The HIV, Adipose Tissue Immunology, and Metabolism (HATIM) Study

Vanderbilt University Medical Center

Clinical Visit Protocol

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1.0 Background and Rationale

With the introduction of effective antiretroviral therapy (ART), HIV-infected persons can now survive for decades, but this success has been accompanied by an increased risk of developing metabolic disease compared HIV-negative persons. In the Multicenter AIDS Cohort Study, HIV-infected men had a greater than 4-fold increased incidence of a new diabetes diagnosis compared to HIV-negative men after adjusting for age and body mass index (BMI).[1] Prevalence studies of diabetes in HIV-infected individuals on ART have reported incidence rates of 3.1 to 14 per 1000 patient-years.[2, 3] Furthermore, treated HIV infection appears to act synergistically with other risk factors, and diabetes prevalence is especially high among HIV-infected individuals with obesity and advanced age.[2, 4]

While early studies found inflammation (i.e., innate immune activation) was associated with diabetes risk in persons with HIV, more recent data suggest chronic cellular immune activation may have a central role in diabetes pathogenesis. Recent studies from HIV-negative subjects identified several associations between adaptive immune cell populations and impaired glucose tolerance. Peripheral T regulatory (T_{reg}) cells are significantly lower in patients with type-2 diabetes and inversely proportional to HbA1c levels, while the numbers of CD25+ (activated) T cells and the proportion of T_H1 (pro-inflammatory) cells are higher in diabetics.[5-7] More recently, an analysis of the Multi-Ethnic Study of Atherosclerosis (MESA) cohort found a higher proportion of circulating memory CD4+ T cells and T_H1 cells was associated with insulin levels and prevalent type 2 diabetes.[8] Our group found a similar association between memory CD4+ T cells and incident diabetes for HIV patients in the Veterans Aging Cohort Study (VACS; see preliminary data).

T cells and macrophages are present in the stromal fraction of adipose tissue and affect adipocyte function. The striking increase in adipose tissue $CD4^+$ T_H1 cells and $CD8^+$ T cells, and a decrease in T_{reg} cells, observed in obesity may have an important role in the development of insulin resistance.[9-12] Secretion of the proinflammatory cytokines IFN-γ and IL-17 by T_H1 and T_H17 cells are implicated in the induction of proinflammatory M1 macrophages, which express IL-6 and tumor necrosis factor alpha (TNF-α) and inhibit adipocyte insulin signaling by promoting phosphorylation of insulin receptor substrate 1.[13-1515, 17, 18] T cell infiltration into adipose tissue appears to be an early and necessary step preceding macrophage recruitment into adipose tissue, and in murine models antibody-induced CD8+ T cell depletion resulted in reduced M1 macrophage infiltration.[9]

HIV offers a unique model to study linkages between peripheral T cells, adipose-resident T cells, adipose tissue function, and glucose tolerance. The changes in peripheral T cell subsets observed in chronic HIV-related immune activation are similar to those associated with insulin resistance in obese HIV-negative individuals. Immune cells translocate from the peripheral circulation into adipose tissue in a dynamic process, and we hypothesize that the chronic, HIV-related activation of peripheral CD4+ and CD8+ T cells may be accompanied by the accumulation of activated T cells in adipose tissue with adverse effects on metabolic activity. In this study, we will test the hypothesis that the oligoclonal expansion of chronically activated peripheral T cells in adipose tissue is a primary driver of macrophage inflammation and reduced adipocyte insulin sensitivity. Furthermore, we propose that this represents a central mechanistic linkage underlying the association between circulating T cell activation and incident diabetes risk observed in HIV-infected and HIV-negative individuals.

2.0 Preliminary Studies

2.1. Adipose tissue from HIV+ persons is enriched for activated CD8+ T cells and there is a correlation between the percentage of circulating and adipose-resident memory CD4+ T cells: To assess the relationships between circulating and adipose tissue T cell subset distributions, we conduced pilot study to compare T cell populations in adipose tissue biopsies and peripheral blood in 10 HIV infected subjects on long-term ART with sustained viral suppression. We collected 5-10 ml of abdominal subcutaneous adipose tissue using a 2.7 mm liposuction cannula. All subjects were fasting. Adipose tissue biopsies were repeatedly washed to eliminate any blood contamination and T cells extracted. Flow cytometry was performed on the adipose tissue T cells in addition to paired peripheral blood samples to measure CD3, CD4, and CD8 surface marker expression, and markers of activation (CD38, HLA-DR, CD69), senescence (CD57, PD-1), and memory cells (CD45RO).

