IMPACT OF SEMAGLUTIDE (LONG-ACTING GLP1 AGONIST) ON PERIPHERAL BLOOD DERIVED CD34+ ENDOTHELIAL PROGENITOR CELLS (EPCS) AND SUBCUTANEOUS FAT DERIVED MESENCHYMAL STROMAL CELLS (MSCS) IN TYPE 2 DIABETES SUBJECTS

INVESTIGATOR-SPONSORED STUDY PROPOSAL

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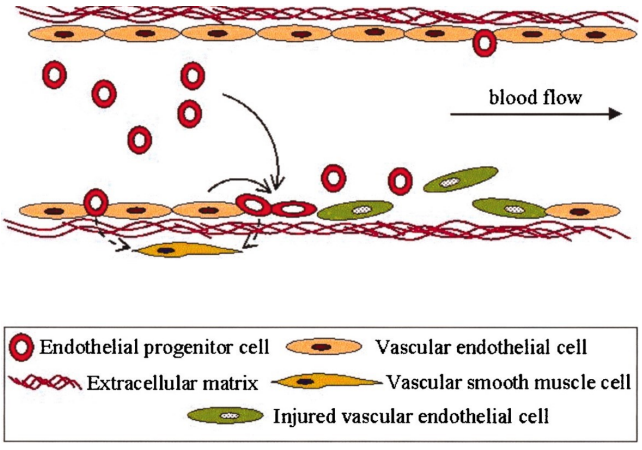
1. BACKGROUND AND SIGNIFICANCE:

Diabetes affects more than 9% of adults in the United States and this is projected to nearly double by 2025[1]. Both diabetes and obesity are associated with endothelial dysfunction, oxidative stress, endothelial cell inflammation, cardiovascular pro-thrombotic states and are the most common causes of kidney disease and blindness [2,4]. Endothelium and its progenitors, meaning endothelial progenitor cells (EPCs), are an established surrogate of cardiovascular risk outcome measures [4, 6]. EPCs have been defined as CD34+ cells [6] thereby identifying a defined homogenous population from a heterogeneous peripheral blood derived mononuclear cells.

We and others, have previously shown that EPCs can act as a cellular biomarker that is *more* reliable than serum based markers for CVD risk estimation [6, 10]. We demonstrated that gene expression in EPCs change within two weeks of an intervention such as aerobic exercise [10]. On the other-hand serum biomarkers usually take much longer time to change secondary to an intervention [4, 16, 17]. Also the paracrine effect of damaged endothelium is secondary to gene expression changes that have been altered in the progenitor cells several months ahead of discernable changes in serum based biomarkers such as endothelium based inflammatory markers. When serum inflammatory markers are elevated that may mean that the endothelium is already damaged/ inflamed and possibly irreversibly. [16, 17].

EPC are the future endothelium, therefore studying EPCs may help us to predict the effect of an intervention (such as a medication or exercise) on the future of endothelium and endothelial function. [5] In normal course of events, the EPCs transition to mature endothelium and replace endothelial cells after normal cell death cycle or programmed apoptosis. However, unfortunately, type 2 diabetes being a pro-inflammatory, high ROS disease [4] process, chronically depletes the EPC population by up-regulating apoptotic pathways mediated by p53 [4]. As an apoptotic condition, hyperglycemia even mild (such as prediabetes) affects immature EPCs more so than the mature endothelium. Hence, the damaged and inflamed mature endothelium, with time, is not replaced by EPCs [10] as the progenitor pool has been depleted. This maybe one of the reasons why vascular damage takes 4-5 years to develop following onset of hyperglycemia. [2-4]



Endothelial Progenitor Cells (EPCs, defined as CD34+ cells) replace injured/ apoptotic mature endothelial cells [5]

It is known that GLP1 agonist has positive effect on oxidative stress, and endothelial function, therefore semaglutide can be hypothesized to have a positive effect on EPC and endothelium and possibly reduce fat inflammation. [8,9]. It may also reduce transformation of multipotent mesenchymal stem cells (MSCs) towards more fat formation (prevent adipogenesis) which may explain weight reducing capability seen in semaglutide studies (SUSTAIN trials). The use of

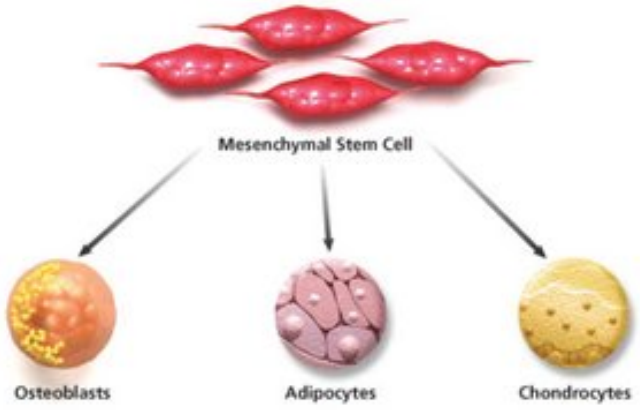
CD34+ cells and MSCs as a biomarker is novel. One can obtain CD34+ cells from a simple peripheral blood draw (without doing an invasive procedure). The blood is then sorted for a homogenous progenitor/stem cell population [6]. Role of CD34+ve EPCs in vascular biology, heart regeneration and collateral vessel formation as an endothelial progenitor cell is well established. It's role as a biomarker is also being developed [7,10,12]. CD34+ cells are the most studied cardiovascular progenitor cells and its efficacy has been established in chronic diseases such as diabetes by Werner et al in 2005. [6,17]

Similarly, one can obtain fat derived MSC from fat biopsies, particularly from overweight and obese individuals. Diabetes is not only a state of endothelial dysfunction, it is also a state of fat hyperplasia, insulin resistance at the level of muscle and fat and is associated with high ROS. Improvement of endothelial health is most likely paired with healthier fat. A state of healthier fat will be associated with healthy adipocytes, pre-adipocytes and healthy MSCs [3,13-15].

The weight reducing data from SUSTAIN 6 trial [16] using semaglutide at 0.5mg and 1.0mg, is encouraging. It has also shown significant improvement in blood pressure and HbA1C within 8 weeks and definitely by 16 weeks even at a lower FDA approved dose of 0.5mg once a week. These finding prompted us to use MSC as a fat surrogate and EPCs as an endothelial surrogate to establish a cellular mechanism behind the clinical trial findings. It may also shed light on cross-talk between these two important insulin responsive tissues that contribute towards cardiovascular health.

We believe EPC is the ideal **cellular** vascular outcome biomarker while MSC is the ideal adipocyte health bio-marker. Based on our recently published data on saxagliptin's effect on EPC of subjects with Type 2 Diabetes, we are confident that EPC is a robust endothelial marker with quick changes in number, function and gene expression, after appropriate intervention. [18].

The purpose of the present study is to study the effect of a long-acting GLP-1 agonist, over a period of 24 weeks and understand how it influences two different yet related cell types such as endothelium and adipocyte, both of which are key players in insulin resistance/sensitivity in the body [14,17].



Mesenchymal Stem Cells (MSCs) differentiate into osteoblasts, adipocytes and chondrocytes. MSCs is a multipotent stem cell that can be obtained from bone marrow, fat and any mesenchymal tissue [17] and should be able to differentiate in these 3 lineages in normoglycemic environment. Hyperglycemia and certain environments, such as PPARG agonists, presence of xeno-estrogens, accentuates adipogenic lineage thereby prevents osteogenesis and chondrogenesis. We would like to investigate how GLP1 influences this differentiation pathway

2. SPECIFIC OBJECTIVES:

Primary Objective: semaglutide modification of fat derived mesenchymal stem cells and CD34+ cell number, function, and gene expression.

This will help identify genes and pathways that are responsive to semaglutide in the two different stem cells and identify associated proteins and pathways that may be quickly responsive and important in two different cell lineages

Secondary Objective: Investigate mitochondrial metabolism of undifferentiated cells such as MSCs derived from subcutaneous fat of subjects treated with or without semaglutide.

This will Evaluate whether semaglutide prevents adipogenic differentiation of fat derived stromal cells which will explain clinical observation of weight loss (SUSTAIN- trial).

Tertiary Objective: To determine whether semaglutide improves vascular reactivity (arterial stiffness), change body habitus parameters, serum endothelial inflammatory markers and levels of appetite controlling hormones.

This will investigate whether semaglutide improves endothelial dysfunction.

3. RESEARCH DESIGN AND METHODS

Study Hypotheses:

We hypothesize that GLP1 agonists, like semaglutide, have a positive effect on the EPC number, function, targeted gene expression, arterial stiffness and endothelium specific inflammatory markers.

Additionally, we hypothesize that semaglutide therapy will reduce adipogenesis and increase bone and cartilage formation by increasing cellular metabolism, as evidenced by increased mitochondrial biogenesis and increased cellular oxygen consumption rate (OCR, measured by SeaHorse).

