PROJECT SUMMARY (See instructions):

Excess dietary fat and obesity-associated perturbations (such as inflammation, hyperlipidemia, hyperinsulinemia, or diabetes) produce an overabundance of certain lipid metabolites implicated in impaired cellular survival and function. Sphingolipids accumulate in muscle and liver of diabetic rodents and humans. These also appear to overaccumulate in neurons, where they can contribute to lipotoxic cell death or dysfunction, and they change as of function of lipoprotein lipase activity. As such, changes of these same lipid species have been implicated in the progression of neurodegenerative diseases and ischemic cell death. While compounds which limit ceramide or glucosylceramide accumulation have become attractive options as pharmaceutical insulin sensitizers, strategies for evaluating sphingolipid measurement in heterogeneous structures have limited advances in understanding their roles in the CNS.

Here we will implement two recently-available mass spectrometry techniques in order to pinpoint the *neuroanatomical location* and *classes* of lipid metabolites which overaccumulate in the obese brain. These studies bolster our preliminary work suggesting that free fatty acids may be liberated by lipoprotein lipase residing in brain sites devoid of a blood-brain barrier known as the circumventricular organs. We anticipate that concentrations of free fatty acids and fatty acyl Co-A will be higher surrounding these brain regions where triglycerides are hydrolyzed and dissipate as they diffuse farther away.

While imaging mass spectrometry techniques have been used to perform lipidomic analyses, and has been applied to neurological samples, these will be the first studies to use this technique to evaluate lipid metabolite changes in the brain during metabolic adversity. Via this method, we can avoid previous surrogate methods of measuring isolated neurons or organotypic slices when determining alterations in metabolites. This technique offers a much more accurate understanding of neuronal metabolite conditions *in vivo.* To do this, we will make use of a Shimadzu 7090 imaging system which was recently installed at UT Arlington in the Shimadzu Institute for Advanced Analytical Chemistry. This is the first machine of this type in the Southwest region and the first 7090 system in North America. This technique holds the potential to transform the field of lipid measurement in the healthy and diseased brain. Following training at the Vanderbilt Advanced Imaging Mass Spectrometry (AIMS) Course we (the Holland lab) have been essentially the only users of this Shimadzu 7090 spectrometer, as we have developed the ability to analyze sphingolipids in pancreatic samples. With the help of the Gautron lab we will begin applying this technology to the brain.

We complement the MALDI imaging, which is biased based on the efficacy of different matrix compounds (DHB, right) to ionize small molecules, with shotgun lipidomics. Using an ABI 5600+, we can simultaneously identify changes in hundreds of distinct lipid species via a nonbiased approach following direct infusion of extracted lipids. This allows us to test for accompanying changes in other potentially neurotoxic lipids-from the same microdissected sample. Developmental software, designed by our collaborator Jeffrey McDonald, tremendously assists with simplifying the data by identifying lipid species. The combination of mass spectrometry techniques will also aid in the complex identification of peaks from MALDI spectra. These are the two most cutting edge approaches for identifying which lipid metabolites are altered, and the precise anatomical location where they are altered. We hope that they will prove useful to future NIH-funding efforts for our labs and the labs of our collaborators, who will also greatly benefit from the technology.

RELEVANCE (See instructions):

The current project represents a new collaboration between our young labs. The Holland lab is new to neuroscience, having focused on lipid-induced insulin resistance in peripheral tissues. The Gautron lab is new to lipid metabolite measurements, having focused on neuroanatomy and protein signals that regulate control of food intake and energy expenditure. Here we join forces to investigate projects of mutual interest based on preliminary results obtained separately from each lab. In so doing, we plan to achieve interdisciplinary training for each of our groups. Dr. Holland will aid the Gautron lab to learn measurement of lipid metabolites using mass spectrometry to aid their ongoing studies of PPARγ function in the Brain. Dr. Gautron will help train the Holland lab how to correctly identify anatomical structures in the brain. Our combined efforts will establish the capacity to perform broad-spectrum, non-biased shotgun lipidomic analysis of lipid metabolites on discrete brain regions. Additionally, we will apply our mass spec imaging approaches to brain sections. These techniques hold the potential to transform the field of lipid measurement in brain structures, which we can use to aid our colleagues throughout the UT System.