**AN *IN VITRO* MODEL OF HEPATIC STEATOSIS TO STUDY ISCHAEMIA- REPERFUSION INJURY**

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**Lay Abstract**

There are numerous limitations to liver transplantation including the scarcity of donor livers and a rise in livers that are unsuitable to transplant, such as fatty livers. Due to the obesity epidemic, many potential donor livers will have excess fat. Fatty livers tend to fail or are susceptible to complications after transplantation. We are studying a form of cell death called necroptosis, which is particularly important during liver transplant when there is an interruption to the blood supply during surgery. Using a cell culture model, we hope to examine this cell death and try to inhibit it. We hope to develop markers of injury that can be used to determine which livers might be suitable for transplantation and more importantly to predict those that may fail during transplantation. Our studies will help to improve the numbers of successful liver transplants and reduce deaths on the liver transplant waiting list.

**Scientific Abstract**

Steatotic donor livers are at a high risk of graft non-function due to susceptibility to ischaemia-reperfusion (I/R) during transplant. Necroptosis is associated with I/R injury and receptor-interacting protein kinase 3 (RIPK3) is instrumental in execution of necroptosis. We developed an *in-vitro* hepatic steatosis model undergoing I/R injury to study necroptosis during I/R injury in steatosis hepatic liver.

Methods: Hepatic steatosis was induced in AML-12 cells by culturing in 2mM free-fatty acid (FFA) for 24 hours and further incubated in oxygen-glucose deprivation (OGD) conditions (1% O2, 5% CO2, and 94% N2) for 12 hours to mimic ischaemia.

Results: Hypoxia-sensitive genes *Slc2a1* and *Vegf* were upregulated in OGD cells (2.69-fold and 2.55-fold respectively). RIPK3 protein was increased in OGD treated cells compared to control FFA treated cells (2.4-fold; p=0.01). Cell viability was reduced after 12 hours of OGD. Lack of cleaved-CASPASE3 expression indicated apoptosis was not an active pathway in our model. *NF-κB,* a regulator of the DNA damage repair system and *Pgam5*, a gene that protects cells from necroptosis decreased in OGD cells (4.55-fold and 1.5-fold respectively).

Conclusion: Our findings suggest that necroptosis may contribute to I/R injury in our model. Our model may be used for further investigation of necroptosis inhibition as a potential therapeutic option for I/R injury.