**Title: Ultrasonographic analysis of risks and complications in obesity and diabetes in Mexican Americans living in South Texas**

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## A. SPECIFIC AIMS

The United States (US) Hispanic population almost doubled between 1990 and 2007 (from 9% to 15%), and it is projected to reach 30% of the total population by the year 2050.(1) Of the Hispanics residing in the US, Mexican Americans represent the largest and most ethnically distinct subgroup, and many live in communities with notable health disparities.(2) Hence, understanding prevalent diseases in this population and designing innovative community-based interventions will be of enormous societal interest. Hispanics and Mexican Americans in particular, are at high risk for obesity, which in turn predisposes to type 2 diabetes. In our border population, the situation is worse: 50.2% are obese (vs. 39.3 % Mexican Americans nationally).(3) Similarly, the prevalence of diabetes nationally is twice as high in Mexican Americans (10.4%) as in non-Hispanic whites (6.5%), and it is even higher in border populations (19.2%).(4) The risk of diabetes and its complications is enormous with implications for both human suffering and healthcare costs.

In our border population, with over 90% Mexican Americans, we have the two poorest counties for their size in the U.S.(5) Health disparities are severe; 48.5% of these Mexican Americans are obese (vs 39.3 % Mexican Americans nationally).(3) Similarly, nationally the prevalence of diabetes is twice as high in Mexican Americans (10.4%) as in non-Hispanic whites (6.5%), but in our randomly selected cohort of Mexican American border residents, diabetes is nearly 3 times higher than among Mexican Americans nationally.(4;6) Using the 2010 definition, 29.7% of our population over the age of 18 has diabetes. The risks of diabetes and its complications are huge in this population, with profound implications for human suffering and healthcare costs.

Complications of obesity and diabetes are a major threat to health and the economy for Mexican Americans. Cardiovascular disease is one of the major complications of diabetes, and CVD is a leading cause of mortality; therefore cardiovascular disease is an important consideration in a population with high diabetes rate.

Understanding the disease process in Hispanics will help to elucidate the mechanisms associated with ethnic differences observed in CVD morbidity and mortality. Previous research suggests that Hispanics may have lower rates of vascular disease than non-Hispanic whites.(12) For example in the Multi-Ethnic Study of Atherosclerosis (MESA), Hispanics had a substantially lower prevalence of coronary artery disease than non-Hispanic whites.(13). What is not known however is whether the disease has a different presentation in Hispanics, specifically cardiac failure which may be overlooked in mortality data records, rather than myocardial infarction or cerebral vascular accident which are more dramatic and obvious events.

Another common complication of obesity and diabetes is non-alcoholic fatty liver disease (NAFLD). There is a growing body of evidence suggesting that cardiovascular disease is the leading cause of death in patients with advanced NAFLD and that nonalcoholic fatty liver disease is associated with an increased risk of cardiovascular disease independent of the risk conferred by traditional risk factors and components of the metabolic syndrome. Although the underlying mechanisms linking nonalcoholic fatty liver disease, diabetes and cardiovascular disease are not completely elucidated, possibilities include visceral obesity and inflammation. Another important mechanism may be development of insulin resistance which is also an important risk factor for cardiovascular disease.(14)

Other complications of diabetes are also common, particularly kidney disease. Both obesity and diabetes also contribute to obstetric and other complications. In this study we will offer ultrasound examinations to participants of our Cameron County Hispanic Cohort (CCHC). These examinations will be used to determine the prevalence of cardiovascular, renal, liver and other complications of disease by evaluating sonograms in addition to the physiological, clinical, biological and sociodemographic risk factors we already collect in our CCHC participants. Since Cameron County is ethnically homogeneous, with the vast majority of its residents being of Mexican origin, we have a unique opportunity to evaluate Mexican ethnicity, cardiovascular, NAFLD renal and other diseases. Previous studies that have included Hispanics have combined genetically diverse ethnic groups (Mexican, Puerto Rican, and Cuban) which limit our capacity to understand the process of a disease in a specific ethnic group. This study will provide an opportunity to measure objectively the prevalence of complicated disease in Mexican Americans in the United States.

