PROTOCOL TITLE: Fenofibrate in Type 2 Diabetes- Novel Biomarkers and Mechanisms

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1.0 Objectives / Specific Aims

Fenofibrate, a safe and long-established lipid-lowering agent, was recently discovered to have unexpected yet dramatic therapeutic effects on diabetic retinopathy (DR) in two large clinical trials (1,2). Intriguingly, the retinal benefits were independent of plasma lipids, suggesting novel mechanisms of action. Furthermore, evidence indicates that fenofibrate's beneficial effects extend to many other microvascular complications of diabetes.

Our aims are: 1) to recruit a cohort of 40 well-characterized fenofibrate-naïve patients with type 2 diabetes who will receive oral fenofibrate 160 mg/day for six weeks; this work will establish a clinical sample repository, collected immediately prior to and after treatment, to enable us to 2) characterize the global plasma proteome before and after fenofibrate treatment and future follow-up studies. We expect to gain novel insights into the systemic effects of fenofibrate on the pathophysiologic processes that drive diabetic vascular complications. This will generate new hypotheses about the common root causes of diabetic complications and complement data being accrued in ongoing studies of retinopathy, nephropathy, atherosclerosis, and complications of pregnancy that integrate human data with and animal/cellular model systems. The ultimate translational impact of the work will be the development of proactive means to block a range of complications early in their evolution, either through the development of more selective targeted therapies, and/or finding new disease indications for existing drugs.

To translate this into new preventive and/or therapeutic measures, we hypothesize: 1) that fenofibrate has mechanisms of action beyond PPARα agonism that attenuate microvascular complications of diabetes; and 2) that observable treatment-induced changes in the constituents of plasma will reflect the underlying effects of the drug on target tissues.

2.0 Background

Work in my laboratory is directed at improving our understanding of the vascular complications of diabetes, and our work relates to vascular disease in general (i.e. to cardiovascular, cerebrovascular, and peripheral vascular disease).

The complications of diabetes, which range from well-recognized conditions such as DR, to less obvious ones such as hepatic steatosis and fibrosis, remain challenging and without truly satisfactory therapies. For example, DR is a leading cause of blindness worldwide, and with the ongoing epidemic of diabetes, promises to constitute a major global health problem for the foreseeable future. Current standard of care includes anti-VEGF antibodies and steroids (both via invasive intraocular injection), laser photocoagulation, and vitrectomy, all of which are useful only in the late stages of the disease. By that time, irreversible retinal damage has occurred, and some therapies actually involve further retinal destruction. Similar considerations apply to diabetic renal disease and neuropathy. Such 'crisis management' is necessary and valuable, but it would be vastly preferable to stop complications at an earlier stage using a convenient, safe, orally available drug. The recent discovery that fenofibrate is beneficial for DR and other microvascular complications (1,2) brings hope, and tantalizingly, the drug's efficacy across different complications suggests unrecognized common disease pathways (3-7). Still, the lack of correlation between its microvascular protective effects and its lipid-lowering activity raises questions (1,2). Presumably, the relevant mechanism(s) of action are unique to fenofibrate, a PPARα agonist, and not shared with either PPARγ agonists, e.g. rosiglitazone and pioglitazone, that enhance insulin sensitivity, or HMG-CoA reductase inhibitors (statins), that lower lipid levels by

upregulating hepatic LDL receptors. It is also unclear whether the benefits of fenofibrate are mediated via PPARα or some novel mechanisms of action (8-13). In fact, recent data from our group using a human retinal pigment epithelial (RPE) barrier model showed that fenofibrate, but not other PPARα agonists (WY14643, gemfibrozil, bezafibrate), attenuated the toxic effects of oxidized LDL on barrier integrity (14). Thus, fenofibrate may or may not be the answer, but it certainly provides important clues, and our lack of understanding of its systemic effects on the diabetic milieu is a barrier to the development of new treatments. Discovery of the mechanisms and pathways of action could yield a new generation of drugs that can protect multiple tissues from the effects of diabetes, blocking disease development proactively instead of reacting to end-stage complications.

3.0 Intervention to be studied

Fibrates belong to a class of drugs that exert their effects by activating peroxisome proliferator activated receptor α (PPARα) (15). These drugs reduce the concentration of plasma triglyceride (TG) by 30-50% and raise the level of high-density lipoprotein cholesterol (HDL-C) by 2-20% (15-17). The first member of the class, clofibrate, was introduced in the 1960's and became available in the US in 1967 (18). Fenofibrate (the original generic name of which was procetofene), a third-generation fibrate, was introduced in clinical practice in 1975 (19). Fenofibrate is now one of the most commonly prescribed fibrates worldwide, indicated for the treatment of hypercholesterolemia, combined dyslipidemia, remnant hyperlipidemia, endogenous hyperlipidemia (hypertriglyceridemia) and mixed hyperlipidemia (Friedrickson type IIa, IIb, III, IV, and V dyslipidemia, respectively) (19).

