SP2* Non-invasive detection of white adipose tissue inflammation with Raman spectroscopy
Abigail Haka, Weill Cornell Medical College
Funding Source and Period: 5RO1 HL093324-07 (Maxwell, PI)
Associated With: TRD3

Significance: Dr. Haka is an expert in the application of optical spectroscopy and imaging tools to elcuidate the role of macrophage lipid clearance in disease. In particular, her lab is interested in how macrophages catabolize objects that cannot be internalized by standard phagocytic mechanisms and how this novel method of degradation effects the biology of conditions such as atherosclerosis and white adipose tissue inflammation. Data from her and others have defined a process, termed exophagy, in which large moieties or species tightly bound to the extracellular matrix are initially digested in an extracellular, acidic, lytic compartment [1-3]. Exophagic catabolism of aggregated LDL results in uptake of cholesterol by the macrophage leading to foam cell formation, an integral part of atherosclerotic plaque formation. Working with Dr. Dannenberg, a leader in the field of chronic inflammation and cancer, Dr. Haka further investigates the use of spectroscopic techniques for the non-invasive diagnosis of white adipose tissue inflammation (WATi). It is imperative to identify those subjects who harbor this chronic, low-grade inflammation prior to the development of disease. While WATi is common in the obese, it is recognized that as many as 30% of phenotypically obese individuals may be metabolically healthy [4-7], while significant metabolic abnormalities occur in others despite having a normal body mass index (BMI) [8, 9]. Hence, precisely defining the population most likely to benefit from targeted intervention(s) to reverse WATi is a challenge and requires a more sophisticated assessment than BMI alone.

Approach: For this purpose, sensitive spectroscopic characterization based on endogenous contrast mechanisms offers a powerful tool. Detection strategies currently under investigation, such as positron emission tomography (PET) and magnetic resonance imaging, require the administration of exogenous contrast agents, involve exposure to high-dose radiation, and have not been rigorously tested in humans [10, 11]. To this end, leveraging the capabilities of the portable Raman spectroscopy system developed by the LBRC, the Haka laboratory has demonstrated the ability of sponateous Raman spectroscopy to detect WATi and shown its diagnostic ability in diverse tissue specimens both *ex vivo* (**Fig. 1**) and *in vivo* [12]. The diagnoses are based primarily on changes in fatty acid saturation that occur in association with adipocyte hypertrophy, which is a known correlate of WATi.

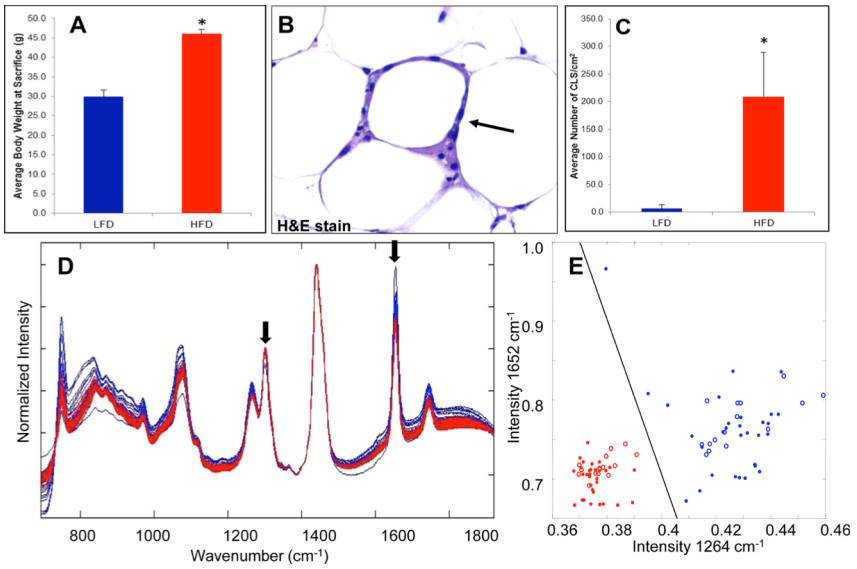

Fig. 1. Raman spectroscopy detects WATi in epididymal fat from a mouse model of diet-induced obesity with perfect accuracy. (A) Average body weights of mice on the low fat diet (LFD) (noninflamed) and high fat diet (HFD) (inflamed) at the time of sacrifice (n = 10/group). (Asterisk (*) denotes P < 0.001.) (B) A crown like structure (CLS) in WAT from a mouse on the HFD stained with H&E. (C) Average number of CLS/cm^2 in mice on the LFD and HFD. (D) Raman spectra from inflamed (red, HFD) and noninflamed (blue, LFD) epididymal WAT. Clear differences in the spectra of inflamed and noninflamed WAT can be seen, particularly in the intensity of the Raman peaks at 1264 and 1652 cm^{-1} (arrowed features). (E) Diagnostic scatter plots based on the peak heights of the 1264 and 1652 cm−1, which can separate inflamed (red) from noninflamed (blue) WAT with 100% accuracy (data shown as closed circles). Prospective application on 10 mice also accurate identification of inflammatory These data are shown as red and blue open circles on the diagnostic scatter plot in panel (E).

