with type 2 diabetes at CNMC and illustrates that ~ 60-70 youth would meet our eligibility criteria and the majority of these youth are seen within the monthly type 2 diabetes clinics at CNMC. We anticipate that recruitment and enrollment will occur over a 3-year period.

Table 4: Demographics of Eligible Patients with Type 2 Diabetes seen at CNMC, 2014-2015

1. New-onset T2D patients:	
a. # of newly diagnosed patients with T2D seen each year (within 3 mos of dx)	**40-50**
b. Approximate % pediatric (<18 yrs)	**89%**
c. Approximate %adult (>18 yrs)	**11%**
2. Established T2D patients:	
a. # of established patients currently seen at least once a year	**195**
b. Approximate % pediatric (<18 yrs)	**86%**
c. Approximate %adult (>18 yrs)	**14%**
3. Insulin Use:	
a. Approximate % of patients using insulin	**41%**
4. Race/ethnicity of T2D Patients: *Indicate approximate distribution of the T2D patients you see:*	
a. Approximate % Non-Hispanic White	**10%**
b. Approximate % African American	**66%**
c. Approximate % Hispanic	**2%**
d. Approximate % Asian	**3%**
e. Approximate % American Indian / Alaska Native	**0%**

T2D – type 2 diabetes

To improve retention in this study we will employ the following strategies:

1) Promote research and health literacy through community outreach.
2) Daily to weekly telephone and text message reminders.
3) Monthly follow-up visits to monitor medication and glycemia.
4) Strategize with individual families regarding their unique socio-economic barriers and offer alternative and supportive strategies to encourage study adherence (e.g. finding creative solutions to transportation issues).
5) Offer study visits on the weekends and holidays to encourage participation without interrupting the children's

academic schedule and performance.

Risks/Benefits Analysis including Considerations of Alternatives to Participation

Evaluation of Benefits

This research protocol is designed to elucidate the physiologic targets of metformin and liraglutide. Both agents are anti-diabetic agents and are expected to improve overall glycemia in individuals. Metformin and liraglutide are approved for the treatment of youth with type 2 diabetes 10 years or older as an adjunct to diet and exercise.. Therefore, participants may benefit from participating in the protocol with the use of these 2 agents. Participating in the protocol procedures may also be beneficial to the individual because it may provide specific information about the individual's glycemic control and diabetes management, as well as more frequent interaction with the study team. If a secondary genetic finding is identified during genetic testing, a participant may learn valuable information about their health which could lead to treatments or monitoring that could significantly improve their health outcomes. Such a finding could also have implications for family members who may share the risk variant.

Evaluation of Risks

The specific risks and our approach to minimize risks are outlined below.

1. Stable Isotope Administration: Stable isotopes are naturally occurring and non-radioactive and are not associated with any toxicity at the doses used in these studies. However, rapid administration of deuterated water (^2H_2O) can be associated with mild and transient dizziness. Administering the deuterated water in 3 divided doses overnight while the patient is lying down significantly minimizes this risk. If the subject needs to get out of bed (e.g. to use the bathroom), the patient will first sit upright in bed for a few minutes prior to standing and a nurse will be available to supervise and assist. The isotopes will be prepared by Pine Pharmaceuticals and tested to certify sterility and freedom from bacteria and substances that may cause pyrogenicity. The NIH Clinical Center Pharmacy will determine final approval of isotope products after careful review of sterility and stability records.

2. Blood Sampling: Peripheral blood draws (venipuncture) performed during this study for research will not exceed 10.5 mL/kg, or 550 mL (whichever is smaller) per 8-week period for adults. For pediatric patients, blood draws will not exceed 5 mL/kg in a single day, or 9.5 mL/kg or 550 mL (whichever is smaller) per 8-week period. The total blood volume for this study is 520 ml in 12-week period. Patients may experience some discomfort at the site of the needle entry, and there is a risk of bruising at the site. There is a remote risk of fainting or local infection.

3. Intravenous Catheter Placement: The placement of intravenous catheters can be uncomfortable and pose the potential risk of bleeding, bruising and infection. All catheters will be placed under sterile conditions and universal precautions will be observed. Should any complications occur, they will be addressed immediately.