We found adipose tissue to be enriched for activated CD8+ T cells (CD38+ and HLA-DR+) compared to blood – a cell type shown to promote adipose-resident macrophage activation in animal studies (**Table 1**). Furthermore, the relative percentages of CD4+ and CD8+ T cells were highly correlated between blood and adipose tissue, as were the percentages of activated (DR+) CD8+ T cells and memory (CD45RO+) CD4+ T cells. This finding supports our hypothesis that the distribution of T cell subsets in blood reflects the distribution of these T cell subsets in adipose tissue.

Table 1. Pilot study comparing T cell subsets in blood and adipose tissue from HIV+ patients with long-term (>4 years) ART treatment and viral suppression (n=10)

	Comparison of median values in blood vs. adipose tissue				Correlation between blood and adipose tissue	
T cell subset	**Median in blood**	**Median in adipose tissue**	**p-value**		**Spearman rho**	**p-value**
CD4+ %*	43.2	34.0	0.06		0.95	**<0.01**
CD4+ PD1+%	24.6	24.8	0.93		0.42	0.27
CD4+ CD57+%	2.2	5.4	0.06		0.95	**<0.01**
CD4+ CD38+%	4.0	2.4	0.44		0.16	0.68
CD4+ DR+%	1.0	2.5	**0.001**		0.52	0.15
CD4+ CD69+%	0.25	0.80	0.08		0.23	0.56
CD4+ CD45RO+%	50.3	57.4	0.86		0.83	**0.005**
CD8+ %*	50.4	60.9	0.11		0.85	**0.004**
CD8+ PD1+%	22.1	21.8	0.99		0.80	**0.01**
CD8+ CD57+%	22.7	35.1	**0.01**		0.38	0.31
CD8+ CD38+%	0.50	0.90	**0.02**		0.32	0.40
CD8+ DR+%	0.90	5.3	**<0.001**		0.67	**<0.05**
CD8+ CD69+%	0.55	1.00	0.44		0.59	0.10
CD8+ CD45RO+%	31.3	33.6	0.73		0.23	0.55

*Represents the percentage of CD3+ T cells that expressed CD4 or CD8. All other values represent the percentage of CD4+ or CD8+ population expressing selected surface markers.

2.2. Adipose resident CD8+ T cell density and TCR clonality is higher in obese mice fed a high-fat diet for 16 weeks compared to mice fed a low-fat diet. To understand whether adipose-resident CD8+ T cell receptor oligoclonality varies with changes in adipose tissue, we assigned C57BL/6 mice to a chow, low-fat, or high-fat diet for 16 weeks (n=9 total) and then collected epididymal fat samples for T cell extraction, sorting, and T cell receptor surveillance

sequencing and clonality score calculation (a measure based on Shannon's entropy of the distribution of all TCR sequences – a higher clonality score indicates less entropy). Mice fed a high-fat diet were more obese and demonstrated the greatest number of CD8+ T cells per gram of adipose tissue (10,254 CD8+ cells/gram in high-fat; 9,940 cells/g in low-fat; and 3,922 cells/g in chow diet) and high-fat diet adipose tissue had greater expression of CD11b and F4/80 macrophage markers and IL-6 compared to low-fat diet ($p<0.05$ for all). More striking, adipose-resident CD8+ T cell density was significantly correlated with T cell receptor clonality score ($r=0.78$, $p=0.01$, **Table 2**), suggesting the increased CD8+ density in the obese mice represented an oligoclonal expansion.

Table 2. Correlation between adipose-resident T cell density and clonality score in mice fed a chow, low-fat, or high-fat diet for 16 weeks (n=9)

	Pearson's r	p-value
CD8+ cells	0.78	**0.01**
CD4+ cells	0.82	0.06

Density is calculated as the number of cells per gram of adipose tissue. CD4+ T cell recovery from chow mice was below the limit for sequencing (<1000 cells)

2.3. Greater circulating CD4+ memory T cells are associated with the odds of incident diabetes in HIV+ persons on ART: In pilot data from 76 male HIV+ Veterans Aging Cohort Study Biospecimen Cohort (VACS-BC) subjects, a higher proportion of memory T cells (CD4+ CD45RO+) was associated with a higher odds ratio for incident diabetes (OR= 10.0 for highest tertile of CD45RO+ cells versus lowest tertile; **Table 3**). There was also a positive relationship between T_H1 cytokine producing cells and the odds of incident diabetes that was not statistically significant ($p=0.16$), which may have been due to insufficient power. There were no significant findings for CD4+ or CD8+ T cell markers of activation or senescence. This finding, and similar results from the MESA cohort in non-HIV infected persons, supports our hypothesis that chronic immune activation of circulating T cells affects the insulin sensitivity of adipose and other metabolically active tissues.