Specific Aim 1: Determine the evidence for pathological progress of cardiovascular, renal, liver and other diseases using ultrasonography. In collaboration with relevant expertise from the UT Health Department of Medicine in Houston we will train staff to perform non-invasive ultrasound examinations on consenting cohort participants. Ultrasound images will be reviewed and graded in Houston. These examinations will include carotid artery, brachial reflexivity, liver, renal and include obstetric images if appropriate. A side of that we will also take retinal images using a digital camera. Subjects will be recruited from the ongoing Cameron Country Hispanic Cohort (CCHC) and will be asked to undergo non-invasive carotid and brachial artery ultrasounds to evaluate cardiovascular function and pathology.

Specific Aim 2: To identify risk factors for cardiovascular, renal and liver disease in Mexican American adults over the age of 18 living in South Texas. Over 40% of the CCHC have evidence of liver disease suggesting but not diagnostic of NAFLD evidenced by alanine transaminase levels over 40 mg/dl and over a third have evidence of cardiovascular disease. Renal disease is common particularly in association with diabetes. We will compare ultrasound assessment of the architecture of coronary arteries and liver and assess arterial flexibility with the range of biological and other variables already being collected. These data will form the basis of future clinical trials. .

## B. BACKGROUND AND SIGNIFICANCE

### B.1 Cameron County, Texas

The Lower Rio Grande Valley (LRGV) is the southernmost tip of Texas and borders Mexico. Although it is one of the fastest growing regions in the country, it is home to the poorest of the U.S. poor, with its two major metropolitan areas ranking last in the nation in per capita income.(16) Cameron County, located in the LRGV, is the site for this proposed study. Table 1 presents US Census Bureau socioeconomic characteristics of Cameron County compared to Texas and the United States (17). These data present an unusual demographic dynamic as the vast majority of the residents are Hispanic or Latino and speak a language other than English in their homes. Moreover there is greater socioeconomic disadvantage as can be observed by the greater household sizes, a lower high school graduation rate, a lower median household income and more than three times the nation average of families living below the poverty line. In terms of health, this translates into a lower overall capacity to utilize and navigate health care services through, lower health literacy, communication barriers between doctors and patients, and lower rates of insurance coverage.

Table: Socioeconomic characteristics of Cameron County, Texas*

| | Cameron County | Texas | United States |
|---|---|---|---|
| **Social** | | | |
| Median Age (years) | 28.8 | 33.1 | 36.4 |
| 65 years or older (%) | 11.1 | 10.0 | 12.5 |
| Average Household Size | 3.29 | 2.82 | 2.60 |
| High School Graduates (%) | 61.6 | 78.6 | 84.0 |
| Foreign Born (%) | 25.4 | 15.8 | 12.5 |
| Speak a language other than English in the home (%) | 73.7 | 33.5 | 19.5 |
| Hispanic/Latino (%) | 86.0 | 35.5 | 14.7 |
| **Economic** | | | |
| Median Household Income | 28,026 | 46,248 | 50,007 |
| Families below the poverty line (%) | 32.2 | 13.3 | 9.8 |

*Source: US Census Bureau 2000 Population Characteristics.

Table 1

Added to the general demographic picture of Cameron County is an unusual age distribution. What can be seen from table 1 is that although the median age is substantially lower than Texas and the United States, the proportion of the population that is 65 years or older is 11.1 %, greater than that of Texas (10.0%) and approximately within one percentage point of the United States overall (12.5%). As fertility rates decline, the proportion of persons over the age of 65 in Cameron County is expected to grow. It is forecast that there will be a greater burden of disease in Cameron County, since a larger proportion of the population will be at risk for chronic diseases, therefore creating more demand on the healthcare system. Since a large percent of the Cameron County population is uninsured, this will place a higher demand for already overburdened care services. Furthermore, understanding how cardiovascular disease and NAFLD evolve in adult years will help us design appropriate intervention.