4. Timed urine collection: Urine collection is not associated with any health risk but may be inconvenient for subjects.

5. Resting Energy Expenditure: There are no significant risks associated with measurement of resting energy expenditure. Some patients may feel uncomfortable from lying still with a plastic hood over their head for approximately 30 minutes.

6. Risk associated with metformin
    i. Gastrointestinal upset: Nausea, vomiting, diarrhea, flatulence, abdominal discomfort and indigestion are common side effects associated with metformin use. The following actions will be taken to minimize the risk for gastrointestinal disturbance:
        a. Metformin dosing will be gradually increased over 4-week period (Table 3).
        b. Participants who experience mild to moderate gastrointestinal symptoms will contact the study team and the dose of metformin decreased incrementally for 1 week. If symptoms have resolved, the dose will be incrementally increased to achieve maximum dose of 1gram twice daily.
        c. Participants who experience severe and/or recurrent gastrointestinal symptoms will be withdrawn from the study.

    ii. Boxed Warning – Lactic acidosis (rare): The risk of lactic acidosis with metformin use is increased in elderly individuals with renal dysfunction or congenital heart failure. Participants with chronic renal or heart failure, or other chronic metabolic illnesses will not be recruited for this study.

7. Risks associated with liraglutide (Victoza®)
    i. Gastrointestinal upset: Nausea, vomiting and/ or diarrhea are common adverse reactions associated with liraglutide (Victoza®) occurring in 5-20% of patients (FDA labeling insert). Combination therapy of Victoza® and metformin slightly increases the incidence rate of nausea, vomiting and diarrhea to 9-24%[62]. In a 52-week randomized double blind controlled trial of liraglutide vs. placebo in 135 youth with type 2 diabetes treated with metformin, liraglutide was associated with a higher rate of serious adverse events although these were not associated with any specific finding or patter. The most common adverse event noted in at least 5% of patients was: nausea (29%), vomiting (26%), diarrhea (23%) and abdominal pain (18%) and nasopharyngitis (17%)[63]. The majority of all adverse events were mild in severity and resolved. There were no deaths reported. The symptoms were mild and resolved spontaneously within 1-4 days. The following actions will be taken to minimize the risk for gastrointestinal disturbance:
        a. Liraglutide dosing will be gradually increased over 4-week period (Table 3).
        b. Participants who experience mild to moderate gastrointestinal symptoms will contact the PI and the dose of liraglutide decreased incrementally for 1 week. If symptoms have resolved, the dose will be incrementally increased in 1-week intervals to achieve maximum dose of 1.8mg once daily.
        c. Participants who experience severe and/or recurrent gastrointestinal symptoms will be withdrawn from the study.

    ii. Hypoglycemia: Victoza® lowers glucose by increasing glucose-dependent insulin secretion, lowering glucagon secretion and delaying gastric emptying. These actions result in lower fasting and postprandial glucose concentrations in the circulation; in adults, Victoza® can increase the risk of hypoglycemia at a rate of 0.24 episodes/ patient years. In youth treated with liraglutide and metformin, minor hypoglycemia occurred in 24% of patients and there were no cases of severe hypoglycemia[63]. We will monitor the participants closely and the following precautions and actions will be taken to minimize the risk for hypoglycemia:
        a. Blood glucose monitoring. Patients will monitor their blood glucose by either checking their fingerstick blood glucose levels at least twice daily (pre-breakfast and bedtime) or wearing an FDA-approved CGM device for the duration of the study.

b. All participants will be educated on the signs and symptoms of hypoglycemia: dizziness or lightheadedness, sweating, confusion, headache, blurred vision, slurred speech, hunger, weakness, anxiety or irritability, fast heartbeat.
c. Blood glucose values <70mg/dl with or without signs and symptoms of hypoglycemia (described above) will be immediately reported to the principal investigator and dose of liraglutide decreased.
d. If a participant experiences recurrent severe hypoglycemia, defined as blood glucose <50mg/dl with or without symptoms, the need for a third party to resolve a hypoglycemic episode or loss of consciousness or seizure on 2 or more occasions, liraglutide will be stopped and they will be withdrawn from the study.