Study Endpoints:

Primary Objective (Peripheral blood based Endothelium directed outcome measures): *semaglutide modification of fat derived mesenchymal stem cells and CD34+ cell number, function, and gene expression.*

1. **CD34+ cell number, function, and gene expression.** The CD34+ cell number will be measured as % of total mononuclear cell (MNC) population. The function will be assessed as migratory potential of the CD34+cells in response to SDF1a. We will examine the cellular expression of genes that control endothelial function and cell survival and apoptosis genes such as SOD1-3, catalase, peroxidase, eNOS, vWF, hsCRP, IL-6, IL1β, TNF-alpha, COX2, endothelin 1, p53, p21, and caspases. This endpoint will be obtained by a venous blood draw. (Week 0, 8, 24).

Details: Upto 95 ml of peripheral blood will be obtained by venipuncture of which 65-70 mL of whole blood will be used to obtain CD34+ cells from the mononuclear cell (MNC) population. 20-25ml of whole blood will be used for biochemistry and serum ELISA assays.

MNC will be obtained from whole blood prior to CD34 column sorting following protocols described before and currently used in our laboratory for clinical studies [18]. A portion of the

MNCs will be plated to obtain and count colony formation units (CFU) at day 5 and an additional portion will be analyzed to determine increased or decreased presence of stem like cells (marked by CD34+, CD133+) by Fluorescence-activated cell sorting (FACS) The remaining MNCs will be sorted and separated by magnetic bead column method to obtain *CD34+ cells* (Miltenyi Biotec).

Post-CD34 column sort we will undertake migration studies and mRNA isolation:

First, we will count the number of viable CD34+ve cells obtained post column separation and divide that number by the total MNCs obtained to note the percentage of CD34+ve cells.
Next we will set up the migration assays using a Boyden's chamber using SDF1 alpha at 0, 10ng/ml and 100ng/ml concentration. The migration will be assessed at 24 hours using standard published methods [18].
We will undertake targeted gene expression by RT-PCR on CD34 positive cells from mRNA:
a. Anti-oxidant genes (SOD 1-3, catalase and peroxidases), glucose transporters [4,13]
b. Endothelial function assay genes: endothelial NOS (eNOS), von-Willebrand's factor (vWF), VEGF and KDR [17]
c. Inflammatory genes such as IL6, IL1β, TNFα, COX2 and endothelin-1 [4,17]
d. Apoptosis pathway genes such p53, p21, Bax-Bcl2 and caspase-3 & 9 [4,17]

Secondary Objective (Fat and fat derived MSC based outcome measures):

1. Investigate targeted gene expression of subject's subcutaneous fat.

SEMAGLUTIDE'S EFFECT ON FAT METABOLISM. **THIS ENDPOINT WILL BE OBTAINED BY A SUBCUTANEOUS FAT BIOPSY OBTAINED FROM THE SUBJECT'S LEFT ABDOMEN. (WEEK 0 AND 24).** We will evaluate mRNA gene expression and western blot protein estimation for mature fat and fat related transcription factors: leptin, perilipin, adiponectin, PPARG, CREBP-α and β, inflammatory markers: TNFα, IL-6, antioxidants: SOD-1 to 3 and Catalase in antioxidant MSC groups. We will estimate PGC1α, UCP-1 and PRDM-16 gene expression in the fat depots in order to verify browning or beiging of fat Genes, that will be tested are categorized as follows:
Genes associated with
Inflammation: IL-6 (interleukin 6), IL-10, IL1β, TNFα (tumor necrosis factor- alpha),
Cellular Apoptosis p53, p21, caspase-3 and 9,
Cellular Anti-oxidants: SOD1-3 (superoxide dismutases), CAT (catalase), GPX-1 & 3 (glutathione peroxidase),
Genes associated with mitochondrial complex subunits such as NDUF1 and SDHB [15,31],
Genes associated with lipid formation and accumulation: CEBPα and CEBPβ (cholesterol ester binding protein α and β), PPARG, FABP4, Leptin, FGF21, Adiponectin and Perilipin;
Genes associated with mitochondrial activity such as COX2, COX4, TFAM, ATP5B, NRF-1, NRF-2, UCP1, PGC1A, PRDM-16, Sirtuin-1 and Sirtuin-3
Genes associated with glucose transport and taste receptors: GLUT1, GLUT4, TAS1R3, TAS2R3.

2. **Fat derived mesenchymal stromal cells (MSCs).** We will investigate changes (up or downregulation) in bone, muscle, cartilage and adipocytes associated gene expressions in adipose tissue- derived MSCs. This endpoint will be obtained by a subcutaneous fat biopsy obtained from the subject's left abdomen. (Week -8, 16)

Along with the genes noted above we will look at MSC differentiation [21-26]

Adipogenic Genes: CEBPA AND CEBPB (CHOLESTEROL ESTER BINDING PROTEIN A AND B), PPARG, FABP4, PLIN.
Myogenic Genes: MYOD **(**Myogenin D)
Osteogenic Genes: RUNX2, BGLAP (osteocalcin), ALPL (alkaline phosphatase), BMP2, BMP6, SMADS3, TGF-B.
Chondrogenic: COL1A, COL2A COLLAGEN TYPE II (CO-II), COLLAGEN TYPE XI (CO-XI), ACID PHOSPHATASE (ACP), CARTILAGE OLIGOMERIC MATRIX PROTEIN (COMP) AND ELASTIN

Tertiary Objective: To determine whether semaglutide improves vascular reactivity (arterial stiffness), change body habitus parameters, serum endothelial inflammatory markers and levels of appetite controlling hormones.

- **Arterial Stiffness:** We will acquire Pulse Wave Analysis and Vascular Flow using SphygmoCor CP system from ATCOR [19] as a measure of central arterial pressure and arterial stiffness. Vessel health will be assessed by degree of arterial stiffness, using arterial tonometry.
- **Body Composition Scale:** Height and weight will be measured and the body mass index (BMI=kg/m^2) used as an indicator of relative weight. The body composition scale calculates body fat%, total body water%, fat free mass, etc., in addition to BMI.
- **Hip & Waist Measurements**
- **Blood biochemistries and Urine microalbumin ratio:** Patient blood biochemistries will be collected as a part of standard of care, and to monitor the overall health of the subjects throughout the protocol. We will be measure HbA1C, Lipid Panel, CRP, comprehensive metabolic panel (including liver panel), leptin, adiponectin, glucose, insulin, IL-1B, IL-6, IL-10, TNFα, leptin, ApoB, Apo A1 and urine micro-albumin /creatinine ratio.
- **Appetite controlling hormones:** We plan to investigate changes in GLP1 (via LabCorp) and ghrelin (via ELISA).

Study Type:
This is a single center, double-blind, placebo-controlled, parallel group, randomized phase IV clinical trial. There will be two treatment arms (Placebo and semaglutide), each with 20 patients. Subjects will be undergoing treatment for 16 weeks.

Study population:
We will randomize **40 subjects** to start on study medications, with approximately 20% drop out rate over two years in a hope to retain approximately **32 individuals (16/group)**. We plan to screen approximately 60 subjects. This is a single site study, with the sole site being the GW Medical Faculty Associates in Washington, D.C.

We will attempt to recruit patients from across all races and ethnicities as much as possible to minimize ethnicity factors affecting results. Subjects only on metformin as diabetes medication will be included. Subject cohort with at risk for CVD being diabetic and obese but subjects with known history of myocardial infarction and cerebrovascular accident will be excluded. We want to study semaglutide without the confounding effect of any other anti-diabetic agents, other than metformin, in a patient population at risk of CVD, so that there is room for endothelial function improvement in this cohort.

CVD risk in addition to type 2 diabetes will be an added as an inclusion criteria as determined by the investigator such as microalbuminuria or proteinuria (as defined by ADA), hypertension (labile and uncontrolled hypertension) and left ventricular hypertrophy, left ventricular systolic or diastolic dysfunction, or an ankle–brachial index [the ratio of the systolic blood pressure at the ankle to the systolic blood pressure in the arm] of less than 0.9, low HDL with hypertriglyceridemia (as defined by NCEP ATP III) , strong family history of CHD (as defined by NCEP ATP III) [27, 28]

The primary outcome measures are based on endothelium, secondary outcome markers on fat/ fat derived MSCs and tertiary outcome markers are based on body composition, arterial stiffness, serum and urine biochemistries. All three outcome measures as elaborated above, are crucial in order to corroborate our cellular findings with currently accepted [17] clinical efficacy outcome measures such as arterial stiffness [19] and serum biochemistry. This design is similar to our recently published manuscript on Saxagliptin and cellular outcome measures [18].