**Inflammation: the 'common soil' hypothesis:** Dysregulation of adipose metabolism in type 2 diabetes and pre-diabetes has for some time been thought to be at the root of a characteristic low level inflammatory state. This state includes insulin resistance and altered cytokine and adipokine responses. It has been described as the 'common soil' for many of the complications of diabetes through multilevel interactions (18-22). In this proposal we focus on three complications of diabetes that have been little studied in health-disparity populations: cardiovascular disease (CVD), non-alcoholic liver disease (NAFLD), and immune responses to infections. We have found all three of these disabling and life-threatening complications of diabetes in our Mexican American population on the US-Mexico border.(6;23-28) Our understanding of the underlying mechanisms and the potential to modify inflammation has advanced in recent years, opening the prospect that not only treatment but also prevention might be amenable to simple medications.

**Cardiovascular Disease in diabetes: 'The Grim Reaper':** CVD is documented to carry a high risk of mortality in Mexican Americans with diabetes, particularly in those with poorly controlled disease.(29) The prevalence of obesity was much higher in our cohort than the most recent estimates for Mexican Americans nationally (50.7% vs. 33.8%), bringing a high risk of CVD.(30;31) In our cohort we also documented elevated rates of resting ischemic electrocardiographic (EKG) abnormalities known to be associated with increased CVD mortality.(26) The Framingham Risk Score (FRS) developed using a white Caucasian population may be inaccurate for some minority populations.(32) When we examined a small subset of 101 cohort participants using ultrasound to determine carotid intimal medial thickness (cIMT) and carotid atherosclerotic plaque presence, we re-classified 10% of participants identified as low risk using the FRS as in fact at high risk of CVD.(24) Our goal is to predict, and then prevent CVD in Mexican Americans with high rates of obesity and diabetes.

Although Hispanic Americans have a disproportionate burden of coronary risk factors, in national data, they have a lower prevalence of coronary heart disease than other ethnic groups. **We hypothesize that this may be accounted for by a different disease pattern leading to chronic, silent heart disease, such as heart failure (HF) that is unrecognized and underdiagnosed in this medically underserved and understudied minority**. Our preliminary data showed that 34% of a sample of this cohort have N-terminal pro-brain-type natriuretic peptide (NT pro-BNP) levels ≥300 pg/ml (age-independent cut-point) suggestive of HF diagnosis; 59% of whom have NT pro-BNP >900 pg/ml (very high likelihood of HF). NT pro-BNP can detect cardiac dysfunction with a high accuracy. These preliminary findings are supported by our studies demonstrating a high burden of cardiovascular disease (CVD) in this minority cohort.

To test our hypothesis, we will perform a longitudinal study to confirm the high risk of HF in the CCHC, and determine underlying mechanisms that modulate the development or progression of HF. Echocardiography is an important and simple noninvasive tool to assess ventricular function, both systolic and diastolic, as well as underlying cardiac structural abnormalities.

To examine the association between diabetes and peripheral arterial disease (PAD) in a population of subjects that is at high risk for this disease, we are planning to add a questionnaire for PAD and a noninvasive testing to perform segmental pressure vascular testing to identify arterial narrowing interfering with circulation. Understanding the factors that influence diabetes and PAD may lead to early detection and prevention of CVD in Mexican Americans living in border cities.

**Non-alcoholic fatty liver disease in diabetes: 'The Sleeping Giant':** NAFLD has only recently attracted scientific attention as a debilitating and potentially fatal complication of diabetes. NAFLD affects 10-35% of the population worldwide, and is the most common cause of liver disease in the US.(33;34) Population based studies show a higher prevalence of NAFLD in Hispanics than other ethnicities (35-38), and this is thought to be due to the higher prevalence of obesity and insulin resistance in Hispanics.(35) In a recent study of outpatients of mixed ethnicity, the prevalence of NAFLD was 46%, and non-alcoholic steatohepatitis (NASH) was confirmed in 12.2%; Hispanics and diabetes patients had the highest prevalence.(38) Data from the Third National Health and Nutrition Examination Survey (1988–1994) also showed that ALT elevation was more common in Mexican Americans (14.9%) than non-Hispanic whites (7.1%). NAFLD is not only commonly seen

in patients with type 2 diabetes (69%) but is also associated with CVD.(39-41) In our own CCHC cohort, nearly 40% had biochemical evidence of liver injury.(23)