iii. Pancreatitis: In post marketing reports, Victoza has been associated with acute pancreatitis. In a large 52-week randomized trial of liraglutide vs. placebo in 135 youth with type 2 diabetes on metformin, liraglutide was associated with higher lipase concentrations, but there were no cases of pancreatitis. The following measures are being taken to minimize the risk of pancreatitis in this study:

a. Subjects at increased risk of pancreatitis are excluded, as follows:
   i. Triglycerides over 500 mg/dl. This will exclude patients with extreme hypertriglyceridemia at baseline, who have a higher risk for pancreatitis.
   ii. Prior history of more than one episode of pancreatitis.
   iii. Current lipase > 60 units/L (the upper limit of normal in the NIH assay, 02/08/2014 to present). While both amylase and lipase are often used clinically in the assessment of pancreatitis, amylase is a non-specific test that may be elevated for other reasons. Subjects with elevated lipase at study entry will be considered to have laboratory findings consistent with pancreatitis and will be excluded.
b. If pancreatitis is clinically suspected (either based on signs/symptoms consisting of moderate to severe abdominal pain with nausea or vomiting, or based on lipase), the following will take place:
   i. Amylase and lipase levels will be drawn.
   ii. Gastroenterology (GI) consultation will be obtained.
c. If a diagnosis of pancreatitis is likely or confirmed based on labs and/or GI consultation, the following additional actions will be taken:
   i. The patient will be referred to CNMC for further management and admission.
   ii. Appropriate treatment will be initiated based on GI recommendations (usually, NPO and pain medications).
   iii. The patient will be withdrawn from the study.
   iv. The IRB will be notified per guidelines for serious adverse effects.

iv. Kidney problems: In individuals with kidney failure, diarrhea, nausea and vomiting may cause a loss of fluids and dehydration. Subjects with impaired renal function at baseline evaluation will not be recruited for this study.

v. Thyroid C-Cell Tumors: Victoza® has a black box warning for the risk of thyroid c-cell tumors. Liraglutide causes dose-dependent and treatment-duration-dependent thyroid C-cell tumors (adenomas and/or carcinomas) at clinically relevant exposures in both genders of mice. It is unknown whether Victoza will cause thyroid C-cell tumors and in humans, the relevance of liraglutide-induced rodent thyroid C-cell tumors has not been determined. There is insufficient evidence to establish or exclude a

causal relationship between medullary thyroid carcinoma and Victoza use in humans. The following measures are being taken to minimize the risk of thyroid c-cell carcinoma and Victoza use in this study:

a. Patients with a personal or family history of medullary thyroid carcinoma, and patients with multiple endocrine neoplasia 2 will be excluded from the study because the use of Victoza in these patients is contraindicated.
b. We will monitor subjects clinically for signs of thyroid nodules or neck swelling.
c. Serum calcitonin concentrations will be measured at baseline and at the end of the study.
d. If a thyroid nodule or neck swelling is observed, further evaluation will be pursued with thyroid/ neck imaging and measuring serum calcitonin concentrations.

vi. Hypersensitivity Reactions: There are no studies linking Victoza® to allergic reactions; however, in post-marketing reports, Victoza has been associated with serious allergic reactions (e.g. swelling, hives, difficulty breathing). Individuals who are known to have an allergic reaction to Victoza® will not be enrolled in this study.

8. Risk associated with drug-free run-in period
   i. Severe Hyperglycemia with metabolic decompensation: During the 5-7-day period between Visit 1 and Visit 2, youth may be at increased risk for severe hyperglycemia since the youth will be asked to stop their anti-diabetic medication. This risk is reduced because we will only enroll individuals who are in relatively good glycemic control (HbA1c <9%) who will be at lower risk for decompensation. The 5-7 day period was chosen to minimize risk, and diet and lifestyle treatment is in keeping with current standard of care clinical guidelines as an important treatment modality for youth with type 2 diabetes [95]. For youth previously on metformin (the majority of eligible patients will be in this category), 5-7 days is sufficient to ensure complete elimination of metformin from the plasma and erythrocyte compartments; elimination half-life of 4-6 hours [96]and 18 hours respectively [97]. Therefore, sufficient drug elimination will occur after ~ 5 half-lives i.e. plasma: 30hrs (1.25 days) and erythrocyte: 90 hrs (3.75 days).