Inclusion Criteria:

1. Age 30-70
2. Diagnosed with Type 2 diabetes mellitus
3. Body Mass Index (BMI) between 25.0-45.0 (both inclusive)
4. eGFR ≥ 30 mL/min/1.73 m^2 by MDRD
5. HbA1C 7.0 – 10.0 %
6. Subjects on a stable dose of Metformin (1-2 grams), only, for 3 months prior to screening.
7. Ability to provide informed consent (and document informed consent by signature) before any trial-related activities are conducted.
8. Additional CVD risk factor such microalbuminuria or proteinuria (as defined by ADA, UACR > 30 mg/g), hypertension (labile and uncontrolled hypertension) and left ventricular hypertrophy, left ventricular systolic or diastolic dysfunction, or an ankle–brachial index [the ratio of the systolic blood pressure at the ankle to the systolic blood pressure in the arm] of less than 0.9, low HDL with hypertriglyceridemia (as defined by NCEP ATP III) , strong family history of CHD (as defined by NCEP ATP III and ATP IV).
9. Retinal examination within last 18 months of enrollment, showing no proliferative retinopathy

Exclusion Criteria:

1. Uncontrolled hyperglycemia with fasting glucose >240 mg/dL (>13.3 mmol/L)
2. Liver disease with ALT, AST or ALP ≥ x3 ULN
3. Planned CV surgery or angioplasty in the past 1 month
4. History of established CVD
5. Known personal history of cerebral stroke or heart attack (myocardial infarction)
6. All other diabetes medications other than metformin
7. Personal or family history of medullary thyroid cancer (MTC)
8. Personal or family history of Multiple Endocrine Neoplasia Syndrome Type 2 (MEN 2)
9. GFR <30 mL/min/1.73 m^2 by MDRD

10. Prior surgery with chronic malabsorption (eg, bariatric) in prior 1 year
11. Clinically significant RBC disorders such as hemoglobinopathies
12. Diagnosis of Type 1 diabetes mellitus or history of GAD antibody positive status
13. Chronic use of anti-inflammatory drugs for the last 3 months
14. Beginning statin medications or change in statin dose in the past 1 month
15. Use of consistent long-term steroid medication (oral, inhaled, injected) within the last 1 month

16. History of pancreatitis
17. Known or suspected allergy to GLP-1 agonists, excipients, or related products.
18. Active smokers
19. Active wounds (i.e. diabetic ulcers) or recent surgery within 1 month
20. Untreated hyper/hypothyroidism
21. Implanted devices (eg. Pacemaker) that may interact with Tanita scale
22. Any other clinical condition that would jeopardize patients safety while participating in this clinical trial
23. Women of child bearing potential who are not willing to use a contraceptive method to avoid pregnancy for the 16 weeks of study duration plus 2 months post treatment (for semaglutide washout).
24. Women who are pregnant or breastfeeding
25. Chronic or persistent alcohol or drug abuse
26. Prisoners or subjects who are involuntarily incarcerated
27. Subjects who are compulsorily detained for treatment of either a psychiatric or physical (eg. infectious disease) illness
28. Participation in another trial with an investigational drug within 30 days prior to informed consent.
29. Untreated or active hemorrhagic proliferative diabetic retinopathy

Exclusionary Laboratory Findings

30. Chronic Kidney Disease (CKD) stages 4 and 5 (estimated CrCl less than 30 mL/min)
31. Serum creatinine levels ≥1.8 with estimated CrCl < 60 mL/min
32. Triglycerides > 500 mg/dL
33. Low hematocrit (<28 Units)

Withdrawal Criteria:

This research study is entirely voluntary, and whether or not a subject takes part is entirely up to them. Subjects can withdraw at any time. If subjects withdraw after being randomized, all their data will be kept up to that date, unless a letter is written to the sponsor-investigator explicitly indicated against this. If subjects withdraw due to health reasons, they may be followed for safety reasons.

Any subject with active or bleeding hemorrhagic proliferative diabetic retinopathy as a side effect, will be taken off the Investigational product but they can continue with the study till completion.

Subjects who become pregnant during the study or have the intention of becoming pregnant will be removed from the study for safety reasons. There may be other reasons, up to the investigator's discretion, for early removal of a research subject.

All medications and treatments not permitted during the trial are outlined in the inclusion / exclusion criteria.

Study Drug Interruption

Subject who interrupt their study drug dosage will be allowed to stay in the study if they resume their dosage within two weeks.

The target for this trial is to keep the patient on maximum dosage of semaglutide, but if the patient can't tolerate the maximum dosage of 1.0 mg /week, we will allow the patient to stay on the trial on the maximum tolerable dosage.

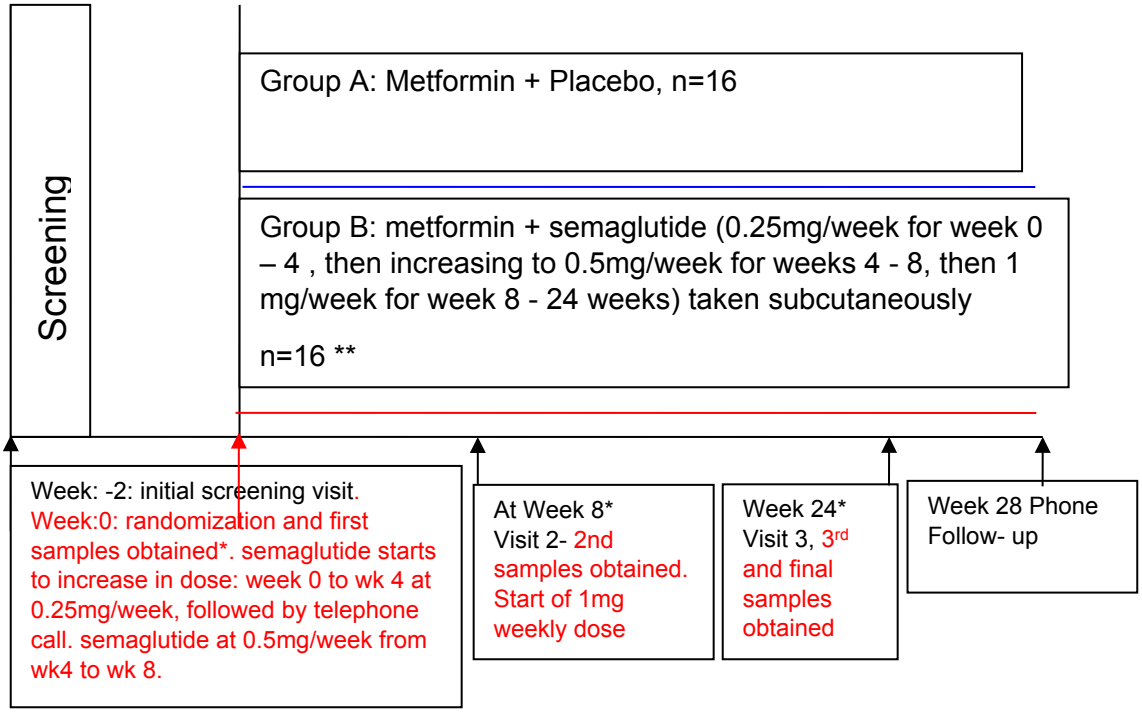
Subject Replacement:

If a subject withdraws or is removed from the research study, they will not be replaced as we are already accounting for attrition with 40 subjects being randomized.

Rationale for Study Population:

This is a high impact clinical and translational project looking at the mechanism of how semaglutide along with metformin can improve number and function of endothelial progenitor cells (as a cellular marker of endothelial function) and fat derived mesenchymal stem cells (as a surrogate of fat metabolism and differentiation). The study population has been carefully selected to match the overall patient population that would most likely be receiving treatment with semaglutide. Patients have type 2 diabetes (HbA1C 7–10%), above normal BMI, and are at risk for CVD. We choose a population that at risk of CVD, rather than known history of CVD, as we believe this is a population that can have the greatest benefit from this medication, with a certain degree of positive chance in reversing endothelial dysfunction and deranged fat metabolism. We hope to corroborate our cellular findings with patient blood biochemistries gathered as used for regular standard of care for diabetes. We also hope to establish that monitoring function and gene expression of endothelial progenitors and fat derived MSCs will allow us to quantify CVD risk, monitor obesity at a stem and progenitor cell level, post GLP-1 treatment in patients with type 2 DM. Effect on stem/progenitor cells will help us to predict how semaglutide would affect cardiovascular health in the near future in a specific subject/subjects. The exclusion criteria have been carefully selected to align with the prescriber information for semaglutide and ensure that this drug would be safe for study participants to take.

4. STUDY DESIGN



*Assessed at week: 0, 8 and 24: Biochemical and cellular markers to be obtained.
Blood for EPCs will be harvested at weeks 0, 8 and 24
MSCs from fat biopsies will be harvested at weeks 0 and 24.
Week 28 - A telephone call to subjects will be made 4 weeks after last dose of study medication to determine if there have been any adverse events.