Table 3. Relationships of CD4+ memory cell and T_H1 cell populations with incident diabetes in a pilot study of HIV+ VACS subjects

CD4+ T cell subset	Odds ratio for incident diabetes	p-value
Memory (CD45RO+)	10.0	**0.01**
T_H1 (helper cell)	2.9	0.16

• Odds ratio for highest tertile of cells versus lowest tertile
• Logistic regression models adjusted for age, race, BMI, hepatitis C status, alcohol use, cocaine use, CD4 count, HIV-1 RNA, and CT visceral fat quantity.

3.0 Hypotheses and Specific Aims

This study will assess the relationships of peripheral T cell distribution, adipose-resident T cell distribution, and adipose tissue inflammation in HIV-infected individuals, with the goal of understanding how chronic peripheral and tissue T cell activation may affect the risk of HIV-associated metabolic disease. If our hypotheses are correct, we will demonstrate that chronic peripheral immune activation (i.e., high memory T cells, low naïve cells, and increased expression of activation surface markers) is associated with greater adipose-resident CD4+ and CD8+ T cell expression of activation markers, the oligoclonal expansion of T cells expressing a restricted TCR repertoire, and adipose tissue inflammation.

Aim 1: To test the hypothesis that a higher percentage of activated CD4+ and CD8+ T cells in circulating blood is associated with a higher percentage of activated T cells in adipose tissue.

Aim 2: To test the hypothesis that activated T cells in adipose tissue contribute to macrophage activation, abnormal adipocyte gene expression, and glucose intolerance in HIV+ persons.

Aim 3: To test the hypothesis that adipose tissue from HIV+ patients with greater glucose intolerance is characterized by higher CD8+ T cell receptor oligoclonality, potentially reflecting an antigen-driven process.

4.0 Study Methods

4.1 Study Overview: The study design incorporates a 3 year, two-visit longitudinal cohort study of 96 HIV+ subjects who are non-diabetic at enrollment but either at high risk (i.e., pre-diabetic) or lower risk (i.e., not pre-diabetic) of progressing to clinical diabetes, in addition to a cross-sectional study which will compare these non-diabetic HIV+ subjects to an additional 40 HIV+ diabetics and 40 HIV-negative diabetics (see **Figure**). The baseline and follow-up study visits will include fasting peripheral blood collection, glucose tolerance testing, CT quantification of adipose tissue, and subcutaneous abdominal adipose tissue biopsies using a liposuction cannula system to collect approximately 10 ml of tissue.

4.2 Participant Recruitment: HIV+ participants will be recruited from the Vanderbilt Comprehensive Care Clinic (VCCC), the largest HIV provider in central Tennessee and a principal study site for the AIDS Clinical Trials Group (ACTG). In 2014, a total of 2,954 HIV+ patients had at least one VCCC visit, including 358 new patients (up from 297 in 2013), and over 250 antiretroviral therapy-naïve adults start treatment yearly. Diabetic patients without HIV will be recruited from the Vanderbilt *ResearchMatch* cohort, an electronic clinical study volunteer registry. In March 2016 there were 180 adult volunteers with Type 2 diabetes mellitus within 50 miles of Vanderbilt University.