   The following steps will be taken to minimize the risks of severe hyperglycemia:

   a. Participants at higher risk for severe hyperglycemia defined as those with HbA1c ≥9% will not be eligible to participate.
   b. All participants will receive up-to-date education on diet and lifestyle management by the PI and study staff.
   c. During the run-in drug free period all individuals will monitor their blood glucose levels with a FDA-approved CGM device or a home glucometer 2-3 times daily. Study staff will review blood glucose logs and CGM tracings daily. For participants with persistently elevated FSBG without metabolic decompensation, the physician may recommend admission to the NIH metabolic unit for close monitoring of their blood glucose levels prior to Visit 2.
   d. If FSBG is >200mg/dl fasting, or 300mg/dl bedtime is noted during the run-in period, they will notify the study PI immediately. Individuals with persistently elevated FSBG and-signs and symptoms of metabolic decompensation (e.g. vomiting, dehydration, lethargy, abdominal pain) will be withdrawn from the study and restarted on his/ her medication.
   e. If vomiting, abdominal pain or dehydration is noted, participant will be evaluated by a medical professional or PI immediately for metabolic evaluation of acidosis and ketosis. If metabolic decompensation (acidosis or ketosis) is noted, participants will be promptly treated and withdrawn from the study.

9. Stool sampling: Stool sampling is not associated with any health risk but may be uncomfortable for some participants.

10. Radiation Exposure: Dual energy absorptiometry (DXA) scan. The DXA scan is a reliable and reproducible method to measure body composition, specifically body fat and lean body mass. The patient lies on a flat table with the x-ray source below the table and the detector above. Each scan takes about 3 minutes. For the DXA scan the effective dose of radiation is 0.00006 rem, which is below the guideline of 5 rem (or 0.5 rem in children) per year allowed for research subjects by the NIH Radiation Safety Committee. The average person in the United States receives a radiation exposure of 0.3 rem per year from natural sources, such as the sun, outer space, and the earth's air and soil. If participants want to learn more about radiation, they will be given the pamphlet: An Introduction to Radiation for NIH Research Subjects.

11. Genetic Testing: Genetic and phenotype data will be placed in a dbGaP or similar database in accordance with NIH policy on sharing of genetic data. Through this database, researchers with approved proposals will be able to access these data; however, the data will be de-identified prior to data-sharing. As genetic information is unique to an individual, there is a small chance that an individual may be identified through these data, and it is possible that this risk will grow over time. Additionally, there is a small risk that genetic information could lead to discrimination, for instance for employment or insurance; however, there are laws in place that protect against this sort of discrimination. Genetic information may lead to statements about an individual's ethnic group that they do not agree with. If a secondary genetic finding is identified, learning about that finding may be upsetting for the participant. Their relatives may also be upset if the variant has implications for their health. Participants who do not receive a report of a secondary finding may falsely interpret this lack of finding as assurance of absence of any medically serious genetic variants, though the genetic testing conducted is very limited and cannot be interpreted in this way.

Alternatives to participation
Participation in clinical trials is completely voluntary. Refusal to participate will not affect a subject's ability to participate in other studies at NIH or elsewhere.

Adverse Event Reporting

Event Characterization and Reporting to the IRB
Adverse events, non-compliance both serious or continuing, protocol deviations both major and minor, as well as unanticipated problems are defined & described by the NIH Office of Human Subjects Research Protection policy #801 and will be reported in accordance with this policy.