Fenofibrate is FDA-approved for oral administration. The primary indication is for use in adult patients with hypercholesterolemia or mixed dyslipidemia. The standard dose of fenofibrate, 160 mg/day that was used in the ACCORD study (2) will be replicated in the current study. Fenofibrate is not FDA approved for the treatment of diabetic retinopathy.

4.0 Study Endpoints (if applicable)

Specific endpoints have not been predetermined.

5.0 Inclusion and Exclusion Criteria/ Study Population

Type 2 diabetes is highly prevalent in our endocrine clinic population and recruitment of sufficient numbers of volunteer subjects should be easily achieved. We anticipate recruitment of subjects to occur over six months, such that all study samples will be available for LC-MS/MS analysis within eight months of start-up. We have a long track record of collaboration with the SCTR NEXUS facility, e.g. through DCCT/EDIC, and are familiar with their policies and procedures. While our sample size is adequate for discovery research related to the systemic effects of fenofibrate treatment, any human study of outcomes would require a much larger cohort and longer period of follow-up. All subjects will be screened by their provider to ensure the eligibility for the study according to the criteria outlined below.

Inclusion criteria:

- Male or female aged ≥18 and ≤ 70 years

- Type 2 diabetes of at least one year's duration; stable glycemic control (no more than 1.0% change in HbA1c in the previous six months, but no limit on HbA1c)
- Triglycerides >150 mg/dL (in the previous six months)

Exclusion criteria:

- Previous use of Fenofibrate or other fibrates
- Pregnancy/lactation
- Active malignancy
- Recent cardiac event or congestive heart failure
- Active liver disease (LFT's > 2x's normal value)
- Significant renal impairment (serum creatinine > 2mg/dl)
- Current warfarin use

6.0 Number of Subjects

For this study, 40 patients (20 males and 20 females) aged 18-70 years, matching the ethnic composition representative of the demographics of SC, will be recruited from the cohort attending our Endocrinology Clinics at MUSC.

7.0 Setting

Participants will be recruited and consented at MUSC's Endocrinology clinic. Procedures at both visits will be performed at SCTR NEXUS nursing clinic.

8.0 Recruitment Methods

Participants will be approached by their treating physician (in this case either the PI or one of the co-investigators) from the patients attending the MUSC Endocrinology clinics. Advertisements will be placed in MUSC Endocrinology clinics and MUSC elevators. ResearchMatch.org will be utilized as a recruitment tool for this protocol. ResearchMatch.org is a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009.

9.0 Consent Process

Potential participants will be identified by treating physicians (PI/Co-PI's) from the patients attending the MUSC Endocrinology clinics. The PI and/or IRB-approved personnel will explain the nature of the study to individuals who meet criteria. Individuals who take part in this study must choose to participate voluntarily and must provide signed consent. Potential participants will be provided IRB-approved consent forms, the opportunity to read it, and the opportunity to ask questions. They will be consented before undergoing any study-related procedures. They will be encouraged to take time to discuss study participation with family and friends. Once the informed consent form is signed, participants will be actively enrolled in the study.

By an analogous process, written permission will be obtained for the HIPAA document. IRB approved personnel will give a copy of the signed informed consent form and the HIPAA form, to the participant. Informed consent forms will be signed, and copies distributed as usual (PI files and to the participant), with strict regard for confidentiality.

10.0 Study Design / Methods

Regularly scheduled Endocrinology clinic visit:

- If participants qualify and agree to participate, they will be provided an informed consent form, and a HIPAA form and provided the opportunity to sign agreement to participate.
- Visit one will then be scheduled for participants at the SCTR NEXUS clinic by the research coordinator.
- Participants will be instructed on fasting protocol following consent process and prior to visit one.

Visit 1:

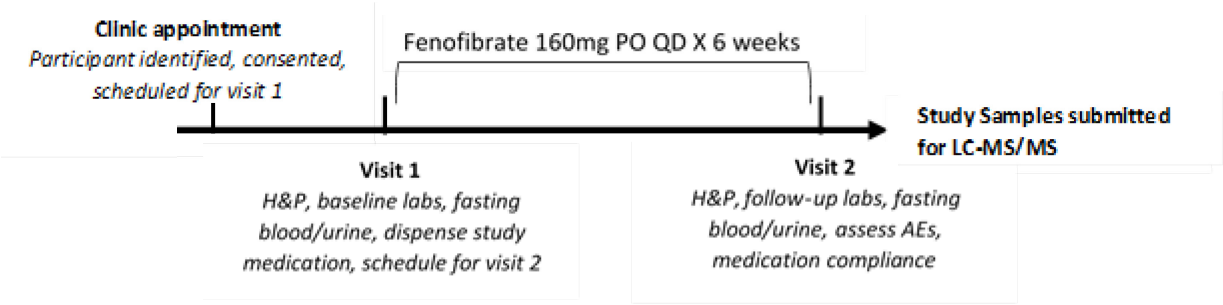
- Upon arrival at the SCTR NEXUS clinic, a physical examination will be performed by either the PI or co-investigators. Clinical data will be recorded to include demographic information, diabetes complication status and medications, and anthropometric information.
- Women of childbearing potential will have urine β-HCG measured (+pregnancy outcome is criteria for exclusion) and will be required to use the following as acceptable methods for birth control throughout the study: 1) barrier methods including male and female condoms, diaphragm, cervical cap and contraceptive sponge, 2) hormonal methods including birth control pills, vaginal ring (NuvaRing), contraceptive implant (Nexplanon), contraceptive injection (Depo-Provera) and birth control patch, 3) intrauterine devices (IUDs) such as the copper IUD (ParaGard) and the hormonal IUD (Mirena, Skyla, Kyleena, others), 4) sterilization to include tubal ligation or the Essure system for women, and vasectomy for men, or 5) avoiding sexual activity that could cause you to become pregnant.
- Fasting blood (80ml) will be obtained for baseline labs (BUN, Cr, urine microalbumin, LFTs, CPK, Lipid Profile, HbA1c, TSH, C-peptide) and processed to yield heparin, EDTA, and citrate-plasma, serum, and pellets (sufficient for all planned analyses). A timed morning urine sample will be collected with the volume recorded. Samples will be stored in ultra-low freezers for proteomics and subsequent confirmatory assays (by ELISA).
- Participants will then be given the study medication (160 mg fenofibrate to be taken orally daily for six weeks) by the research physician. As well, they will receive instruction on possible side effects and proper protocol for reporting unexpected symptoms.
- Following venipuncture, participants will be given a choice of breakfast foods from which to choose by the IRB approved personnel and a place to eat.
- Participants will receive a gift card at the completion of visit one in the amount of $25.
- Participants will be scheduled for visit 2 in six weeks.

Visit 2:

- Upon arrival at the SCTR NEXUS clinic, a physical examination will be performed by either the PI or co-investigators. Clinical data will be recorded to include demographic information, and medications, and anthropometric information.
- Fasting blood (80ml) will be obtained for follow-up labs (BUN, Cr, urine microalbumin, LFTs, CPK, Lipid Profile, HbA1c, TSH, C-peptide) and processed to yield heparin,

EDTA, and citrate-plasma, serum, and pellets (sufficient for all planned analyses). A timed morning urine sample will be collected with the volume recorded. Samples will be stored in ultra-low freezers for proteomics and subsequent confirmatory assays (by ELISA).

- Participants will then undergo an assessment of adverse events and study drug compliance, which will be determined by pill count.
- Following venipuncture, participants will be given a choice of breakfast foods from which to choose by the IRB approved personnel and a place to eat.
- Participants will receive a gift card at the completion of visit two in the amount of $25.
- The plasma proteomic signature will be generated by LC-MS/MS (n=80 proteomes) of each subject before and after fenofibrate treatment. Bioinformatic approaches will then be employed based on geneset enrichment analysis to identify the biological processes/signaling networks impacted by treatment.



11.0 Collection and Banking (if applicable)

For this work, fasting blood samples (80 ml) will be collected at each of two visits, and processed via centrifugation to yield heparin, EDTA, and citrate-plasma, serum, and pellets (sufficient for all planned analyses). A timed morning urine sample will be collected with the volume recorded. Samples will be alphanumerically coded and correlated to the stored samples. Codes have no association to the participant's identity. Samples will be stored in ultra-low freezers for proteomics and subsequent confirmatory assays (by ELISA). Links to participant identity (including demographic and clinical information) will be accessible to the PI and research coordinator only and links will be destroyed at the study's conclusion. Consent forms and any associated hard documents will be kept in a locked cabinet, in the locked office of the PI.

Specimen/Banking for Future Use

The samples will be stored in ultra-low freezers maintained by the Lyons lab on the 9th floor of the Clinical Science Building (CSB). The freezers are housed in room 912, which remains locked and accessible only to members of the Lyons lab. Samples will be alphanumerically coded and linked to clinically pertinent information. The samples will be stored indefinitely or until completely used.

Future research may be conducted by Dr. Lyons or by other researchers who obtain IRB approval for their research. There is potential in the future for genetic analyses of samples and therefore the option to opt out will be made available to participants in the informed consent form.

12.0 Data Management

Participants' privacy will be protected via alpha-numeric coding of the data once obtained. Names of participants will not be entered in the database, instead they will be identified by assigned code. Information linking the code to the participant will be kept securely by the PI. There will be no link to the participant after study completion. The consent forms, which are the possible way to breach confidentiality, will be stored in the PI's office as well in a secure location. All clinical data pertaining to the study will be stored online in REDCap.