4.3 Inclusion Criteria for HIV+ Participants:

- On antiretroviral therapy for at least 18 months
- HIV-1 RNA <50 copies/ml for the prior 12 months
- CD4+ count >350 cells/μl in the prior 12 months
- HbA1c in prior 6 months within specified limits (See Figure)
- Pre-menopausal by self-report
- No known inflammatory or rheumatologic conditions
- No heavy alcohol (>11 drinks per week) or cocaine, amphetamine, or illicit (non-

prescribed) opiate abuse by self-report
- Not on on GLP-1 agonists or DPP-4 inhibitors (diabetic participants only)

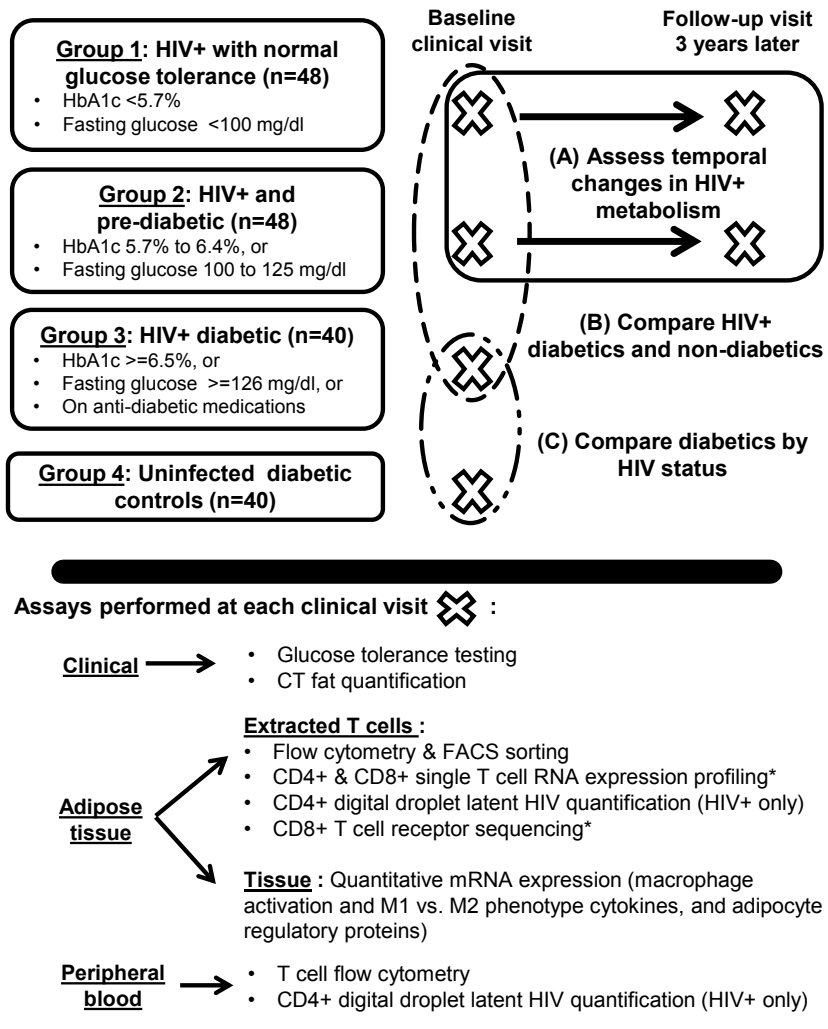
4.4 Inclusion Criteria for HIV-negative Participants:

- A HbA1c >6.5% or a fasting glucose >126mg/dl, or on anti-diabetic medications for at least 6 months
- Pre-menopausal by self-report
- No known inflammatory or rheumatologic conditions
- No heavy alcohol (>11 drinks per week) or cocaine, amphetamine, or illicit (non-prescribed) opiate abuse by self-report
- Not on a GLP-1 agonist or DPP-4 inhibitor

The fasting glucose, hemoglobin A1c (HbA1c), and/or anti-diabetic medication requirements for inclusion in one of the three HIV+ groups or the one HIV-negative group are shown in the **Figure**. The fasting glucose of 100mg/dl and HbA1c of 5.7% separating the two HIV+ non-diabetic groups are based on American Diabetes Association cutoffs for pre-diabetes.[16]

In selecting HIV+ participants, we will maintain a similar median age in each group, and we will recruit roughly equal numbers of white and non-white participants (reflecting the VCCC demographics), equal men and women, and an equal number of obese and non-obese (BMI >30 kg/m^2) in each group. We will also strive to enroll at least half our participants on an integrase strand transfer inhibitor (INSTI)-based regimen. INSTIs are becoming a preferred antiretroviral agent to the exclusion of some older agents. This strategy will aid in understanding

Figure: Subject groups, study design, and proposed assays



*Single T cell RNA expression profiling and CD8+ T cell receptor sequencing will only be performed at the baseline visit. Cells will be stored from the follow-up visit.

potential racial, sex, and antiretroviral medication factors in our analysis.

