**Protocol Tile: Adipose tissue aspiration by subcutaneous needle biopsies for gene**

**expression.**

**Rationale for the procedure:**

We have been studying gene expression through RNASeq in the Cameron County Hispanic Cohort (CCHC) for the last two years with interesting findings implicating genes associated with obesity and diabetes which are therefore potential targets for new therapeutics. However, peripheral blood does not provide the detailed information we need about gene expression in adipose tissue, which is metabolically highly active in obesity. As a first step to trying to gather more precise data, we propose to add subcutaneous fat biopsies to our protocol so that we can perform single-cell sequencing. This is a rapidly evolving field that allows a detailed understanding of how cells react and communicate in disease states. In the first instance, we are looking at gene expression changes in obesity to start to understand these processes in detail. The importance of this information is the excessive obesity (51%) and diabetes (27%) in our study population, which we do not sufficiently understand to impact at the population level. In this protocol we are working with well-established genetics collaborators at Vanderbilt University (Dr. Jennifer Below), the University of North Carolina (Dr. Kari North), and adding a Professor and Director of Translational Adipose Biology and Obesity in the Diabetes, Obesity and Metabolism Institute, Mount Sinai (Dr. Susan Fried), who has special expertise in single-cell sequencing and analysis of the data.

**Inclusion criteria:**

* 100 obese CCHC case-participants (BMI>35kg/m2)
* 100 non-obese CCHC control-participants (BMI>30kg/m2)
* Wight stability of +/- 3% over past 2 months.

**Exclusion criteria:**

* Illness considered to be severe by the Principal Investigator or the radiologist (cancer, respiratory, renal, or cardiac failure)
* BMI<20kg/m2 (to ensure adequate adipose sampling)
* Weight loss or gain of +/- 3% over the past 2 months.
* Use of statins or of medications that may affect glucose or lipid metabolism including beta-blockers, thiazide diuretics, hypolipidemic agents, thyroid hormone, or weight loss medications or formulas;
* Inability to understand the procedure and its implications

**Procedure:**

* Subjects will undergo:
* A study visit for fasting blood and abdominal and femoral subcutaneous adipose tissue collection. Subjects will report to the Brownsville CRU in the morning, having fasted for 8 hours. A blood sample will be collected, about 30 mL, and run A1C, CBC-Diff, Lipid Panel, and CMP.
* No exercise, no excessive stressors, no alcohol, and no active or passive smoking for 72 hours previous to the procedure to avoid complications. Research staff will check for the absence of abnormal coagulation and allergies to local anesthetics, as well as for contraindicated treatments. Abdominal and femoral subcutaneous adipose tissues will be collected approximately 5 cm lateral to the umbilicus and from the lateral mid-thigh area with a 2.5-mm cannula about 1 hour after the blood sample has been collected.
* Potential risks:
* *Adipose tissue aspiration*: There is a very small risk of infection with the adipose tissue aspiration which is minimized by adherence to the aseptic technique and implementation by experienced medical professionals. There may be slight discomfort and burning when the local anesthetic is injected prior to the needle biopsy, but subjects experience minimal discomfort during the biopsy procedure. Following the biopsies, there may be tenderness, bleeding, bruise formation, or infections. Pressure will be applied to the site immediately after the procedure to limit bruising, swelling, and tenderness. An aseptic technique will be used to minimize the chances of infection. Subjects with allergies to lidocaine are excluded. The total amount of lidocaine will not exceed 300 mg per day. The aspirations may cause bruising (black and blue marks) which resolves within several days. This procedure has been done by some of our collaborators that have had experience with hundreds of fat aspirations without encountering any infections or adverse events. In our previous studies, volunteers returned on 4 separate occasions for a total of up to 9 fat aspirations, thus we believe the procedure is tolerated well. Participants are given instructions about dressing and cleaning the site of the biopsy. There is no follow-up visit after the aspiration procedure. However, a phone call from the research team will be placed to the participant within 24 hours after the biopsy to inquire about any problems. Moreover, should a question or problem arise, participants can make phone contact with the research team and the PI any time after the procedure.
* *The risk levels are low to moderate and unlikely to have a significant impact on subjects.* Although no untoward side effects are expected, all tests will be carried out in Valley Baptist Medical Center in Brownsville with an MD available at any time in case of an emergency. Any adverse events will be treated as required and reported to the IRB.

Prior to the appointment, the procedure will be explained to the participant by Clinical Research Unit trained staff, and written informed consent obtained. A one-time incentive for the procedure of $150 will be offered and given to the participant once the biopsies have been obtained.

The participant will be in a fasting state and will be accompanied to the procedure by Clinical Research Unit staff, who will remain available throughout the procedure. Laboratory staff from the School of Public Health will be on-site to receive the specimen and perform immediate flash-freeze procedures according to protocol.

* Only a trained qualified MD, physician assistant, or nurse practitioner will perform the adipose tissue aspirations. We will inquire about lidocaine allergies and adhere to an aseptic technique throughout the procedure. Participants will be given instructions about dressing and cleaning the site of the biopsy. A phone call from the research team will be placed to the participant within 24 hours after the biopsy to inquire about any problems.
* The radiologist/staff will again discuss the procedure with the participant, explaining its potential risks and complication (see above) including the option of not performing the procedure. The identity of the participant and the written informed consent will be checked.

With the participant in the supine position, two suitable subcutaneous sites will be identified in the anterior abdominal wall and the lateral thigh regions as described above. using ultrasound as necessary to guide the procedure. The sites will be marked and prepped and draped in the usual sterile fashion. Infiltration of 1% of lidocaine into the local subcutaneous tissues will be performed to ensure local anesthesia. Two to three passes with a mini cannula will be made into the subcutaneous tissue at each site to obtain about 300 mg of tissue. Following the procedure, the participant will be observed for 30 minutes, and then accompanied by the Clinical Research Unit staff to a suitable departure point indicated by the radiologist or MD after having been given the instructions and advice detailed above. A follow-up phone call will be called within 24 hours and be given details of contacts if problems or questions arise.