Time line:
Week -2: initial screening
Week 0: randomization, start on 0.25mg/week, sc, or placebo, Baseline-Studies: blood, fat biopsy and arterial stiffness.
Week 4: follow up phone-call to ascertain compliance
Week 4: start on 0.5mg/week, sc, or placebo, following phone call
Week 8: start on 1mg/ week, sc, or placebo, Studies: blood, and arterial stiffness.
Week 16: follow up phone-call to ascertain compliance at the mid-point of the study
Week 24: end of study: on 1mg/ week, sc, or placebo, Studies: blood, fat biopsy and arterial stiffness
Week 28: follow-up phone call for patient safety and satisfaction

Initial Screening: week -2
*** 2 weeks of screening period will be used to help subjects adjust to the protocol and suggested lifestyle changes. (**week -2 to week 0, two weeks**)*

At the start of week 0, subjects will be randomized to treatment or placebo group. Treatment group will receive 0.25 mg /weekly semaglutide or placebo taken subcutaneously (week 0 to end of week 4, four weeks). Week 0 will be visit 1

Patients randomized on semaglutide arm, will proceed to be started at 0.5g/week at the beginning of week: 4 and then increased to 1.0mg/week at the beginning of week 8. A matching placebo for either 0.25mg or 0.5mg or 1.0mg will be administered to the subjects in the placebo arm.
At week 8 will be visit 2, when all parameters similar to visit 1 will be tested except fat biopsies.
At week 24, will be visit 3, when all parameters similar to visit 1 will be tested including fat biopsies.

Visit Procedures:
The study will consist of pre-screening, four visits to The GW Medical Faculty Associates, and a follow-up phone call. All procedures will take place at the GW MFA.

Pre-Screening (Within 3 months of Screening Visit), 20 Minutes: Subjects who are interested in the research study will first be asked a series of questions either over the phone, or in-person in the MFA clinics to determine initial eligibility for the research study. See attached "Pre-Screening Form". This is called pre-screening and will be performed either over the phone, or when interested subjects are identified in the MFA clinic. If, when going through the questions, a subject is found ineligible due to the criteria, then the pre-screening will be stopped then. The responses to this study will only be recorded if the subjects are found eligible and are invited to an in-person screening visit, where they will sign the ICF.

Screening Visit (week -2) 90 Minutes: During the screening visit, a trained member of the research staff will explain the study procedures and the participant will have the opportunity to ask questions. See "Consenting Procedures" below. After providing written consent, a copy of the signed ICF will be provided to that patient. Then the following screening procedures will be done:

- Medical history & medications reviewed and physical exam
- Vitals & Anthropometric measures: heart rate, blood pressure, temperature, weight and height
- Biochemistry: Urine will be collected to perform a microalbumin/creatinine ratio, and pregnancy test (if applicable). A blood draw will be performed by trained staff to obtain approximately 22ml of blood (1.5 tablespoons). This blood will be used to check the standard of care labs:
    - Complete Blood Count (CBC)
    - Sedimentation Rate (Infection, anemia, and others)
    - Hemoglobin A1C (HbA1C, Blood sugar),
    - Chemistry panel: ALT/AST (Liver function), Blood Urea Nitrogen (BUN) (Kidney function), Electrolytes (Kidney function), Fasting Glucose (Blood sugar)
    - Lipid Panel (Cholesterol, Triglycerides)
    - Thyroid Stimulating Hormone (TSH, Thyroid Function)
- Dietary advice: Participants will be instructed not to change their eating habits, including alcohol intake and to maintain their weight and unsupervised activity level steady in a log throughout the study.

Randomization Visit (week 0), Visit 1: 4hrs and 20 minutes: If the results of the screening tests show that the subjects are eligible for the study, then they will return in 2 weeks for their study randomization visit.

- Inclusion / Exclusion criteria will be re-reviewed
- Concomitant medications and recent medical changes will be reviewed
- Assessment for adverse events
- Randomize Subject
- Dispense either 0.25mg / Week for week 0 to 4 of semaglutide or Placebo
- Dispense either 0.5mg / Week for week 4 to 8 of semaglutide or Placebo
- Dispense either 1.0mg / Week for week 8 to 24 of semaglutide or Placebo
- Educate patients on drug + delivery by using the direction for use (DFU).

Visit 1 continued (week 0),

- Vitals: heart rate, blood pressure, temperature
- Waist circumference, hip circumference
- Weight and body fat will be measured with bare feet on the body composition scale. This scale sends a mild electrical current through one's body when they step on it. The electrical current is so small that it is not felt. This is a research related procedure only and is not usually done as part of a physical exam. This is NOT an investigational device.
- Urine sample for microalbumin/creatinine ratio, urine pregnancy test (if applicable), and exosome analysis
- A 95ml blood draw for laboratory EPC analysis (Primary outcome, See Appendix A) and for the following standard of care labs:
    - Chemistry Panel (ALT/AST, BUN, Electrolytes, fasting glucose)
    - Hemoglobin A1C(HbA1C) (Blood sugar)
    - Lipid Panel (Cholesterol)
    - C-Reactive Protein (inflammation)
    - Adiponectin (measure of endothelial paracrine function)
    - Fasting Insulin levels (measure of insulin resistance)
    - Interleukin-6 (inflammation)
- Subcutaneous Fat Biopsy (See Appendix B&C for details)
- Arterial Stiffness (vessel health): Measurements of the blood vessels (pulse) in the wrist (radial), neck (carotid), and groin (femoral) area. This is a research related procedure, not routinely done. This is performed with a SphygmoCor Atcor machine, which is NOT an investigational device.
- Flow Mediated Dilation: Endothelium-dependent process facilitating the relaxation of an artery in response to increased shear stress. It is a direct marker of nitric oxide bio-availability. This is a research related procedure, not routinely done.

Week 4: phone call by coordinator to ascertain subject compliance and establish willingness of the subject to move from 0.25mg to 0.5 mg dose

- *Week 8: Visit 2 (start of 1mg dose), 2.5 hours:*
- All procedures performed in visit 1 will be repeated in the exact same manner, **except there will be no fat biopsy**.
- Subjects will be dispensed either 1.0 mg/week of semaglutide or the matching placebo, based on their assigned randomization. Trial product is the same product and strength as V1, only the dose varies and thus the number of pen-injectors to be dispensed will be increased.

Subjects will return all their used, partly used or unused study pens, and their exercise journal so that compliance can be measured.

Week 16: Mid-point of 1mg dose: phone call by coordinator to ascertain subject compliance.

Week 24: Visit 3 (end of 16 weeks of 1mg dose), 4 hours: All procedures performed in visit 1 will be repeated in the exact same manner. There **will be a second subcutaneous fat biopsy performed at this visit (6 months from the initial one)**. Subjects will return their old study pens, and their exercise journal so that compliance will be measured.

Follow-Up Phone Call (week 28), 10 minutes: Subjects will be called 4 weeks after completing their final visit in order to assess any AEs and check general subject health.

X-Chart:
Please See Appendix D

Consenting Process:
Consenting will take place in a HIPAA Compliant, private room in the MFA. The protection of human subjects will be carefully managed in this study and subjects will be fully informed of their rights as research participants. Subjects will have the opportunity to discuss the study with their family and friends or think about it prior to agreeing to participate.Extensive discussion of risks and possible benefits of participation in this study will be provided to the subjects by trained study staff. An IRB approved consent form will describe the study procedures and risks in detail. In order to voluntarily participate in the study, informed consent will be obtained at the start of the screening study visit, before any screening or study procedures are conducted except from pre-screening.

Written documentation of informed consent is required for all participants prior to undergoing any screening procedures or enrolling in the study. The subjects may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care or their academic standing will not be adversely affected if they decline to participate in this study.

Screen Failures:
Upon completion of the screening visit, all of the data collected will be reviewed with the study staff. Ultimately, the investigator will made the decision on whether to enroll a subject who meets all inclusion / exclusion criteria, upon reviewing their PMH, Concomitant medications, laboratory results, and physical exam. Subjects who meet the eligibility criteria may not

necessarily be enrolled, based on the investigator's discretion. If it is decided not to enroll a subject, they are classified as a screen failure.

Subjects who screen fail can re-screen up to 2 times, with a minimum of a month between screenings.

Subject Withdrawal Process:

If a subject, wishes to withdraw from the research study, they must formally inform the research staff. Ideally, the research staff will get this in writing, and document the reason for the withdrawal. If subjects withdraw after being randomized, all their data will be kept up to that date, unless a letter is written to the sponsor-investigator explicitly indicated against this. If subjects withdraw due to health reasons, they may be followed for safety reasons.

Data Handling:

For each enrolled patient, the medical record number, and study ID providing the link will be kept in a password protected file on the secure MFA network. This will be deleted once the study has closed. All study files that contain subjects' de-identified data, as well as files that contain the subject's consent forms will be kept in hard-copy files in the study coordinator's locked MFA office. These will be destroyed after study closure at the appropriately designated time (6 years).

All study data will be recorded on source documents, which will be kept in the subject's study folders. These will be kept locked in the study coordinator's office, and will only be accessible to study staff. All de-identified study data will also be uploaded to RedCap, a password protected database, for data compilation. No PHI will be placed on RedCap.

Data analysis will be completed at either the MFA or Ross Hall. In order to maintain data security, the research staff will ensure that all data is completely de-identified prior to analysis beginning.