As with the HIV+ subjects, we will aim to recruit roughly equal numbers of white and non-white participants, equal men and women, and an equal number of obese and non-obese HIV-negative subjects.

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The enrollment schedule is shown in **Table 4**. We have estimated at least 80% of the HIV+ non-diabetic participants will be retained for the follow-up visit 3 years later (>=40 per group) based on loss and refusal rates in VCCC ACTG longitudinal studies. Participants will be actively followed by the research nurse and asked to complete the second visit early if they plan to move out of the area or start on anti-diabetic medication.

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Table 4. Enrollment by study group and year

	Year 1				Year 2				Year 3				Year 4				Year 5				
Clinical visits	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q4	**Total visits**
Baseline visit: HIV+ non-diabetics		10	20	20	20	20	6														**96**
Baseline visit: HIV+ diabetics							10	10	7	7	6										**40**
Baseline visit: HIV-negative diabetics									6	7	7	10	10								**40**
Follow-up visit: HIV+ non-diabetics														20	20	20	20				**80**

4.5 Study Procedures

Blood collection: We will collect 80 ml of fasting peripheral blood by percutaneous venipunture on the left or right upper extremity. We will collect an additional 5 ml of blood at -15, 0, 30, 60, 90, and 120 min after ingestion of the oral glucose tolerance test (the 90 minute collection may be omitted if CT scan is performed during this time).

Adipose tissue biopsy: We will collect approximately 10 ml of subcutaneous adipose tissue (equivalent to roughly 9.5 g) using a specialized 2.7 mm blunt, side-ported peripheral liposuction catheter (Tulip CellFriendly™ GEMS system, Tulip Medical Products) designed for the efficient extraction of viable adipocytes and stromal vascular cells for outpatient cosmetic re-injection procedures.[19] The GEMS system is ideal for our study due to the quantity of adipose tissue which can be obtained without injury to the cells. The procedure takes less than 15 minutes and blood loss is generally zero. This liposuction procedure is a standard technique for outpatient collection of subcutaneous adipose tissue and has been performed over 300 times at Vanderbilt.

Oral glucose tolerance test: All subjects will be fasting. We will draw blood at -15, 0, 30, 60, 90, and 120 min after oral administration of 75 g glucose syrup for measurement of serum glucose, insulin and C-peptide (the 90 minute collection may be omitted if CT scan is performed during this time). Glucose tolerance will be classified according to current American Diabetes

Association guidelines.[20] Although the primary outcome will be two-hour glucose, we will calculate insulin sensitivity as the homeostasis model assessment (HOMA2) of insulin sensitivity (www.dru/ox.ac.uk).[21-24] We will also calculate the Matsuda Index as 10,000/sqrt($Glucose_0$ x $Insulin_0$ x mean glucose x mean insulin) and the Insulinogenic Index defined as ΔInsulin (0-30, pmol/L)/ΔGlucose (0-30, mmol/L).[25, 26]

CT adipose tissue quantification: A noninvasive CT scanning protocol will be performed using established methods previously used in NHLBI/NIA cohort studies to measure adipose tissue mass and distribution, including abdominal subcutaneous and visceral fat, liver adiposity, pericardial adiposity, and thigh adipose tissue and muscle.[27-36] **Appendix 1** describes the scanning protocol.

Health and behavior assessment: We will assess diet, activity level, alcohol intake, family history, and health behaviors using a modified version of the Wellspring Health Risk Assessment (see **Appendix 2**).[37] Selected variables will be included in the statistical models if associated with the outcomes.

5.0 Risks to Study Participants

5.1. Potential Risks: The procedures presenting potential risks of this clinical study include phlebotomy, percutaneous adipose tissue biopsy, radiographic imaging, oral glucose tolerance testing, pregnancy testing and a breach of confidential information. Common risks of phlebotomy include pain, local skin irritation, and formation of a superficial hematoma; more severe risks would include local or systemic infection or accidental cannulation of an artery (all uncommon).

Risks from the adipose tissue biopsy include pain, local skin irritation, bruising, bleeding, and formation of a hematoma in the subcutaneous tissue; more severe risks would include local or systemic infection, or difficult to control bleeding (all uncommon). Lidocaine may cause local discomfort during injection or a rash, redness or soreness at the injection site, and in rare cases could induce a transient alteration in heart rhythm (suction is applied to the syringe prior to injection to ensure the lidocaine is not being injected into a vessel).