Center Offering: First, LBRC provides a fiber-probe based portable Raman spectroscopy unit that is ideal for WATi measurements both *ex vivo* and *in vivo*. Second, since these studies are performed in the absence of specific contrast agents, the acquired Raman signal presents a composite signature of numerous tissue constituents. Hence, transcutaneous measurements, in particular, necessitate the development of advanced chemometric models, which have been developed by the LBRC. Together, these offerings empower noninvasive data acquisition and WATi recognition in clinical settings that would represent a critical milestone in translation. Future studies will also evaluate the feasibility of this tool for assessing the efficacy of therapeutic interventions (behavioral, dietary, pharmacological) aimed at attenuating WATi and subsequent disease.

Literature Cited:

1. Haka AS, Singh RK, Grosheva I, Hoffner H, Capetillo-Zarate E, Chin HF, Anandasabapathy N, Maxfield FR. *Monocyte-Derived Dendritic Cells Upregulate Extracellular Catabolism of Aggregated Low-Density Lipoprotein on Maturation, Leading to Foam Cell Formation.* Arterioscler Thromb Vasc Biol, 2015. **35**(10): p. 2092-103.
2. Singh RK, Barbosa-Lorenzi VC, Lund FW, Grosheva I, Maxfield FR, Haka AS. *Degradation of aggregated LDL occurs in complex extracellular sub-compartments of the lysosomal synapse.* J Cell Sci, 2016. **129**(5): p. 1072-82.
3. Haka AS, Barbosa-Lorenzi VC, Lee HJ, Falcone DJ, Hudis CA, Dannenberg AJ, Maxfield FR. *Exocytosis of macrophage lysosomes leads to digestion of apoptotic adipocytes and foam cell formation*. J Lipid Res, 2016. **57**(6): p. 980-92.
4. Klöting N, Fasshauer M, Dietrich A, Kovacs P, Schön MR, Kern M, Stumvoll M, Blüher M. *Insulin-sensitive obesity.* Am J Physiol Endocrinol Metab, 2010. **299**(3): p. E506-15.
5. Xu XJ, Gauthier MS, Hess DT, Apovian CM, Cacicedo JM, Gokce N, Farb M, Valentine RJ, Ruderman NB. *Insulin sensitive and resistant obesity in humans: AMPK activity, oxidative stress, and depot-specific changes in gene expression in adipose tissue.* J Lipid Res, 2012. **53**(4): p. 792-801.
6. Denis GV, Obin MS. *Metabolically healthy obesity: origins and implications.* Mol Aspects Med, 2013. **34**(1): p. 59-70.
7. Blüher S, Schwarz P. *Metabolically healthy obesity from childhood to adulthood—does weight status alone matter?* Metab Clin Exp, 2014. **63**(9): p. 1084-92.
8. Chen S, Chen Y, Liu X, Li M, Wu B, Li Y, Liang Y, Shao X, Holthöfer H, Zou H. *Insulin resistance and metabolic syndrome in normal-weight individuals.* Endocrine, 2014. **46**(3): p. 496-504.
9. Deepa M, Papita M, Nazir A, Anjana RM, Ali MK, Narayan KM, Mohan V. *Lean people with dysglycemia have a worse metabolic profile than centrally obese people without dysglycemia.* Diabetes technology & therapeutics, 2014. **16**(2): p. 91-6.
10. Bucerius J, Mani V, Wong S, Moncrieff C, Izquierdo-Garcia D, Machac J, Fuster V, Farkouh ME, Rudd JH, Fayad ZA. *Arterial and fat tissue inflammation are highly correlated: a prospective 18F-FDG PET/CT study*. European journal of nuclear medicine and molecular imaging, 2014. **41**(5):934-45.
11. Luciani A, Dechoux S, Deveaux V, Poirier-Quinot M, Luciani N, Levy M, Ballet S, Manin S, Péchoux C, Autret G, Clément O. *Adipose tissue macrophages: MR tracking to monitor obesity-associated inflammation.* Radiology, 2012. **263**(3): p. 786-93.
12. Haka AS, Sue E, Zhang C, Bhardwaj P, Sterling J, Carpenter C, Leonard M, Manzoor M, Walker J, Aleman JO, Gareau D. *Noninvasive Detection of Inflammatory Changes in White Adipose Tissue by Label-Free Raman Spectroscopy.* Anal Chem, 2016. **88**(4): p. 2140-48.