Assessments for Safety

At each visit, the subject's laboratory results will be assessed by the investigators. All laboratory samples will be collected during the screening and regular visits (1-3). They will be drawn by a trained member of the study staff, and processed and stored according to the guidelines for the specific tests being requested, and while adhering to OSHA guidelines. All laboratory results will be acquired from LabCorp, and results will be reported to the study coordinator.
Laboratory results will be compared to the pre-determined "normal ranges", and will be indicated if any value falls above or below this normal range. The investigator will then determine for all laboratory results, and especially for all values outside of the normal range, if it is clinically significant.
Vitals and laboratory results will be monitored at every visit to see if any value classifies as an adverse event, in which case it will be reported via the appropriate manner (see AE Reporting Section)

Other Assessments

All samples being collected will be identified with the subjects' specific study ID. Samples being sent to LabCorp will also include the subject's DOB. Samples will be stored in the appropriate manner for the respective test being run. Samples that are planned to be sent to LabCorp will be sent same day via LabCorp courier. Samples being brought to GW Ross Hall for basic science lab processing will be transferred between clinical and basic science research staff at the GW

MFA. After transfer of care, samples will be maintained in the investigator's lab, and only be accessible by members of the study staff. Samples will be destroyed upon completion of the study, and after a "data lock" has occurred. Some samples may be stored in the -70*C freezer of the MFA for later processing.

Subject Compliance

Subject compliance will be measured by subjects returning the semaglutide pens at visits 2 and visit 3. A compliance calculation will then be done. If subjects have taken 70% of the dosage they were supposed to, they will be considered compliant with the study medication.

5. STATISTICAL CONSIDERATIONS:

Sample Size Calculation

Sandri et al. (2005, Study A, patients with peripheral arterial occlusive disease) [11] found that in the control group, CD34+ cells increased from 372 cells per mL blood at baseline (SD 156) to 402 (SD 183) at 4 weeks. In their exercise training group, the increase was from 458 (SD 252) at baseline to 2977 (SD 852) at 4-weeks.

We created a simulated data set with these parameters in order to calculate the percent of CD34+ cell variation that was explained by the group x time interaction. We tested two random effects mixed models, one without and the other with, a group x time interaction (group is treatment vs control; time is pre vs. 4-weeks post). The percent variance explained = (Vno-int – Vint)/Vno-int, where Vno-int is the residual covariance parameter estimate without a group x time interaction term in the model, and Vint is the residual covariance parameter estimate with a group x time interaction term in the model. We found that based on the baseline and 4-week mean and standard deviation of CD34+ cell concentrations reported by Sandri et al. (2005), the interaction explained 84.5% of the variance in CD34+ cell concentration (a very large effect size). The correlation among repeated measures was r=.62. Using these parameters in G-Power3 (version 3.1.3), with 2 groups measured at 2 time points, in order to achieve power >.95 would require a total sample size of 6 subjects (3 per group) each measured at 2 time points.

Assuming 25% subject loss, we would need to start with 4 per group. If the group x time interaction explained only 50% of the variance in CD34+ cell count (still a large effect), we would need total sample size of 8 (4 per group) in order to achieve power >.95. In order to account for possible 25% drop-out, 6-8 per group should be randomized.

In Sen et al. (2015), [10] in a sample of patients with pre-diabetes (n=11), CD34+ cell number increased from 0.8 (SEM 0.1) before exercise, to 1.4 (SEM 0.2) after exercise, a pre-post effect size (Cohen's d) of 1.81. In order to detect an effect of this size using a 2-tailed paired t-test, with alpha=.05, for power >.80, >.90, and >.95, the number of subjects required would be 5, 6, or 7, respectively. However, these two quoted studies are different from our proposed study on patients' with diabetes. It is well known that diabetes can affect CD34+ number and function, therefore in our proposed study number of CD34+ cell availability may be a limiting factor.

Our recently concluded study also confirms that our power calculations are satisfactory [18]. Therefore, we propose to use double the number suggested by power calculation and use 8x2=16 patients per group.

Data Analysis Methods

We will use generalized estimating equations (GEE) with robust standard error estimates to model within-subject and between-group differences over time. First we will investigate whether

an unstructured correlation matrix is best fitting for the error term based on examination of the working correlation matrix output. If an exchangeable structure fits the data, we will consider using a random-effects mixed model with restricted maximum likelihood, due to its weaker assumption for missing data on the response variable. Using either approach, of primary interest will be the group (drug treatment) by time interaction, which will tell us whether the change in the dependent variable over time differs between groups. We will include as covariates any pre-treatment variables (e.g., demographics, baseline levels of the dependent variable, comorbidities, laboratory test values) that differ between groups even at a trend level of significance ($p<.10$).

Interim Analysis
No interim analysis will be performed, in order to keep all members of the research staff blinded, and to not introduce any bias.

6. DATA HANDLING AND RECORD KEEPING:

For each enrolled patient, the medical record number, and study ID providing the link will be kept in a password protected file on the secure MFA network. This will be deleted once the study has closed. All study files that contain subjects' de-identified data, as well as files that contain the subject's consent forms will be kept in hard-copy files in the study coordinator's locked MFA office. These will be destroyed after study closure at the appropriately designated time (6 years).

All study data will be recorded on source documents, which will be kept in the subject's study folders. All contact and communication with subjects will be recorded in progress notes, and maintained in the pateint folders. These will be kept locked in the study coordinator's office, and will only be accessible to study staff. All de-identified study data will also be uploaded to RedCap, a password protected database, for data compilation. No PHI will be placed on RedCap.

Data analysis will be completed at either the MFA or Ross Hall. In order to maintain data security, the research staff will ensure that all data is completely de-identified prior to analysis beginning.

7. ETHICS:

This study will be reviewed and approved by the GW MFA and the GW IRB Full Board prior to beginning any recruitment or study procedures. A copy of the IRB approval letter and all IRB approved documents will be provided to the sponsor, upon approval. This study will be conducted in accordance with the Declaration of Helsinki, the ICH GCP Guidelines, and all other regulatory and legal requirements. Informed consent will be provided to subjects prior to conducting any study procedures.

Study Risks
Blood Draw: This may result in discomfort at the site of the needle entry or bruising at the site. There is also a remote risk of fainting or local infection associated with drawing blood. To address this, only skilled and well-trained phlebotomists will carry out these procedures. In addition, peripheral blood draws (venipuncture) performed during this study for research will not exceed 95 mL per visit in visits 1-3. **The total amount of blood drawn for the study, including 22 mL from screening, is 307 mL.**

Urine collection: Participants will be asked at some visits to collect a urine sample. Some people do not like the idea of providing a urine sample and may experience difficultly with the collection as a result.

Medical Information: Participants will be asked to provide a medical history. Some participants may find this uncomfortable or embarrassing. We have minimized this risk by de-identifying the data obtained to ensure that this information is not linked to the subject's identity. In addition, we will ensure that interviewers are properly trained to obtain this information in a comfortable and non-judgmental manner. There is, however, always a risk of loss of confidentiality. While all efforts will be dutifully undergone to maintain study confidentiality, it is a consideration.

Tanita Body Composition Scale may cause electrical devices inside the body to malfunction. It should not be used by people with pacemakers or other similar devices.

Subcutaneous fat biopsies: Collection of subcutaneous adipose tissue biopsies may result in pain, bruising, hematoma, infection, and scarring. The procedure will be performed under sterile technique to minimize the chances of infection. Local anesthetic will be used to minimize pain. Ice will be applied to the site immediately after the procedure to limit bruising, swelling and tenderness. After each biopsy, patients will be monitored by the study physician. Post-biopsy the incision site will be cleaned and closed with adhesive wound closures and covered with gauze and translucent dressing tape. Study participants will be instructed to report to the study phyisician any changes at the biopsy site including bleeding, secretion, erythema, pain, and signs and symptoms of infection. Study participants will be instructed to self-monitor after their study visit. An MFA phyisican who is both trained and has experience with performing fat biopsies will be performing the procedure.

Study Medication: semaglutide. The following warnings and precautions are listed:

- The most common adverse reactions, reported in ≥ 5% of patients treated with semaglutide, are: Nausea, Vomiting, Diarrhea, Abdominal pain and Constipation.
- Pancreatitis has been reported in clinical trials. If pancreatitis is suspected, study medication will be discontinued immediately.
- Diabetic Retinopathy Complications have been reported in a clinical trial. We will monitor patients with a history of diabetic retinopathy.
- Hypoglycemia is a risk with concomitant use of insulin secretagogues or insulin. semaglutide itself does not cause hypoglycemia Blood sugars will be closely monitored, and insulin dose titrations will be made, if necessary.
- Thyroid C-cell tumors were found in rodents. It is unknown if semaglutide causes C-cell tumors in humans (including MTC).
- Acute Kidney Injury: There have been post-marketing reports of acute kidney injury and worsening of chronic renal failure, in patients treated with GLP-1 receptor agonists. Renal function will be monitored throughout the study, especially in patients reporting severe gastrointestinal reactions.

Steps Taken To Minimize Risks

The inclusion/exclusion criteria have been created to only allow a patient population that is safe to take semaglutide (within the clinical indication). Throughout the study subject's health will be

monitored at all study visits, and regular Adverse Event assessments will be performed. If a subject does have an adverse event during the study they are instructed to seek medical care, and then to notify the study staff. They can be seen in an interim visit if necessary to asses any adverse events. If patients cannot tolerate semaglutide they will be removed from the study.