The risks of the oral glucose tolerance test include nausea or vomiting following ingestion of the 75 g glucose solution.

The CT scan will include imaging of the abdomen, the thigh, and the chest (pericardial fat). The average amount of radiation a person will receive from the CT scan is estimated to be 7 mSv (range 3.5 -14 mSv), or 14 mSv for those patients seen at both the baseline and follow-up visits (separated by more than 3 years). This can be compared to the radiation people in the U.S. receive every year from natural sources (about 3 mSv). By contrast, people who work with radiation have a yearly exposure limit of 50 mSv. Therefore, the estimated total radiation exposure at the initial and follow-up visits will be approximately equal to slightly more than four years of background radiation from natural sources.

All female patients will receive a pregnancy test, and a negative test result will be a prerequisite for any further study procedures. The major risk of the pregnancy test is disclosure of a previously unknown pregnancy.

To reduce the risk of potential disclosure of identifying information, study participants will be

assigned a study number (not derived from any personal information) to identify all samples and study records, and the key linking study numbers to medical record numbers will be maintained in a locked file in the locked, private office of the PI. We believe this system will provide a high degree of security to maintain the confidentiality of personal health information.

5.2. Biohazard Risks: Participants in this study will not be treated with any biological agents or administered any substances that could present a biohazard risk (aside from lidocaine). However, the serum samples, adipose tissue biopsies, and mononuclear cells collected from participants in this study represent biohazardous specimens and will be handled according to biohazard protocols in place at Vanderbilt University. Since each participant will undergo study procedures individually, study volunteers are not expected to be at risk of exposure to biological samples from other participants at any time. Samples will be collected by trained research staff and needles and other equipment will be disposed of in the appropriate containers for processing by the Medical Center waste facility.

5.3. Protections against Risk: The planned study procedures are all commonly used in immunologic and metabolic clinical research and will be performed by personnel with specialized training. The VCCC ACTG research facility has a long history of successful participation in complex multi-center clinical studies and a competent, professional research staff. To reduce the risk of loss of confidentiality, each study participant will be assigned a unique study ID number that is not derived from any personal identifier. All participant computer data entry forms, imaging results, and samples will be labeled with this unique study number; no specimens or data forms will include any personal identifiers. Only the PI will have access to the key linking the study ID to a VUMC medical record number, and this information will be maintained in a locked filing cabinet in the locked office of the PI (who will be responsible for maintaining the confidentiality of this document). All study data will be collected and stored in the REDCap database, a centralized, password protected electronic research database created and maintained by the Vanderbilt Institute for Clinical and Translational Research. Data collection at the time of the visit will be computer-based to the extent feasible, reducing the need for paper data reports or forms. The electronic data will be uploaded to REDCap. The only patient identifiers included in the REDCap database (as defined by HIPPA) will be dates (month/date/year) related to clinical care (e.g., date of treatment start). Only the PI and the project biostatisticians (Bryan Shepherd PhD and Cathy Jenkins MS) will have access to the REDCap study database.

5.4. Potential Benefits of the Research: While the proposed research will not have direct benefits to the study participants, the risks of the study are reasonable in relation to the benefit to HIV-infected adults as a population. The study findings will be directly relevant to the design of intervention trials to reduce metabolic disease risk in HIV-infected adults, which could lead to changes in clinical practice that benefit study participants in the future.

6.0. Data and Safety Monitoring Plan

A data safety monitoring board (DSMB) will be convened prior to the study start, preliminarily comprised of clinical researchers and a statistician not affiliated with the study. All adverse events will be submitted to the DSMB and IRB in progress reports (at required time intervals) and serious adverse events will be reported immediately to the DSMB and IRB.

7.0 Reporting of Adverse Events

Appropriate medical personnel will be present during study procedures and adverse events will be recorded. Adverse events will be reported according to IRB policy.

An Adverse Event (AE) is any untoward medical occurrence in a patient during the conduct of this study, and which does not necessarily have a causal relationship with the study. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with participation in the study.

Any AE that results in any of the following outcomes will be considered a Serious Adverse Event (SAE). The following outcomes would represent examples of SAEs.