In order to gather additional and better liver images we will add a liver elastography. Elastography is based on liver ultrasound, but has a huge difference to the normal imaging technique of ultrasound of the liver. It uses mechanical waves that are sent through the liver. The speed of these waves through tissue provides data about the condition and stiffness of the liver and thus can indicate a liver fibrosis. Elastography might become a completely non-invasive substitute for Biopsy. This method provides a particular benefit for populations at risk.

## C. PRELIMINARY STUDIES

### C.1 Hispanic Health Research Center (HHRC), Brownsville, Texas

The current study will be conducted at the clinical research unit in Brownsville, TX. The Hispanic Health Research Center (HHRC), a satellite clinic of UTHSC-H Center for Clinical and Translational Sciences (CCTS) that is funded through the NIH Clinical and Translational Science Award program will be used as the data collection site. This satellite CRU, located in Brownsville, TX is operated by UTHSC-H School of Public Health (SPH)-Brownsville Regional Campus and adheres to NIH protocols for safe handling and best practices for clinical research. There a number of ongoing – NIH funded – investigations that address diabetes, obesity and cardiovascular disease from different directs as the proposed study. The current study will complement these ongoing studies by cardiovascular, liver, renal and other components to understanding the effects of diabetes on longevity and quality of life, an essential area and one that was lacking in the CRU. Below is a brief description of some of the related ongoing studies at the CRU.

Table 1. Key socio-demographic and anthropomorphic characteristics of the CCHC population comparing participants who fulfill the 2010 definition of diabetes and those who do not. Overweight = BMI ≥25 to <30 kg/m$^2$0; Obese ≥30 kg/m$^2$; Obese category I ≥30 to <35 kg/m$^2$; Obese Category II ≥35 to <40 kg/m$^2$; Obese Category III (morbidly obese) >40 kg/m$^2$. (n=2000 unless otherwise indicated. Percentages are weighted column percents.)

| | Total n=2000 | Diabetes n=593 | No diabetes n=1407 | |
|---|---|---|---|---|
| **Categorical variables** | **n (%)** | **n (%)** | **n (%)** | **RR (95% CI)** |
| Self-reported diabetes | 294 (13.7) | 294 ( 44.5) | | |
| Diabetes (2010 definition) | 593 (30.7) | | | |
| Diabetes (2006 definition) | 380 (17.9) | 380 (58.2) | | |
| Impaired fasting glucose (2010) | 365 (19.2) | | | |
| Impaired fasting glucose (2006) | 511 (28.0) | 146 (28.9) | 365 (27.6) | 1.05 (0.87, 1.27) |
| Females | 1343 (55.3) | 393 ( 54.4) | 950 (55.7) | 0.97 (0.81 1.15) |
| Born in Mexico | 1311 (66.5) | 405 (63.9) | 906 (61.9) | 1.06 (0.88, 1.28) |
| Health insurance | 570 (31.4) | 221 (43.1) | 349 (26.2) | **1.66 (1.38, 1.99)** |
| BMI: Overweight (n=1990) | 634 (33.2) | 155 (27.6) | 479 (35.6) | **.0.77 (0.63, 0.95)** |
| BMI: Obese (n=2000) | 1017 (48.5) | 367 (60.1) | 650 (43.3) | **1.60 (1.33, 1.93** |
| Obese Cat I | 590 (28.8) | 197 (33.9) | 393 (26.5) | **1.27 (1.06, 1.53)** |
| Obese Cat II | 257 (12.1) | 94 (14.2) | 163 (11.2) | **1.20 (0.95, 1.52)** |
| Obese Cat III (morbid obesity) | 170 (7.9) | 76 (12.5) | 94 (5.9) | **1.66 (1.32, 2.09)** |
| Current Smoker (n=1999) | 596 (33.8) | 186 ( 35.4) | 410 (33.1) | 1.07 (0.90, 1.28) |
| Alcohol User | 823 (41.2) | 218 (36.08) | 605 (45.5) | **0.76(0., 0.92)** |