All study files will be kept in a locked office in the study coordinator's office. All study information will be transferred from the paper source documents to the secure website, RedCap. All other study information, for example the subject link, will be kept in a password protected excel on the MFA network, only accessible to study staff. Research personnel are trained regarding how to collect, enter, and code subject information and the information gathered in this study will not become part of the participants' medical records. Any samples sent to outside laboratories for future analyses will be identified only with the participants' study code number, and not their name or other identifying information.

Potential Benefits

- Improvement in diabetes condition
- Improved cardiovascular health and possible decrease in body weight.
- Continual monitoring of their diabetes progression and overall health every 8 weeks through standard of care labs.
- Information gained from this research study may help other people with diabetes in the future.

8. STUDY SCHEDULE:

As the study involves both blood draw and fat biopsies recruitment may be slow.
The project will be performed over a 2.5 years period: 3 months start up, 24 months recruitment and intervention, 3 months data compilation, analysis and publication. We estimate a total of 40 subjects will be enrolled to achieve 32 completed subjects (16 in each group).

Start of Study: March 2019
FPFV: August 2019
LPFV: August 2021
LPLV: Dec 2021
Final Study Report: June 2022
Publication: Dec 2022

9. STUDY DRUGS AND MATERIALS:

Definition of Investigational Product: A pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical study, including products already with a marketing authorization but used or assembled (formulated or packaged) in a way different from the authorized form, or used for an unauthorized indication, or when used to gain further information about the authorized form. In this protocol, the investigational product is semaglutide.
This study is being conducted under FDA mandated guideline for use in type 2 diabetes. Though in this particular study semaglutide is referred to as the investigational drug, it is not a novel drug and we should not need a separate IND for execution of this study.

Definition of Non-Investigational Product: Other medications used in the study as support or escape medication for preventative, diagnostic, or therapeutic reasons as components of a given standard of care. In this protocol, the non-investigational products are Metformin. Patients will continue on their anti-diabetic therapy of metformin as per their study entry dose. Doses may be altered if laboratory results indicate this is necessary.

Study medication(s) / devices(s)
semaglutide 0.25mg/week for 4 weeks followed by 0.5mg/week for 4 weeks, during initial 8 week period,
with Matching Placebo
semaglutide 1.0mg/week for week 9 to week 24,
With Matching Placebo
Novo Nordisk will supply the investigator with 0.25mg/week, 0.5mg/week and 1.0mg/week of semaglutide, and their respective matching placebo. Global clinical supply will provide active and placebo study drug, which may be packaged differently from commercial product

Packaging and Labelling of Study Medication(s)
Packaging for the medication will be in the following format:
A single trial product (Name: semaglutide, Strength: 1.34mg/ml) will be provided for all three doses along with its matching placebo, it will be in the form of 1.5 ml prefilled pen injector containing solution for sc injection. Subject will be supplied with sufficient trial product at dispensing visits to cover entire period between two visits.

NovoFine® Plus needles (not provided by Clinical Supplies, requested separately in ISS website)

The pens will be labelled by Novo Nordisk as "Either semaglutide or Placebo". Trial product are packed blinded from Novo Nordisk and will not be re-labelled locally. On the label the following or similar will be stated "semaglutide 1.34 mg/ml or placebo" to maintain blinding of staff and subject. All packs are uniquely numbered and a total DUN (Dispensing Unit Number) list providing the information about which numbers are active and which are placebo will be provided to unblinded IDS staff. This list is to be used when dispensing trial product according to treatment allocation.

Storage and Drug Accountability of Study Medication(s)

Prior to first use	After first use	
Refrigerated 36°F to 46°F (2°C to 8°C)	Room Temperature 59°F to 86°F (15°C to 30°C)	Refrigerated 36°F to 46°F (2°C to 8°C)
Until expiration date of drug	For up to 56 days (8 weeks)	
Additional Condition	DO NOT FREEZE and PROTECT FROM LIGHT	

Storage of the study medication prior to dispensation will be done by IDS. Calibrations are performed continuously, and any temperature excursions are extensively recorded, with

accompanying alarms to the IDS Pharmacist. The investigator will ensure the availability of proper storage conditions and record and evaluate the temperature.

No trial medication will dispensed to any person not enrolled in the study. Used trial medication (study pens) will be returned to IDS for compliance purposes, but will be kept separately from the un-used, non-dispensed, drug. All returned drug will be promptly disposed of upon recording compliance, in the appropriate manner.

Extensive logs will be kept by IDS pharmacy documenting subject randomization, and dispensation logs, as well as product return.

Auxiliary Supply
The budget is inclusive of all other auxiliary supplies will be necessary. All supplies will be purchased by the investigator using the study budget funds, and do not need to be provided by the Novo Nordisk.

Randomization and Blinding
This study is a double-blind trial. All study staff will remain blinded to the treatment allocation, in addition to the patients. The only individuals who are un-blinded are IDS staff, who never have any patient contact or interaction. Treatment allocation is assigned by a block randomization method performed by the Investigational Drug Services (IDS) Pharmacy Staff.

Upon receiving a prescription from the investigator for a new subject to be enrolled, IDS computes what their randomization will be. They then prepare the appropriate study product, and re-label all items to be dispensed to ensure that it is blinded.

Breaking of Blinded Codes
Blinding is critical to the integrity of this clinical study. However, in the event of a medical emergency or pregnancy in a subject, in which knowledge of the investigational product is critical to the subject's management, the blind for that subject may be broken.
Code break will be provided by the study staff who was responsible for randomization in the first place. Before breaking the blind of an individual subject’s treatment, the investigator should have determined that the information is necessary, i.e., that it will alter the subject’s immediate management. In many cases, particularly when the emergency is not investigational product-related, the problem may be properly managed by assuming that the subject is receiving active product without the need for un-blinding. When the a code is broken, the person breaking the code must record the time, date and reason as well as his/her initials in the source documents. All codes (whether broken or not) will be kept throughout the trial period. Accountability of all broken or unbroken codes (hard copy or electronic) will be performed at or after trial closure.

End of Study Un-Blinding Protocol
Upon completion of the research study, we will undergo the following protocol in order to unblind the research data.

Unblinding is the process by which the allocation code is broken so that the investigator, clinical staff and the trial statistician becomes aware of which intervention each subject enrolled in the research study was taking.
Unblinding at the end of the study is required in order to make unmasked analysis in accordance with the study analysis plan. It is also conducted in order to inform the participants of which investigational product they were assigned to.

Time to unblind:
Unblinding shall be conducted when all subjects enrolled in the research study have finished treatment, and all follow up visits. There must be no plan to recruit any more subjects in the research study. Additionally, all data points and outcome measures for each research subject must have been collected, and ideally compiled. Prior to unblinding there will be a data lock on clinical outcome measures and basic side outcome measures and associated research data collected for the study.

Procedure to unblind
Once data (from both the clinical and the basic science side) and has been compiled and is data locked, the investigator can choose to unblind. The Principal Investigator must contact the study sponsor and receive permission to un-blind. If, for unforeseen reasons, at the pre-determined date for full study unblinding the data analysis on the cellular or basic aspects of the study is lagging behind the clinical data outcome measures (though the data has been acquired) the Principal Investigator in consultation with the study sponsor may choose to un-blind the clinical outcome measures before the basic side data has been analyzed but compiled. Upon confirmation from the study sponsor, the principle investigator must make a written request to the designated party to unblind, hereto referred at the "unblinder". The unblinder is the bio-statistician of the MFA, **Dr. Richard Amdur**. Upon receipt of an instruction to unblind, the unblinder will sign the request form, indicating their agreement to unblind. This form will then be taken to the pharmacy, **MFA's IDS**, where a member of the IDS staff will take the form, and will give the unblinding study binder to the unblinder. The pharmacy will sign to indicate their release of the binder, and the unblinder will sign to indicate receipt of the binder. At this point in time the chain of custody of the pharmacy unblended binder has been transferred to the designated party to unblind. The form with all of the signatures will be provided to the study coordinator to be kept in the regulatory binder.

10. CONCOMITANT ILLNESSES AND MEDICATIONS:

Definitions:
Concomitant illness: any illness that is present at the start of the trial (i.e. at screening).
Concomitant medication: any medication other than the trial product(s) that is taken during the trial, including the screening and run-in periods.
After signing the informed consent, all concomitant illness and medications are recorded in a study log. Throughout the trial, at every visit these two lists are reviewed with the subject, and any changes / updates are made on the log. If any change would alter eligibility for the study, the investigator will inform Novo Nordisk.

Information gathered regarding concomitant illness includes: Onset date, Diagnosis, Date of resolution / ongoing, concomitant medications taken for the illness

Information gathered regarding concomitant medications includes: Medication name, Start date, dosage/frequency, indication, stop date (if applicable)

11. ADVERSE EVENTS:

Adverse Event Collecting and Reporting

The collection of adverse event (AEs) information will begin at initiation of signed informed consent. Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. To prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more adverse events. AEs will be followed to completion of the study, if considered not related, or until resolution or stabilization if considered related. They will be reported as SAEs if they become serious. The investigator will comply with all local legal, regulatory, and IRB requirements.