- Death
- Life-threatening situation (subject was at risk of death at the time of the event; this does not refer to an event that might have caused death if it was of greater intensity).
- Inpatient hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Important medical events that may not result in death, be life-threatening, or require hospitalization but may jeopardize the subject and may require medical or surgical intervention to prevent one of the above outcomes.

8.0 Study Withdrawal/Discontinuation

Study withdrawal will be based on subject decision. All study procedures will cease if the subject elects to withdraw at any time during the study visit. There are no indications for PI withdrawal.

9.0 Privacy/Confidentiality Issues

Each potential study participant will be assigned a unique study ID number not derived from any personal identifier. All participant computer data entry forms, imaging results, and samples will be labeled with this unique study number; no specimens or data forms will include any personal identifiers. Only the PI will have access to the key linking the study ID to a VUMC medical record number, and this information will be maintained in a locked filing cabinet in the locked office of the PI (who will be responsible for maintaining the confidentiality of this document). All study data will be stored in the RedCap database, a centralized, password protected electronic research database created and maintained by the Vanderbilt Institute for Clinical and Translational Research. All electronic data (e.g., lab results) will be uploaded to RedCap or, if necessary, re-entered in RedCap by the PI. The only patient identifiers included in the RedCap database (as defined by HIPPA) will be dates (month/date/year) related to clinical care (e.g., date of treatment start). Only the PI and the co-investigators will have access to the study database. At the conclusion of the study all identifying information will be destroyed. The de-identified data will be maintained in the RedCap system.

10.0 Follow-up and Record Retention

The study will last approximately 5 years, including recruitment, data collection, and data analysis. De-identified research records and/or limited datasets will be maintained by the PI indefinitely as outlined in the IRB application and informed consent document.

11.0. ClinicalTrials.gov Requirements

This application does not include a clinical trial of a drug, biologic, or device.

12.0 References

1. Brown TT, Cole SR, Li X, Kingsley LA, Palella FJ, Riddler SA, et al. Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study. Arch Intern Med. 2005;165(10):1179-84.
2. De Wit S, Sabin CA, Weber R, Worm SW, Reiss P, Cazanave C, et al. Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. Diabetes Care. 2008;31(6):1224-9. PMCID: 2746200.
3. Capeau J, Bouteloup V, Katlama C, Bastard JP, Guiyedi V, Salmon-Ceron D, et al. Ten-year diabetes incidence in 1046 HIV-infected patients started on a combination antiretroviral treatment. AIDS. 2012;26(3):303-14.
4. Butt AA, McGinnis K, Rodriguez-Barradas MC, Crystal S, Simberkoff M, Goetz MB, et al. HIV infection and the risk of diabetes mellitus. AIDS. 2009;23(10):1227-34. PMCID: PMC2752953.
5. Zeng C, Shi X, Zhang B, Liu H, Zhang L, Ding W, et al. The imbalance of Th17/Th1/Tregs in patients with type 2 diabetes: relationship with metabolic factors and complications. J Mol Med (Berl). 2012;90(2):175-86.
6. Wagner NM, Brandhorst G, Czepluch F, Lankeit M, Eberle C, Herzberg S, et al. Circulating regulatory T cells are reduced in obesity and may identify subjects at increased metabolic and cardiovascular risk. Obesity (Silver Spring). 2013;21(3):461-8.
7. Viardot A, Heilbronn LK, Samocha-Bonet D, Mackay F, Campbell LV, Samaras K. Obesity is associated with activated and insulin resistant immune cells. Diabetes Metab Res Rev. 2012;28(5):447-54.
8. Olson NC, Doyle ME, Psaty BM, De Boer I, Jenny NS, Kronmal R, et al. Abstract P433: Associations of CD4+ T Lymphocyte Subpopulations with Insulin Levels and Type 2 Diabetes: The Multi-Ethnic Study of Atherosclerosis (MESA). Circulation. 2013;127.
9. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med. 2009;15(8):914-20.
10. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med. 2009;15(8):930-9.
11. Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, et al. Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med. 2009;15(8):921-9. PMCID: 3063199.
12. Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, et al. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat Med. 2011;17(5):610-7. PMCID: 3270885.
13. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science. 1996;271(5249):665-8.