‡ Student's t-test for equality of means

### C.2 Cameron County Hispanic Cohort Study (CCHC)

The purpose of the Cameron County Hispanic Cohort (CCHC) study is to provide insight into the relationship between individual and community level socioeconomic status and diabetes onset, prevalence and comorbidities in a sample of Mexican-origin adults ages 35 to 64 living in Cameron County, Texas. The sample was drawn at random using multi-cluster design from the first and third socioeconomic quartiles in the city of Brownsville, Texas. In total, 2558 are now participating in the cohort. Data from the first 2000 are presented in table 2

### C.3 Cameron County Hispanic Cohort (CCHC)

Our Exploratory Center of Excellence houses the CCHC. It has targeted type 2 diabetes with 3 existing coordinated research projects. These projects use resources built up over the past 3 years through our Hispanic Health Research Center (HHRC) established by the EXPORT program; specifically the CCHC together with its field and clinic infrastructure. This key item is a well characterized cohort of 2558 Mexican Americans, randomly selected from the Brownsville community. This study includes cohort participants, using common field and laboratory protocols and shared data collection and management systems.

All ultrasound studies and tests focus on different aspects of the physiology of diabetes and its complications; moreover, the projects examine the risk factors influencing disease status including social, behavioral, environmental and genetic components. Common methodology and resources increase efficiency and allows us to compare results across research projects.

## D. RESEARCH DESIGN AND METHODS

**Overview of the Study Plan and Design:** Mexican Americans in the United States have unusual health and mortality outcomes that are not fully understood. Previous research seems to suggest that Mexican origin persons that are immigrants or living in lower socioeconomic circumstances are the recipients of a cardiovascular mortality advantage. Cardiovascular disease, NAFLD, and diabetes are coexisting conditions with biologically related mechanisms that lead to mortality, yet Mexican Americans have higher rates of diabetes than non-Hispanic whites, while at the same time having lower rates of coronary disease and cardiovascular mortality. These data, however, are based on mortality and what is missing is a measure of the prevalence of vascular disease in the Mexican origin population in the US. This study sets out to explain this paradoxical pattern by investigating the cardiovascular function and health of low-income Mexican Americans living in South Texas border cities. This study makes use of pre-existing data collected as part of the Cameron County Hispanic Cohort (CCHC) study. In addition, participants in this study will be invited to undergo ultrasound testing of their liver and a liver elastography, carotid and brachial arteries, an echocardiogram, and segmental pressure vascular testing in order to assess vascular functioning. As well as retinal imaging.

**Recruitment:** We will recruit subjects 18 years and older who are already enrolled in our ongoing CCHC and who consent to this new study. In addition we will invite new enrollees to the CCHC to participate in this new addition to the CCHC. Currently there are 2,558 activity participants in the CCHC study and we are anticipating the addition of 1,000 more subjects in the coming year. We are also in the phase of 5-year follow-up of participants and will also recruit from this pool.

**Clinic Visit:** All data will be collected at the CRUs. The field and clinic staff are supervised by the Clinical Research Center (CRU) staff. Participants were asked to participate in a questionnaire-guided interview, provide blood samples and anthropometric measures, which are already in place and approved as part of the ongoing cohort study. We are proposing to widen the study of our cohort by implementation of a non-invasive cardiovascular function testing utilizing ultrasound technology. We will add a questionnaire specifically for peripheral arterial disease (PAD). Questionnaires and consent forms were administered in English and Spanish by trained staff at our CRU.