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all AEs: onset, duration, intensity, seriousness, relationship to investigational product, action taken, and treatment required. If treatment for the event was administered, it should be recorded in the medical record. The investigator will report these to the IRB and Novo Nordisk upon continuing review (annually). The investigator must supply Novo Nordisk and the IRB with any additional information requested.

Serious Adverse Event Collecting and Reporting

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, will be collected, including those thought to be associated with protocol-specified procedures. All SAEs will be collected that occur within 30 days of discontinuation of dosing or within 30 days of the last visit for screen failures.

The investigator will report any SAE to the IRB, within 6 business days of knowledge of the event. An SAE report will be completed for any event where doubt exists regarding its status of seriousness. If the investigator believes that an SAE is not related to study drug but is potentially related to the conditions of the study (such as withdrawal of previous therapy, or a complication of a study procedure), the relationship will be specified in the narrative section of the SAE Report Form.

The sponsor-investigator will report to Novo Nordisk all SAEs, SUSARs, and SADRs at the same time such events are reported to regulatory authorities or within 15 days from the sponsor-investigator becoming aware of such adverse events, whichever comes first.

Minimum Information to Report for any SAE, SUSAR, SADR

The sponsor-investigator will collect the following information at minimum for each of these events:

1. Study Name, and PI
2. Patient ID
3. Patient demographics (age, sex, race/ethnicity, etc.)
4. Event (diagnosis if possible)
5. Drug involved, and any actions take with the study drug
6. Reporter identification (e.g. Name, or initials)

7. Investigator determined causality of event
8. Outcome of the event, if possible

Definitions

Adverse Event (AE):

An AE is any undesirable medical event occurring to a subject in a clinical trial, whether or not related to the trial product(s). This includes events reported from the first trial related activity after the subject has signed the informed consent and until post treatment follow-up period as defined in the protocol. The following should not be recorded as AEs, if recorded as medical history/concomitant illness on the CRF at screening:

• Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the subject has signed the informed consent
• Pre-existing conditions found as a result of screening procedures

Clinical Laboratory Adverse Event:

All laboratory test results captured as part of the study will be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported as such.
The following laboratory abnormalities should be documented and reported appropriately:

- Clinical laboratory abnormality regarded as clinically significant i.e. an abnormality that suggests a disease and/or organ toxicity and is of a severity, which requires active management, (i.e. change of dose, discontinuation of trial product, more frequent follow-up or diagnostic investigation).
- Any laboratory abnormality that required the subject to have study drug discontinued or interrupted
- Any laboratory abnormality that required the subject to receive specific corrective therapy.

Serious Adverse Event (SAE):

A serious AE is an experience that at any dose results in any of the following:

• Death
• A life-threatening* experience
• In-patient hospitalisation or prolongation of existing hospitalization
• A persistent or significant disability/incapacity
• A congenital anomaly/birth defect
• Important medical events that may not result in death, be life-threatening*, or require Hospitalisation may be considered an SAE when, based upon appropriate medical judgement, they may jeopardise the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition
• Suspicion of transmission of infectious agents

*The term life-threatening in the definition of SAE refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe.

NOTE: The following hospitalizations are **not** considered SAEs:

- A visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an "important medical event" or a life-threatening event)
- Elective surgery planned or anticipated before signing consent
- admissions as per protocol for a planned medical/surgical procedure
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative).

Serious Adverse Drug Reaction (SADR):
An adverse drug reaction (ADR) is an adverse event (AE) for which a causal relationship to the trial product is at least possible i.e. causal relationship is conceivable and cannot be dismissed. Serious adverse reaction (SAR): Adverse event which fulfils both the criteria for a Serious Adverse Event and the criteria for an Adverse Reaction.

Suspected Unexpected Serious Adverse Reaction (SUSAR):
An SAE which is unexpected and regarded as possibly or probably related to the trial/study product by the investigator.

Medical Events of Special Interest (MESI): A MESI is either

1. A medication error (e.g. wrong drug administration or wrong route of administration) o
2. A suspected transmission of an infectious agent via the product

Non-Serious Adverse Event:
A non-serious AE is any AE which does not fulfil the definition of an SAE.

Severity Assessment Definitions:
• Mild: Transient symptoms, no interference with the subject's daily activities
• Moderate: Marked symptoms, moderate interference with the subject's daily activities
• Severe: Considerable interference with the subject's daily activities, unacceptable

Relationship to study medication Assessment Definitions:
• Probable: Good reasons and sufficient documentation to assume a causal relationship
• Possible: A causal relationship is conceivable and cannot be dismissed
• Unlikely: The event is most likely related to an etiology other than the trial product

The semaglutide Prescriber Information packet will be used to evaluate all unexpected events and adverse reactions.

Outcome Categories and Definitions:

A. Recovered: Fully recovered or by medical or surgical treatment the condition has returned to the level observed at the first trial related activity after the subject signed the informed consent
B. Recovering: The condition is improving and the subject is expected to recover from the event. This term should only be used when the subject has completed the trial
C. Recovered with sequelae: As a result of the AE, the subject suffered persistent and significant disability/incapacity (e.g. became blind, deaf, paralysed). Any AE recovered with sequelae should be rated as an SAE
D. Not recovered
E. Fatal
F. Unknown

Collection, Recording and Reporting of Adverse Events

All events meeting the definition of an adverse event must be collected and reported from the first trial related activity after the subject has signed the informed consent and until the end of the posttreatment follow-up period as stated in the protocol. For specific timelines on reporting, please see above.

Follow-up of Adverse Events

During and following a subject's participation in the trial, the sponsor-investigator and institution will provide adequate medical care to the study subject for any study-related adverse events, including clinically significant laboratory values related to the study. However, the GW Medical Faculty Associates, GWU, GWU Hospital or the sponsor does not have a program to provide compensation or free medical treatment for research-related injury. This would be covered by the subjects' insurance, or whatever route of payment they typically use for medical care.

All adverse events classified as serious or severe or possibly/probably related to the trial product must be followed until the subject has recovered and all queries have been resolved. For cases of chronic conditions follow-up until the outcome category is "recovered" is not required, as these cases can be closed with an outcome of "recovering" or "not recovered".

All other adverse events must be followed until the outcome of the event is "recovering" (for chronic conditions), or "recovered" or until the end of the post-treatment follow-up stated in the protocol, whichever comes first, and until all queries related to these AEs have been resolved.

Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including up to 2 months post product administration, the investigational product will be permanently discontinued in an appropriate manner (e.g., dose tapering if necessary for subject safety). Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. Pregnancy complications should be recorded as adverse event(s). If the infant has a congenital anomaly/birth defect this must be reported and followed up as a serious adverse event.

The investigator or designee will immediately notify the Novo Nordisk of this event immediately (within 24 hours) in accordance with SAE reporting procedures. Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information will be reported.
Any pregnancy that occurs in a female partner of a male study participant should be reported to the sponsor. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

Precautions/Over-dosage
Correct dosing of the investigational product will be overseen by subject drug returns. If a subject does overdose, then this is considered an SAE. Seriousness can be assessed on case by case basis and based on the associated consequences. Appropriate SAE reporting guidelines will be followed.

12. LIABILITY AND SUBJECT INSURANCE:

During and following a subject's participation in the trial, the sponsor-investigator and institution will provide adequate medical care to the study subject for any study-related adverse events, including clinically significant laboratory values related to the study. However, the GW Medical Faculty Associates, GWU, GWU Hospital or the sponsor does not have a program to provide compensation or free medical treatment for research-related injury. This would be covered by the subjects' insurance, or whatever route of payment they typically use for medical care.

The sponsor-investigator will be responsible for the conduct of the study and that the sponsor-investigator agrees to defend, indemnify, and hold harmless Novo Nordisk, any of its parent companies, affiliates, or subsidiaries, and their respective officers, directors, employees, agents, representatives, distributors, salespersons, customers, licensees, and end-users from and against any claim, suit, demand, loss, damage, expense or liability imposed by any third party arising from or related to: (a) any breach of sponsor-investigator's obligations or representations; or (b) sponsor-investigator's negligent or grossly negligent use or willful misuse of the study drug, the results, or services derived therefrom. This indemnification shall not apply in the event and to the extent that a court of competent jurisdiction or a duly appointed arbiter determines that such losses or liability arose as a result of Novo Nordisk's gross negligence, intentional misconduct, or material breach of its responsibilities.

13. EVALUABILITY OF SUBJECTS:

Subjects who meet the eligibility criteria and are deemed suitable by the investigator will be included in the research study. The study statistician will made the determination, upon data analysis about whether to include certain data elements in the conglomerate data set, and outliers may be excluded. Prior to a subject's data been removed, the reasons for their exclusion must be documented and signed by those responsible prior to database release. The documentation must be stored together with the remaining trial documentation.