14. Senn JJ, Klover PJ, Nowak IA, Mooney RA. Interleukin-6 induces cellular insulin resistance in hepatocytes. Diabetes. 2002;51(12):3391-9.
15. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest. 2007;117(1):175-84. PMCID: 1716210.
16. American Diabetes A. Standards of medical care in diabetes--2014. Diabetes Care. 2014;37 Suppl 1:S14-80.
17. Aso Y, Fukushima M, Sagara M, Jojima T, Iijima T, Suzuki K, et al. Sitagliptin, a DPP-4 inhibitor, alters the subsets of circulating CD4+ T cells in patients with type 2 diabetes. Diabetes Res Clin Pract. 2015;110(3):250-6.
18. Chaudhuri A, Ghanim H, Vora M, Sia CL, Korzeniewski K, Dhindsa S, et al. Exenatide exerts a potent antiinflammatory effect. J Clin Endocrinol Metab. 2012;97(1):198-207. PMCID: PMC3251936.
19. Alexander RW, Harrell DB. Autologous fat grafting: use of closed syringe microcannula system for enhanced autologous structural grafting. Clinical, cosmetic and investigational dermatology. 2013;6:91-102. PMCID: 3626419.
20. American Diabetes A. Classification and diagnosis of diabetes. Diabetes Care. 2014;38 Suppl:S8-S16.
21. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care. 1998;21(12):2191-2.
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
23. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004;27(6):1487-95.
24. Caumo A, Perseghin G, Brunani A, Luzi L. New insights on the simultaneous assessment of insulin sensitivity and beta-cell function with the HOMA2 method. Diabetes Care. 2006;29(12):2733-4.
25. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care. 1999;22(9):1462-70.
26. Utzschneider KM, Prigeon RL, Faulenbach MV, Tong J, Carr DB, Boyko EJ, et al. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. Diabetes Care. 2009;32(2):335-41. PMCID: 2628704.
27. VanWagner LB, Ning H, Lewis CE, Shay CM, Wilkins J, Carr JJ, et al. Associations between nonalcoholic fatty liver disease and subclinical atherosclerosis in middle-aged adults: the Coronary Artery Risk Development in Young Adults Study. Atherosclerosis. 2014;235(2):599-605. PMCID: PMC4124046.
28. Mueller NT, Pereira MA, Demerath EW, Dreyfus JG, MacLehose RF, Carr JJ, et al. Earlier menarche is associated with fatty liver and abdominal ectopic fat in midlife, independent of young adult BMI: The CARDIA study. Obesity (Silver Spring). 2014;23(2):468-74. PMCID: PMC4310794.
29. Liu J, Fox CS, Hickson DA, May WD, Hairston KG, Carr JJ, et al. Impact of abdominal visceral and subcutaneous adipose tissue on cardiometabolic risk factors: the Jackson Heart Study. J Clin Endocrinol Metab. 2010;95(12):5419-26. PMCID: PMC2999970.
30. Murphy RA, Register TC, Shively CA, Carr JJ, Ge Y, Heilbrun ME, et al. Adipose tissue density, a novel biomarker predicting mortality risk in older adults. J Gerontol A Biol Sci Med Sci.69(1):109-17. PMCID: PMC3859360.
31. Sacks HS, Fain JN. Human epicardial adipose tissue: a review. Am Heart J. 2007;153(6):907-17.

32. Ding J, Hsu FC, Harris TB, Liu Y, Kritchevsky SB, Szklo M, et al. The association of pericardial fat with incident coronary heart disease: the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin Nutr. 2009;90(3):499-504. PMCID: PMC2728641.
33. Ding J, Kritchevsky SB, Hsu FC, Harris TB, Burke GL, Detrano RC, et al. Association between non-subcutaneous adiposity and calcified coronary plaque: a substudy of the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr. 2008;88(3):645-50. PMCID: PMC3282464.
34. Liu J, Fox CS, Hickson D, Sarpong D, Ekunwe L, May WD, et al. Pericardial adipose tissue, atherosclerosis, and cardiovascular disease risk factors: the Jackson heart study. Diabetes Care. 2010;33(7):1635-9. PMCID: PMC2890373.
35. Schrauwen-Hinderling VB, Hesselink MK, Schrauwen P, Kooi ME. Intramyocellular lipid content in human skeletal muscle. Obesity (Silver Spring). 2006;14(3):357-67.
36. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. J Appl Physiol (1985). 2000;89(1):104-10.
37. http://www.wellsource.com/health-risk-assessments.html.