**Demographics:** We will use the existing CCHC data for the secondary data analysis component of this study. Information was previously collected on CCHC participants such as past medical history and demographic characteristics on themselves and their spouses.

**Anthropometrics:** Height and weight was measured at the time of the CRU visit on all subjects. Weight will be measured using a portable scale to the nearest $10^{th}$ kilogram. In addition, we will measure waist and hip circumference to allow determination of central adiposity. Waist circumference will be obtained at the level of the umbilicus and hip circumference will be obtained at the widest point. In both of these measures, both staff members participate. One takes the measurements while the second assures that the tapes are maintained level on the participant.

**Clinical and Laboratory assessment:** Laboratory assays were collected on CCHC participants. Values that will be used in this study will include total cholesterol, LDL, HDL, triglycerides, HgbA1c, fasting glucose, fasting insulin, and prothrombotic panels. Information on gene expression, inflammatory markers and cytokines will be used in the in the secondary data analysis. mRNA is being collected for studies of gene expression in a separate study, and the DNA from participants is also being sequenced, again in another. We will collect an additional 10 ml of blood for those subjects that the last lab results are more than 3 months old. Blood pressure was obtained on all subjects using our standard protocols and a Welch Allyn automatic blood pressure machine. Participants will be asked to sit quietly for 5 minutes and then a series of 3 blood pressure measurements will be taken. In addition, a resting pulse will be obtained on all subjects using standard protocols. A resting 12-lead standard supine EKG (GE MAC 5500, General Electric), was performed. All EKGs were computer-analyzed and then manually over read and coded using the Minnesota code criteria[17] by a

single cardiologist (S.Q.) to improve diagnostic accuracy. Left ventricular hypertrophy (LVH) was evaluated using both the Sokolow-Lyon and the Cornell criteria.[18-19] The QT interval was corrected for HR using the Bazett's formula using a standard HR of 60 bpm. Prolonged QTc interval was defined as QTc ≥ 460 ms. Abnormal Q/QS waves were defined as Q waves lasting greater than 0.04 seconds and greater than 1 mm in depth (code 1-1-2). ST and T wave abnormalities suggestive of ischemia were defined as horizontal or downsloping ST segments with or without T wave inversion (code 4-1-2). Ischemic T wave changes were defined as symmetric or deeply inverted T waves or biphasic T waves (codes 5-2). Ischemic EKG abnormalities were then defined as the presence of ST and T wave abnormalities suggestive of ischemia, ischemic T wave changes, abnormal Q/QS waves, and the presence of left bundle branch block (code 7-1-1).

**Ultrasound Methods and test:** We will perform ultrasound measurements on participants, during routine cohort enrollment and follow-up visits. Carotid ultrasound, brachial arterial reactivity testing and liver/elastography ultrasound and segmental pressure vascular test will be included, as well as retinal imaging. All will be performed by trained CRU staff or licensed sonographers using the Siemens Acuson X300 and the Siemens S2000 ultrasound systems; and a VF 13-5 linear array transducer or 5-MHz transducer (Ch5-2, Siemens, Mountain View, CA), and interpreted by our expert co-investigators. For the vascular test we will use Koven Smartdop 30 EX; Ankle Brachial Index (ABI) (CPT93922), Segmental Pressures (CPT 93923, Toe Brachial Index (TBI). For the Retinal imaging we will use a Canon CR2 – DVS8 with control software NM 2. A side of the ultrasounds we are implementing some assessments; Time Up and Go (TUG) Test and the Gait Speed test as well. We will also use the "Stay independent" questionnaire to see if the subjects are at risk for falling. These instruments/test are approved by CDC.

- The **carotid ultrasound** protocol follows the guidelines of the American Society of Echocardiography consensus statement on subclinical vascular disease.(50) Both common carotids will be imaged from three different angles for a total of six images. Carotid atheroma will be determined by examining the carotid bulb, its bifurcation and the carotid branch arteries in addition to the common carotid artery. cIMT will be measured using Carotid Analyzer software (Medical Imaging Applications, Coralville, Iowa), a semi-automated border detection program. Measurements will be made at the R-wave of the EKG on a minimum of two clips from each side and results averaged. Carotid atheroma will be defined as an area of wall thickening >50% of the thickness of the surrounding wall.

