NDR is approaching ALS from multiple directions- currently we have 17 active projects! What is fascinating is the emerging overlap between the projects. Many chronic illnesses share common pathologic pathways so it may be anticipated that our paths lead in the same direction. Neurodegenerative disease pathologies disrupt the cell's energy (mitochondria) which disturbs cell-to-cell communication causing inflammation, cell death, and metabolic disturbances.

We initiated a project that examined the adipose cells and adipose stem cells from various sources because fat cells are metabolized to provide energy to the body and nervous system. Fat cells may change in the ALS patient because the metabolic demand increases in disease. Interest was piqued because culture media taken from adipose stem cells that were grown *in vitro*, was successful as a treatment in the ALS mouse model. Fat cells secrete molecules into the blood stream that communicate with the nervous system and the nervous system sends signals back to other tissues including fat cells. Some of these molecules may be measurable in the plasma. The next step was determining the metabolic signals in diseased or stressed cells when compared with healthy subjects.

A snapshot of the metabolic state is obtained by a metabolomic analysis, called a metabolic network. The metabolic network is dynamic in any individual, however some metabolic disturbances in disease should be demonstrably different than the healthy condition. A metabolomics study can target 600 natural metabolites with the hypothesis that the metabolic network in ALS may be a diagnostic feature of the disease.

Because ALS patients are compared with age matched controls, we hope to see a network with a concurrence of molecules abnormal in the ALS group and more typical in the healthy subjects. Metabolomics is a unique opportunity to look for blood biomarkers and then use them as screening tools for disease onset and progression of disease pathology in the ALS patient.

The metabolomics study will identify the lipid signals travelling through the blood to the central nervous system. There are advances in defining the role lipids play at the onset, progression, and lifespan extension of ALS patients. We hope the metabolomics study, secretome analysis study and the literature on lipid biomarkers will be transformative.

It is frustrating that no successful therapy with promising results in animal models has improved the clinical outcome in ALS patients. ALS is defined as a multifactorial neurodegenerative disease caused by genetic and non-inheritable components that lead to degeneration of motor neurons. ALS pathology is not limited to motor neurons. Research shows that ALS is not a single cell type disease but involves the motor neuron associated cells microglia and astrocytes. It was shown that astrocytes can drive motor neuron loss via mitochondrial function, nitric oxide production and changes in nerve growth factors. A hallmark of ALS is neuroinflammation that may be associated with glial cell dysregulation.

Given that there are multiple targets of pathology, ALS has been described as a two-stage process, the early neuroprotective stage followed by the neurotoxic stage. It is difficult to understand ALS from the

standpoint of individual components of dysfunction that result in motor neuron death. It may be more productive to view ALS as a composite syndrome where the culmination of several aberrant cellular pathways reaches a tipping point, and the result is disease. The latter view may argue that one must know the disease process, which pathways have conspired to cause disease because each patient likely have different dysregulated pathways. Developing multi-targeted therapies by combining drugs that are synergistic or using compounds that have multiple targets will be important to combat ALS. The selection of treatment must be preceded by understanding the pathways that are dysregulated. Blood biomarkers are critical for defining the pathology and the selection of appropriate targets in the dysregulated pathway. Metabolomics may define those dysfunctional pathways and reveal biomarkers. Perhaps lipid metabolism holds a key to biomarker discovery for defining the ALS syndrome.

In ALS, dysregulated neuronal lipid metabolism affects energy provided to motor neurons, the structure of cell components that perform housekeeping duties, and signaling the cell's status and needs to other nearby cells as well as cells outside the central nervous system. Lipid metabolism deserves consideration when developing diagnostic tools. Small lipid molecules have already been identified as elevated in ALS patients.

Lipids molecules that are associated with ALS are palmitoleate, a change in palmitoleate to palmitate ratio is important. Another ratio that is associated with ALS is the sterate to oleate ratio and individual lipid levels that are associated include palmitoleate, oleate levels, and stearoyl-CoA desaturase. It is becoming clear that it will take evaluating a panel of these markers and determining a change over time to be meaningful.

Other lipid molecules that are distinctive between ALS patients and control groups are related to metabolism of phosphatidylcholine, sphingomyelin, and triglycerides. Phosphatidylcholine and sphingomyelin are elevated while triglyceride levels are decreased.

Oxidized lipids are relevant to biological and pathway on/off signaling actions, an important compound is prostaglandin E_2. Pharmacological inhibition of PGE_2 receptors can lower neuroinflammation in mouse ALS models, preserve motor functions and extend the survival of the mouse. A complicating issue with PGE_2 is that it exerts various biological actions that depend on specific tissues where it is active. The actions of PGE_2 needs more evaluation to show correlation with ALS pathology. Metabolomics may be informative here.

Increased levels of 5-lipoxygenase were observed in SOD1 mice and administration of 5-lipoxygenase and tyrosine kinase inhibitors extended the lifespan of this animal model. Molecules 12-hydroxyeicosatatraenoic acid and prostaglandin are also increased corresponding to disease in the SOD1 diseased mouse.

Our metabolomics study will include examination of miRNA's, the real time gene expression driving the ultimate metabolic pathways we are investigating. Our greatest hope is to find a panel of markers that are dysregulated in the ALS patient population, differing them from the healthy control population. We hope to identify published neuronal lipid molecules and perhaps find the ALS Rosetta Stone.