

Final project report:

The Role of CD36 in the Obesity-Associated Fatty Liver Disease and Liver Cancer

Investigator: Dmitri Samovski, PhD

Funding Period: 10/01/18- 03/31/20

Current professional status: Assistant Professor in Medicine, Washington University

Project overview

Nonalcoholic fatty liver disease (NAFLD) affects ~30% of adults in the United States and ~70% of adults with obesity, suggesting that obesity is a significant risk factor for NAFLD development. NAFLD encompasses a spectrum of liver abnormalities, manifested by a progression from simple steatosis to steatohepatitis, and fibrosis which can lead to cirrhosis and liver cancer. NAFLD is a robust early predictor of the development of multi-organ IR and often precedes the onset of other indicators of metabolic diseases, including type-2 diabetes (T2D), coronary heart disease, and dyslipidemia. This sequence of events implies that hepatic steatosis has a causal role in the development of systemic IR and the subsequent metabolic complications.

Although the molecular mechanisms underlying the onset and progression of NAFLD are complex, they are often linked to obesity-associated dysfunction in pathways of long-chain fatty acid (FA) utilization and storage. Furthermore, while we do not have a clear understanding as to how NAFLD contributes to the development of multi-organ IR, the emerging data indicate that NAFLD can impact the function of other organs by altering the inter-organ communication between liver and other tissues. The impairment in inter-organ communication is manifested through the changes in the secretion of regulatory factors from the liver, including small extracellular vesicles (sEVs, also known as exosomes) that carry signaling proteins, bioactive lipids, and non-coding RNAs. In this project, we examined the underpinnings of NAFLD development and the associated metabolic complications by a two-pronged approach aimed at the analysis of both the obesity-associated changes in liver FA metabolism and subsequent changes in the secretion of sEVs by the liver.

Results

1. Impact of impaired hepatic FAs sensing on steatosis and IR. CD36, the FA receptor studied in this project, has been linked to metabolic disease. Obesity and T2D were shown to associate with an upregulated hepatic expression of CD36. Using cells in culture and mouse models, we examined how CD36 senses FAs availability in the liver and adjusts their utilization and storage accordingly. We identified a novel molecular pathway that links FA supply to energy regulation by insulin and AMP Kinase. The mechanism involves the direct impact of FA on protein interactions within multiprotein signaling complexes. This effect is mediated by CD36 and is differentially modulated by FA availability and FA type. Our studies in cultured cells revealed that saturated FA (such as palmitic and myristic acids), but not unsaturated FA (such as linoleic and oleic acids) are capable, by modulating protein-protein interactions, to simultaneously enhance AMPK and suppress insulin signaling. These effects would result in FA targeting to oxidation and in minimizing FA storage into lipids. The operation of this mechanism should serve to limit excessive FA accumulation and generation of toxic metabolites. However, it is likely that this FA sensing mechanism is impaired by over-nutrition and obesity resulting in deleterious effects on AMPK and insulin signal transduction.

To validate these findings *in vivo*, we generated a mouse model with a hepatocyte-specific CD36 deficiency (hepCD36^{-/-}). Using these mice, we confirmed *in vivo* our findings from cell culture studies. Our results showed that CD36 promotes insulin signaling in hepatocytes by facilitating the assembly of insulin receptor signaling complex, while saturated, but not unsaturated, FAs suppressed the complex assembly, resulting in inhibition of insulin signaling. The described mechanism provides means for insulin signaling regulation by FAs with differential effects of saturated and unsaturated FAs. Dysregulation of CD36-mediated FA sensing mechanisms, as it occurs in obesity, would lead to impaired lipid metabolism, accumulation of toxic lipid metabolites, hepatic IR and metabolic dysfunction.

2. Differences in circulating sEVs between people with low or high intrahepatic triglycerides

Small extracellular vesicles (sEVs) (also known as exosomes) are nanoparticles with a diameter <200 nanometers that are secreted by most cells in a tissue and provide a pathway for communication with other tissues. sEVs are membrane-bound vesicles that are formed inside the cell and are released from the cell on fusion with the plasma membrane. The composition of the sEV cargo reflects the cell of origin and is believed to mediate paracrine and endocrine communication between the tissue of origin and target tissues. sEVs can exert specific effects on gene expression and function of recipient tissues by delivering miRNAs (the most studied sEV cargo) as well as bioactive lipids, and regulatory proteins.