- The **brachial artery reactivity test** will be performed according to standard procedures.(51) Participants will be asked to abstain from food, consumption of vitamin E or C, and smoking for ≥6 hours before the scan. An occlusion blood pressure cuff will be positioned around the right arm, 2 inches below the antecubital fossa, and the brachial artery of the right arm will be imaged 5 to 9 cm above the antecubital fossa at rest. To induce reactive hyperemia, the brachial artery will be occluded for 5 minutes at an occlusion cuff pressure of 250 mmHg. The occlusion cuff will then be deflated and a 'release' Doppler velocity obtained to verify reactive hyperemia. All brachial artery reactivity studies will be analyzed using a semi-automated border detection software program (Medical Imaging Applications, Coralville, Iowa). FMD will be expressed as the percentage of increase in the brachial artery diameter (media-adventitial interface to the media-adventitial interface) with reactive hyperemia. A change of ≥4% is considered to be significantly greater than natural variability.(52) All measurements will be performed by a single blinded expert reader. In order to monitor intra-reader reproducibility, ultrasound studies from 10% of the participants will be repeated.

- The methods of **liver ultrasound** have been described elsewhere.(38) Briefly, subjects need to be fasting for at least 6 hours prior to ultrasound examination. Liver parenchyma will be examined sub- and intercostally in a decubitus position as well as in modified slightly oblique positions, with the right arm above the head and the right leg stretched, during all respiratory cycles to identify the best approach and to avoid artifacts caused by movement of the thorax. The overall gain, initial gain, and time gain compensation settings will be kept within a narrow range. The liver is considered normal if the echotexture is homogeneous without acoustic attenuation, the portal veins are visible, the diaphragm is well visualized, and echogenicity is similar or slightly higher than the echogenicity of the renal parenchyma. The liver is

characterized as fatty when the liver has areas of significantly greater echogenicity than the renal parenchyma, the ultrasound beam is attenuated and the diaphragm is indistinct, or there is blurring of the intrahepatic vessels (38)**.**

- The **liver elastography** will be performed according to the liver ultrasound method described above. It will provide then additional high quality images with higher resolution. The images will provide additional diagnostic information to detect liver steatosis and fibrosis.

- The **echocardiogram** will be performed following the standard procedure (XX). Subjects are not required to be fasting; they can take any scheduled medication as instructed by their own physician.
  A longitudinal study will be performed to confirm the high risk of Heart Failure (HF) in the Cameron County Hispanic Cohort (CCHC), and determine underlying mechanisms that modulate the development or progression of HF. Echocardiography is an important and simple noninvasive tool to assess ventricular function, both systolic and diastolic, as well as underlying cardiac structural abnormalities. Development and progression to HF may be modulated by specific triggers of increased CV stress such as inflammation. We will measure metabolic and inflammatory markers, and pericardial fat (a metabolically active ectopic fat depot associated with cardiac remodeling and inflammation). We will quantify pericardial fat using echocardiography, and correlate this with left ventricular (LV) structure and function, abdominal adiposity, markers of inflammation and oxidative stress (hsCRP, adiponectin, interleukin-6, MCP-1, and TNF receptor α), dysmetabolic risk profile, and the prevalence, development, and progression of HF. We will use machine learning algorithms to identify modulators of risk association factors among HF phenotypes.

- The **segmental pressure vascular study** will be performed following standard procedure. A specific questionnaire for PAD will be ask before the test (53). Subjects are not required to be fasting; they can take any scheduled medication as instructed by their own physician. It is performed by placing the patient in the supine position and wrapping pressure cuffs around both arms and both legs at various positions. Blood pressure cuffs will be placed on both arms, feet and big toes, ankle, below the knee, above the knee, and thigh. A total of 12 blood pressure cuffs will be connected to the Doppler one at a time. Blood pressure readings will be taken from both arms. Then, readings will be taken for the right foot and leg. Once the right side is complete, the process will be repeated on the left side. Pressures are taken at both arms, and at each position on the legs by inflating the pressure cuffs past the point where Doppler sounds cease, then slowly deflating the cuffs until Doppler sounds return.