Investigational New Drug Application/ Exemption
The FDA expanded the indication for Victoza® (liraglutide) therapy as an adjunct to diet and exercise to improve glycemic control in patients 10 years and older with type 2 diabetes mellitus on June 17, 2019. The FDA approval was based on data submitted from the ELLIPSE clinical trial, which was published in the New England Journal of Medicine in April 2019[63]. Prior to, this approval, we submitted and received an IND exemption (June 2016) for the study drugs used in this clinical study. This trial is not being conducted with the intention to support advertising or to submit data to a marketing application for metformin or liraglutide. In addition, we plan to conduct this clinical trial in accordance with 21 CFR Part 50 and Part 56 and the drugs are used within the labeling requirements.

Data and Safety Monitoring Plan
Study procedures will be subject to audits and/or monitoring visits to ensure compliance with the protocol and applicable regulatory requirements consistent with the NIDDK quality assurance program plan. Audit and/or monitoring visit results

will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

The collection, monitoring and analysis of adverse events will be the responsibility of the Principal Investigator and the investigative team.

As required by FDA 21 CFR 312.50, trial procedures will be subject to review to ensure compliance with the protocol and applicable regulatory requirements. Results will be reported to the Principal Investigator/Sponsor for further reporting to the FDA consistent with applicable regulations. The specific monitoring plan will be developed with the Principal Investigator and frequency of monitoring visits determined by such factors as study enrollment, data collection status and regulatory obligations. Monitoring visit results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

Monitoring Subjects and Criteria for Withdrawal of Subjects from the study

Withdrawal Criteria

1. **Withdrawal of consent**. A subject wish to withdraw from the study as stated in the informed consent (all subjects reserve the right to withdraw from the study without prejudice).
2. **Adverse event**. A subject experience an adverse event that in the investigator's opinion necessitates withdrawal from the study. Specific examples of adverse events that would result in withdrawal are:
    a) Pancreatitis during liraglutide administration. Pancreatitis is a clinical diagnosis and will be suspected in the presence of moderate to severe abdominal pain accompanied by nausea and/or vomiting. If pancreatitis is suspected, amylase and lipase levels will be drawn, and GI consultation obtained.
    b) Gastrointestinal intolerance to study drug (metformin or combination therapy) characterized by persistent and/ or severe nausea, vomiting, diarrhea, abdominal pain or stomach upset.
    c) Diabetic ketoacidosis or severe hyperglycemia requiring insulin replacement.
    d) Severe hypoglycemia defined as: 1) severe symptomatic hypoglycemia with seizures or unconsciousness and blood glucose <50mg/dl or 2) fasting hypoglycemia <30mg/dl on two consecutive days with or without symptoms.
3. **Abnormal screening blood tests.** Subjects who have abnormal blood tests and who meet exclusion criteria will be withdrawn from the study by the investigator.
4. **Investigator decision**. An investigator feels it is in the subject's best interest to terminate participation. The detailed reasoning behind this decision will be documented.
5. **Protocol deviation**. Includes subject noncompliance with fingerstick blood monitoring during the run-in period, pregnancy, study entry criterion deviation, or start of a concomitant medication that would impede accurate study analysis. If a female participant's urine pregnancy test is positive, we will ask the girl's permission to inform her family so that she can get optimal medical care. During the drug-free run-in period, individuals who cannot wear the CGM device or complete fingerstick blood glucose measures or who cannot be reached for ≥3 days during this period will be withdrawn from the study.

Protection of Participant's Privacy and Confidentiality

At the time of enrollment each subject is given a study-specific code name. The coding system is available only to study staff and kept in a secure, password protected database accessible only to study staff. The actual names of the subjects will not be provided or ever made available in any publication.

Compensation

All participants will receive financial compensation for their time per NIH Clinical Center guidelines for on-site visits and additional compensation will be provided for specific procedures based on inconvenience units, as follows:

Table 5: Compensation per Visit – to be given as $ or gift card at the end of each visit

Visit	Compensation to patient ($)
Visit: 1 Screening	$ 30.00
Visit: 2 Inpatient	$ 50.00
Visit: 3 Follow-up	$ 25.00
Visit: 4 Follow up	$ 25.00
Visit: 5 Inpatient	$ 50.00
Total	**$180.00**