Obesity and T2D were shown to associate with increased abundance of circulating sEVs. We postulated that sEVs secreted from liver of people with NAFLD transport disease-promoting signals to skeletal muscle and other tissues and facilitate the development of multi-organ IR and the associated metabolic diseases. To explore this hypothesis, we initiated a pilot human study where we recruited subjects with obesity and either low or high intrahepatic triglyceride (IHTG) content (n=10 subjects/group). Plasma samples and metabolic profiles of human subjects were obtained through a collaboration with the Center of Human Nutrition at Washington University. The following metabolic characteristics were used as inclusion criteria for the study:

1) **low IHTG group**: BMI 30.0-44.9 kg/m², normal fasting blood glucose and oral glucose tolerance and IHTG <5% of total liver volume; and 2) **high IHTG group**: BMI 30.0-44.9 kg/m², impaired oral glucose tolerance and IHTG content ≥10%. Each participant underwent a comprehensive metabolic testing, including a state-of-the-art assessment of liver fat and insulin sensitivity. We collected blood from all the participants and developed and optimized methods for reliable and reproducible isolation of circulating sEVs.

Currently, we are conducting systematic, high-throughput analyses of the isolated sEVs, to identify the specific cargo molecules (miRNAs, proteins and lipids) carried by sEVs of people with high IHTG. The content of signaling molecules in sEVs of people with obesity and low or high IHTG is systematically characterized, to identify the factors that contribute to IR and metabolic dysfunction associated with NAFLD. In parallel, we have been examining the pathophysiological effects of sEVs on IR in cells in culture. Our early findings show that sEVs from people with high IHTG induce insulin resistance in human muscle cells. These findings indicate that plasma sEVs in people with obesity and NAFLD contain molecules that promote metabolic dysfunction in muscle. Later, we plan to examine the effects of sEVs on IR and metabolic diseases in mice infused with the different types of human sEVs that we are collecting.

Significance: Obesity from over-nutrition is one of the leading causes of IR and T2D, two conditions that continue to increase at alarming proportions. IR is also at the core of the metabolic syndrome, which involves at least 25% of the population and is epidemic in some subpopulations such as African Americans. CD36, the FA receptor that was studied in this project, has been linked to the metabolic syndrome in various populations and our findings indicate that its connection to disease might involve its direct impact on insulin signaling pathway. In rodents, abnormal CD36 function contributes to IR and in humans, variants in the CD36 gene associate with the metabolic syndrome and with the susceptibility to coronary artery heart disease. Our findings provide a novel insight into the direct role of CD36 in hepatic FA sensing and insulin responsiveness and further promote our understanding of NAFLD pathogenesis.

As the second, clinical part of our project progresses, we aim to characterize sEVs from a larger cohort of human participants, to gain clinically important new insights into the causes of metabolic diseases associated with NAFLD. This pilot study helped us to establish the methodological framework that allows us now to tackle the link between NAFLD and pathophysiological changes in the content of circulating sEVs in humans. A better understanding of the factors underlying the link between NAFLD and metabolic disease of obesity could aid in the identification of novel biomarkers and clinically relevant therapeutic targets. The generous support by LLF has been instrumental to the success of both parts of this project and allowed us to generate findings that support our pending and future grant application and research papers.

List of resulting publications:

1. Samovski D, Abumrad NA. Regulation of lipophagy in NAFLD by cellular metabolism and CD36. *J Lipid Res.* 2019;60(4):755-7. Epub 2019/03/02. doi: 10.1194/jlr.C093674. PubMed PMID: 30819696; PubMed Central PMCID: PMC6446712.
2. Son NH, Basu D, Samovski D, Pietka TA, Peche VS, Willecke F, Fang X, Yu SQ, Scerbo D, Chang HR, Sun F, Bagdasarov S, Drosatos K, Yeh ST, Mullick AE, Shoghi KI, Gumaste N, Kim K, Huggins LA, Lhaxhang T, Abumrad NA, Goldberg IJ. Endothelial cell CD36 optimizes tissue fatty acid uptake. *J Clin Invest.* 2018;128(10):4329-42. Epub 2018/07/27. doi: 10.1172/JCI99315. PubMed PMID: 30047927; PubMed Central PMCID: PMC6159965.
3. Samovski D, Dhule P, Pietka T, Jacome-Sosa M, Penrose E, Son NH, Flynn CR, Shoghi KI, Hyrc KL, Goldberg IJ, Gamazon ER, Abumrad NA. Regulation of Insulin Receptor Pathway and Glucose Metabolism by CD36 Signaling. *Diabetes.* 2018;67(7):1272-84. Epub 2018/05/12. doi: 10.2337/db17-1226. PubMed PMID: 29748289; PubMed Central PMCID: PMC6014550.

Current funding status: Centene F19-00559: "Adipose tissue-Derived Exosomes and Metabolic Health in Obesity" 04/01/19-03/31/21 \$100,000; Pending: NIH R01 DK127273: "Effect of small extracellular vesicles from adipose tissue on insulin action" 09/01/2020-08/31/2025 \$328,584.