Defining human hepatic insulin resistance

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Background

Efficient fuel selection between glucose and fatty acids in insulin-sensitive organs is a key feature which determines metabolic health in humans. The utilisation of glucose and fatty acids, and their partitioning into intracellular storage pools, such as lipid droplets (LDs), are processes that require tight regulation. The relationship between intracellular triglyceride (TG) droplet storage and insulin sensitivity has been resolved for skeletal muscle; athletic and insulin- sensitive individuals store large TG droplets near mitochondria, whereas similar quantities of TG are stored in distant droplets in insulin-resistant states[(1)]. In this project we want to explore the paradigm of LD size and location within hepatocytes and their relationship to hepatic insulin sensitivity.

Hallmarks of hepatic insulin resistance are increased hepatic glucose output, which leads to an elevation in circulating blood glucose concentrations and increased intrahepatocellular synthesis of new fatty acids from non-lipid precursors (*de novo* lipogenesis (DNL))[(2)], which has been suggested, although not demonstrated in humans, to increase intrahepatocellular TG content. The end product of DNL, palmitoyl-CoA, is also a precursor for ceramide synthesis and intrahepatic ceramides have been associated with liver fat and insulin resistance[(3)]; the relationship between hepatic DNL and ceramide synthesis and content is poorly understood.

Taken together it remains unclear if the intrahepatocellular TG accumulation, which often accompanies hepatic insulin resistance, is a cause or consequence. Specifically, the importance of intracellular LD size and location, DNL, and ceramide synthesis and content, in the development of hepatic insulin resistance remain to be elucidated. Understanding the mechanisms that regulate and impact on hepatic glucose output and fatty acid synthesis will aid in the development of new pharmacological targets/agents for treatment of metabolic disease such as type 2 diabetes and cardiovascular disease.

Hypothesis

The development of hepatic insulin resistance relates to the size and location of intrahepatocellular lipid droplets and the *de novo* synthesis of ceramides, rather than the total amount of TG and the *de novo* synthesis of fatty acids *per se*.

Aims of Project

1) Determine the effect of LD size and location on the regulation of hepatic glucose output.
2) Examine associations between DNL or ceramide synthesis and LD size and location, and glucose output.

3) Explore the translation of *in vitro* cellular findings to *in vivo* human studies.

Description of work

The proposed project will utilise human *in vitro* and *in vivo* models.

***In vitro* approach:** We have developed a physiological *in vitro* model using human hepatocytes that recapitulates the amount of intracellular lipid accumulation seen in human non-alcoholic fatty liver disease (NAFLD). We will further develop this model by manipulation of substrates (fatty acids, sugars and insulin) to create human hepatocyte cell culture models (2D and 3D) of low and high hepatic steatosis. Using these models we will assess cell and lipid droplet morphology and lipid droplet size and location. Using stable-isotope tracer methodologies, fatty acid and ceramide synthesis, along with glucose and TG output will also be determined. Intracellular ceramide composition will be evaluated and molecular phenotype investigated. Nanoscale secondary ion mass spectrometry (NanoSims) and stable-isotope tracer methodologies, will be utilised to determine the influence of fatty acids (exogenous and *de novo*) on the morphology and location of LDs.

***In vivo* approach:** Participants will be recruited from the Oxford BioBank, a 9,000 participant strong cohort, with detailed metabolic and genomic characterisation. Study participants, and appropriate control subjects, will be selected using relevant phenotypic (e.g. high and low liver fat content) and/or genotypic (e.g. mutations in the DNL/ceramide pathway) information. Participants will be studied using stable-isotope tracer methodologies to characterise differences between the groups.

References:

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Supervisor's recent relevant publications:

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