Table 6: Compensation per Procedure – to be given as lump sum at end of study

Visit	Compensation to patient ($)
Visit 2: Inpatient OGTT Genetics DEXA Tracer REE	 $ 50.00 $ 20.00 $ 30.00 $ 100.00 $ 20.00
Visit 5: Inpatient OGTT DEXA Tracer REE	 $ 50.00 $ 30.00 $ 100.00 $ 20.00
Completion Bonus	$ 100.00
Total	**$ 520.00**

Total compensation to participants for entire study is $180 for visits + $520 for procedures = $700. If a participant were to drop out, the participant will only receive payment for whatever visits and procedures they completed up to that point. For example, if a participant drops out after visit 2, he/she will be compensated for Visit 1, Visit 2, 1 OGTT, 1 REE, 1Genetics and 1 tracer = $300.

Total compensation for guardians accompanying minors for the entire study is $40 per visit x 5 visits = $200. The guardians will only receive payments for whatever visits were completed and will be paid as a lump sum at the completion of the study.

Compensation for participating in the making of the video ad is $ 100.00. This is one-time compensation for the subject(s) who will participate in the making of the recruitment video ad when needed.

Conflict of Interest

1. The National Institutes of Health reviews NIH employees at least yearly for conflicts of interest. The following link contains details on this process: http://ethics.od.nih.gov/forms/Protocol-Reveiw-Guide.pdf.

2. This protocol has investigators who are not NIH employees. They are expected to comply with their Institutions' conflict of interest policies.

Appendix A: Sample gastrointestinal questionnaire

Gastrointestinal medication questionnaire.

Confidential

GI symptom survey

How is your general health today?
- ◯ Very good
- ◯ Good
- ◯ Fair
- ◯ Poor

How does this compare to yesterday?
- ◯ Better
- ◯ The same
- ◯ Worse
- ◯ Much worse

Please record any symptoms you may have had today
Absent - I did not have this symptom at all
Mild - I had this symptom occasionally, but it did not really bother me
Moderate - I had this symptom often, it bothered me quite a bit
Severe - I had this symptom very often, it bothered me a great deal

	Absent	Mild	Moderate	Severe
Have you felt any nausea (wanting to vomit) today?	◯	◯	◯	◯
Have you actually vomited today?	◯	◯	◯	◯
Have you had heartburn (burning in chest) today?	◯	◯	◯	◯
Have you had cramping or abdominal pain today?	◯	◯	◯	◯
Have you had a headache today?	◯	◯	◯	◯
Have you been breathless today?	◯	◯	◯	◯
Have you had diarrhea today?	◯	◯	◯	◯
Have you had constipation today?	◯	◯	◯	◯
Have you had bloating today?	◯	◯	◯	◯

How many bowel movements have you had today? _____

Did you take your metformin today?
- ◯ yes, all of it
- ◯ yes, but I missed part of the dose
- ◯ no

Do you think your symptoms are related to the metformin or not?
- ◯ Yes
- ◯ No

Why do you think that it is not related to the metformin?

_____

Appendix B: Continuous glucose monitoring (CGM) and blood glucose management instructions

NAME:

DATE RANGE:

Instructions:

- You will have your continuous glucose monitor (CGM) sensor placed before you go home.
- Check the receiver twice daily, fasting and before bedtime (~ 2 hours after dinner).
- If your CGM sensor malfunctions and you are not able to replace the sensor yourself, we will ask you to either check finger stick glucose levels for the remainder of the week, or you may come in to have the sensor replaced by the study team.
- A member of the study team will review the glucose levels either on the secure web-based application, over the phone or via secure email every week.
- If blood glucose is ever >300mg/dl or <70mg/dl, call study team immediately.

What to do if you have high blood sugars:

- Call the study team and follow the instructions below:

Blood glucose level	Symptoms Excessive thirst, urination or abdominal pain	What to do
Greater than 250 mg/dL	NO	• Drink 16 oz of water and recheck your blood sugar in 30 minutes • If blood sugar is still elevated call study team
Greater than 250 mg/dL	YES	• Call Study team • Drink 16 oz of water and recheck your blood sugar in 30 minutes

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