- The **Retinal Imaging** will be performed using a digital camera (54). Subjects are not required to be fasting. No dilation of the pupil will be necessary. Subject will sit and face a camera, will place the chin on a chin rest and the forehead against a bar to keep the head steady. Subjects will need to keep the mouth closed, open the eyes as widely as possible and stare straight ahead while the photos are taken.

- For the Time Up and Go (TUG) test (55) will be necessary that the subject wear their regular footwear and are going to be able to use a walking aid if needed. Subjects will be asked to stand from a standard arm chair and walk 3 meters (10 feet) at normal peace, turn and walk back to the chair and sit down again. A stopwatch will be used to record the time. For the gait speed test, the subject will be asked to walk a total of 8 meters (26.4 feet) and will record the time that took to walk 5 meters (16.4 feet). Subjects will be asked to answer a set of questions to see if they are at risk for falling (56).

**Statistical Analysis:** We will perform carotid artery ultrasounds (measuring cIMT and atheroma presence) and brachial artery reactivity tests (measuring flow-mediated vasodilation-FMD). Echocardiograms will be performed to determine coronary artery disease. Liver ultrasounds and elastography will be performed to determine percent of fat content of the liver in all participants at baseline and to obtain additional images to

detect liver steatosis and fibrosis, respectively. The segmental pressure test will be performed to determine peripheral artery disease.

All ultrasound readings will be read by a single individual for CVD studies and another single individual for the liver studies. No identifiers will be used. For certification, the readers will assess the tests and will determine if the two studies from the same volunteer are reproducible.

**Data Management:** We will use Access for direct data entry using drop down screens and tablet format. Each visit and follow-up will contain a unique participant identifier. We will use multiple layers of security to ensure database integrity and maintenance of confidentiality. We will use a single server to maintain our centralized data entry system and transfer copies of files via a secure FTP to a second site (i.e., Houston). This FTP procedure is also used to transmit to our Brownsville location the results of any laboratory analyses made in Houston. We will monitor access to specific databases, providing passwords to those authorized to access a given database. Investigators in this project will be authorized and provided passwords to access the relevant database on the server. All analyses will be examined by the principal investigator and questions or disagreements will be resolved by re-examination of the code and datasets, correcting errors where appropriate, and repeating the analyses. When data entry is complete, no personal identifiers (e.g., name, address, phone number, etc.) will be included in the final database for analyses. All analyses will be performed using the SAS software (SAS version 9, SAS Institute, Gary NC)

**Referrals:** In the case of any individual found to have values of clinical significance, we first notify the individual and report their results to the physician or clinic of their choice. If they do not have a physician we refer them to the local Federally Qualified Clinic or other suitable clinic. In addition, participants may request a copy of their ultrasound and report to take to their physician.

**Compensation:** There is no financial compensation for the ultrasound testing.

**Expected Results:** The results from this study will be used to better understand how cardiovascular and liver diseases are characterized and distributed in the Mexican origin population in Cameron County. We anticipate that contributing factors to CHS, CVD, and liver disease in terms of risk that have previously established in other populations will differ in this study. In addition, we expect that diabetic subjects will not differ substantially from non-diabetics in their risk profile. If the preceding expected findings are supported then we will have established potential mechanisms to explain the unusually low rates of CVD and CHD mortality in the Mexican origin population

**Conclusion:** The Mexican American population living in Cameron County, Texas presents an unusual clinical profile with high rates of diabetes and obesity. This study is intended to shed light on the relationships with cardiovascular and liver diseases by collecting sociodemographic, biological and cardiovascular functioning data from a sample of Mexican Americans living in border cities. The results will be disseminated through conference presentations and scientific journal publications.

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