14. PREMATURE TERMINATION OF STUDY:

If, it is deemed by the investigator, Novo Nordisk, the IRB, the FDA, or any other governing body that the study is unfit to continue due to safety, non-compliance, or any other reason, then

all study activity will cease immediately. A formal letter regarding the reason for premature study termination will be submitted to the IRB and Novo Nordisk.

15. PUBLICATION PLAN:

The clinical trial will be registered with clinicaltrial.gov prior to the enrolment of the first subject.
Upon completion of the study, the investigator plans to submit to a peer-reviewed scientific journal within 6 months of last patient, last visit. Results of the study will be reported on clinicaltrials.gov for public access.

16. APPENDIX A: CD34+ CELL ANALYSIS

We will obtain of 95 mL of peripheral blood per visit. Of these 95 mL, 60-70 mL will be used to obtain CD34+ cells from mononuclear cell (MNC) population and 20 mL for biochemistry and serum ELISA assays. MNC will be obtained from whole blood prior to CD34 column sorting following protocols described before and currently used in our laboratory for clinical studies. Some MNCs will be plated to obtain and count colony formation units (CFU) at day 14. Some MNCs will be analyzed to assay increased or decreased presence of stem like cells (marked by CD34+, CD133+) using a Fluorescence-activated cell sorting (FACS) analyzer (part of GW Core facilities). Rest of the MNCs will be put through column to obtain *CD34+ cells* (Miltenyi Biotec).

Post-CD34 column sort we will undertake:

1. CD34+ cell Migration assay using SDF1α conc.at 0, 10, 100ng/ml
2. RT-PCR on CD34 positive cells for:

a. Anti-oxidant genes (SOD 1-3, catalase and peroxidases), glucose transporters
b. Endothelial function assay genes: endothelial NOS (eNOS), von-Willebrand's factor (vWF)
c. Inflammatory genes such as IL6, IL10, IL1β, TNFα, COX2 and endothelin-1
d. Apoptosis pathway genes such p53, p21, Bax-Bcl2 and caspase-3 & 9
e. Mitochondrial genes: TFAM, NRF-1 &2, ATP5B, cytochrome oxidase 2 & 4, PGC1A, PRDM-16, UCP1

17. APPENDIX B: SUBCUTANEOUS FAT BIOPSY PROCEDURE

Two to three grams of subcutaneous adipose tissue from the left abdomen will be collected. This will be done as follows:
Patient will be fasting for 10 hours prior to the procedure. The patient will lie down supine on an exam table. The physican performing the biopsy will perform a minor abdominal exam, and will palpate the abdomen. They will identify the area, between the left iliac crest and the unbillicum where the procedure will be performed, and indciate to the trained assistant. The area will then be thoroughly cleaned with proper anesthetic procedures. A fenestrated drape will be placed over the abdomen, revealed the site of the procedure. Local anesthesia is applied to the area (Lidocaine with or without Biocarbonate). After a few minutes, the physician will palpate the anesthetized area with a scalpal blade to ensure the subejct is apporpriate anestehtized. A small incision of ≤ 1 inch is made with a scalpel. Once the layer of subcutenous fat has been reached, a sterilized small-diameter Bergstrom needle will be inserted into the abdomen, attached to a suction synringe. The physican will manipulate the Bergstrom needle against the fat, while the assistant applies suction. The needle will then be extracted from the abdomen, and the fat will be removed from the needle. A second, sterilized larger-diameter Bergstrom needle will now be

inserted and the technique will be repeated. The fat is deposited into a sterile container. The wound will be closed with any combination of: 1-2 stitches of the physicians choosing, dermabond, steristrips, and bandaids. This will be decided in the moment, based on physician judgement. Local pressure and an ice pack will be applied for 10 minutes.

18. APPENDIX C: SUBCUTANEOUS FAT ANALYSIS

Subcutaneous adipose tissue biopsies: 2-3gms of subcutaneous adipose tissue from the abdomen will be collected. Samples will be stored at -80°C

Transcriptomics (RNA-seq and RT-PCR): Adipose-derived RNA will be sent to a commercial laboratory for RNAseq sequencing at >50 bp SE. Results will be analyzed by Dr. Mazumder to identify changes in RNAseq, using group*intervention ANCOVA and Pearson correlation coefficients in Partek Genomics Suite software (Partek Inc., St Louis, Missouri). Resultant lists of dysregulated transcripts will be tested for representation in known biological pathways via Ingenuity Pathway Analysis Suite (QIAGEN, Valencia, CA).

Adipose tissue MSC for gene expression and SeaHorse OCR Estimation: MSCs will be obtained from fat biopsies, and post culture, will be assessed for gene expression and cellular OCR. Targeted gene expression assays similar to CD34+ genes as described above. To study differentiation program of MSCs post exercise, mRNA gene expression looking at individual lineages as shown in Fig 2 will be interrogated. Other than mRNA analysis we plan to culture MSCs in different mesenchymal tissue (fat, bone, cartilage, muscle) specific culture media as per protocol, followed by staining to confirm differentiation [13].

We have presented similar investigations looking at fat derived MSC metabolism and differentiation obtained from prediabetes subjects, post aerobic exercise. [26].

Appendix E: Optional modified study procedure during COVID19 Pandemic

Due to the COVID19 pandemic and emergency orders in place we have adopted study procedures that will minimize patient and staff exposure to SARS-cov-2 during study procedure. We had many patients who are interested in our trials but does not feel safe to take public transportation to come to site for study procedures hence we have adopted a hybrid approach to keep our studies going. This study process will include virtual study visits, field phlebotomy staff and shipment of drugs via medical courier to subject's home in the DMV area. During screening process subject will be offered both the in site study or the virtual option if they are not comfortable with their transportation options to make their way to Foggy Bottom. We will focus on the core set assessments, which are EPC cell counts, which are vital to the study and answers the primary research questions posed. Any assessment that cannot be done remotely will be considered optional in this format.

Screening: Interview will be done by the study staff and PI virtually to assess eligibility. All blood work done in the past 6 months for standard of care will be considered valid for screening purpose. If the patient does not have the specific blood work to fulfil the inclusion criteria they will not be enrolled. A urine pregnancy test (if female subjects) along with the consent paperwork will be mailed to the patient beforehand with return label. The patient will perform the urine pregnancy test inform the study team of the result. After the informed consent process, the patient will sign it and mail it back with the included return mailer. Some patient may choose to receive the informed consent digitally, sign it and email it back. In those cases, the consent will be received back digitally. The study staff will sign it and file it. The urine pregnancy test will be mailed separately in that case.

Enrollment V1: After the enrollment criteria is fulfilled, Dr Sen will sign the prescription and send it to IDS with planned shipment of the study drugs on the planned enrollment date. IDS at GW MFA has contracted a medical courier service to ship study drugs to patients. Dr Sens lab at GW is currently working on a contract with a mobile phlebotomy company to go to patient's place of residence to draw research blood samples. A mobile phlebotomy order will be sent out for the planned enrollment date. We will be using a third party mobile phlebotomy company called "POW or Phlebotomy on Wheels". They will receive an order form with patient name, address and phone number. After they do the draw they will drop off the blood and urine samples to our GW research staff at GW campus. The research staff will send the appropriate blood tubes to lab corp and basic science lab.

During the zoom call the patient will be counselled on the use of the drug, they will be trained on the use of the injectable study medication. Patient will be instructed to contact study staff if there is any issue or go to the emergency room due to any medical emergency. Secondary outcomes such as arterial stiffness and mesenchymal cell assessment (fat biopsy) will be considered optional in this format.

V2 and V3: Procedure from V1 will be followed.

19. APPENDIX E: X-CHART

	Phone Screening (Before week -2)	Screening Visit (Week -2)	Randomization Visit 1 (Week 0)	Follow up phone call (week 4)	Visit 2 (Week 8)	Follow up phone call (week 16)	Visit 3 End-of-Treatment (Week 24)	Follow up phone call (Week 28)	
Written Informed Consent Obtained		X							
Inclusion / Exclusion Criteria Reviewed	X	X	X						
Medical History	X	X							
Physical Examination		X					X		
Targeted Physical Examination (as needed)			X		X		X		
Vital Signs		X	X		X		X		
Assessment of Signs and Symptoms		X	X		X		X		
Adverse Events Assessment		X	X	X	X	X	X	X	
Laboratory Tests (Biochemical)		X			X		X		
Urine Pregnancy Test (As needed)		X	X		X		X		
Spot Urine Sample		X	X		X		X		
Waist/Hip Measurements			X		X		X		
Body Composition Scale			X		X		X		
Blood Draw for Primary Outcome Measures			X		X		X		
Arterial Stiffness Measurements			X		X		X		
Subcutaneous Fat Biopsy			X				X		
Randomize			X						
Dispense Study Drug			X 0.25 mg/Week or Placebo For 4 weeks AND 0.5 mg/Week or Placebo For another 4 weeks.		X 1.0 mg/Week or Placebo				
Subject compensation			X		X		X		
Handout Direction for use (DFU) Chart			X						
Subject compliance check					X		X		

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