13.0 Provisions to Monitor the Data to Ensure the Safety of Subjects (if applicable)

Adverse event (AE) reporting Adverse events anticipated in this study that involves a fasting blood draw and potential side effects of fenofibrate (for example myalgias, elevation of liver enzymes) are few. Risks associated with drawing blood from the arm include momentary discomfort and/or bruising. Infection, excess bleeding, clotting, or fainting is possible, although unlikely.

Risk minimization These risks will be minimized by allowing only well-trained clinical research staff of the SCTR NEXUS facility to perform study procedures and testing according to standard practice, and by making sure study participants clearly understand study procedures before and during the study protocol.

The blood draw will be performed with the subject resting and recumbent. Adequate rest time will be provided after the blood draw to avoid a hypotensive response. Breakfast will be provided at the end of blood draw. Participants will be instructed to report any concerning symptoms. If necessary, adverse events will be reported to the PI and the IRB. Unanticipated non-serious adverse events must be reported from the investigator to the IRB no later than 10 working days after the event or notification to the investigator that the event has occurred, and serious adverse events will be reported within 24 per MUSC IRB guidelines. In absence of moderate or serious adverse events, reports of study progress will be submitted to the IRB for review on an annual basis.

The potential benefits of the research, which may include lowering of cholesterol and the identification of major new risk factors for complications of diabetes, are considered to outweigh the minimal risks involved.

Monitoring of Study Implementation and Progress Periodic assessments will be performed to assess recruitment and retention of study participants, data quality, and risk-benefit ratio. The recruitment goal for this study is to recruit over the period of 6 months 40 (20 males and 20 females) type 2 diabetic participants with elevated triglycerides. Data checks will be performed by Drs. Lyons and Leyva. If additional information regarding the study's risk-benefit ratio becomes available, the PI will be responsible for notifying the IRB, sponsor, and study participants, as indicated.

A record will be kept of patients who were considered for enrollment but not enrolled e.g., patient screening log to establish that the participant population was selected without bias.

14.0 Withdrawal of Subjects (if applicable)

Participants may be discontinued from study treatment at any time. Specific reasons for discontinuation from this study are:

- Study treatment not tolerated and raising safety concerns. Non-toleration of study drug could include any symptoms of myalgia or at the investigator's discretion.
- Voluntary discontinuation by the participant who is at any time free to discontinue their participation in the study, without prejudice to further treatment.
- Safety reasons as judged by the investigator.
- Occurrence of an adverse event, which in the opinion of the investigator warrants the participant's discontinuation.
- If at any time the participant experiences unexplained muscle pain, tenderness or weakness, study medication should be discontinued.
- Participant lost to follow-up.
- Pregnancy.
- Deterioration in the participant's condition which in the opinion of the investigator warrants study medication withdrawal.

Participants who discontinue will be asked the reason(s) for their discontinuation and the presence of any adverse events. The participant must return all unused investigational products to the investigator. The number of encapsulated tablets returned must be checked against the number dispensed to determine participant compliance.

15.0 Risks to Subjects

Potential risks are low and include:

- Research records will be confidential. There is a small risk of loss of confidentiality, but records will be stored, locked in the PI's office as a means of preventing this loss of confidentiality.
- Hazards of venipuncture: pain, bruising, inflammation or clot formation, syncope. Procedures for minimizing potential risks include the use of aseptic techniques for venipuncture, and drawing blood with subjects in the recumbent position. Subjects will be provided explanation in advance about laboratory test procedures to be performed. The subject's results will be known only to the investigators.
- Fenofibrate use has been associated with an increased risk of muscle pain, abnormal results in tests of liver function and allergic reaction. Participants will be instructed on risks and provided protocol for reporting with medication administration.

16.0 Potential Benefits to Subjects or Others

Fenofibrate lowers triglyceride levels, and has been found to confer unrelated and unexpected therapeutic benefit on diabetic retinopathy in two large clinical trials on type 2 diabetic subjects. Additional clinical data suggest that fenofibrate has beneficial effects in diabetic nephropathy, limb ischemia, diabetic neuropathy, hepatic fibrosis, cancer, and other conditions. However, these studies involve longer-term use of the drug, and there is no evidence that short-term use, as in the present study, will confer any benefit.

17.0 Drugs or Devices (if applicable)

All study medication will be procured from MUSC's Pharmacy Distribution Center. It will be labeled, in its original packaging according to FDA standards, by the research coordinator. Medications will be stored securely at room temperature in the Lyons lab in a locked cabinet. All medications will be dispensed to participants by an IRB-approved,

licensed practitioner. All medication-related procedures will be recorded on a drug accountability form.

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