

# SYSTEMATIC CHARACTERISATION OF *IN VITRO* HEPATOCYTE MODELS FOR STUDYING NON-ALCOHOLIC FATTY LIVER DISEASE

Elaina Maldonado, Ruth Passmore, Ciarán Fisher and J Bernadette Moore

School of Biosciences and Medicine, University of Surrey, Guildford, Surrey GU2 7XH

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is primarily linked with the metabolic syndrome, but the underlying molecular mechanisms and impact on regulatory signalling pathways are poorly understood. As hepatocytes are the predominate cell type in the liver, immortalised hepatocytes treated with fatty acids *in vitro* are models routinely used for studying NAFLD. However, the cell lines, culture media, treatment conditions and monitored endpoints vary in the published literature. Here, we systematically characterised the response of two human hepatocyte cell lines to treatment with saturated (palmitic acid, PA) and mono-unsaturated (oleic acid, OA) fatty acids under physiological or high glucose conditions.

## EXPERIMENTAL DESIGN

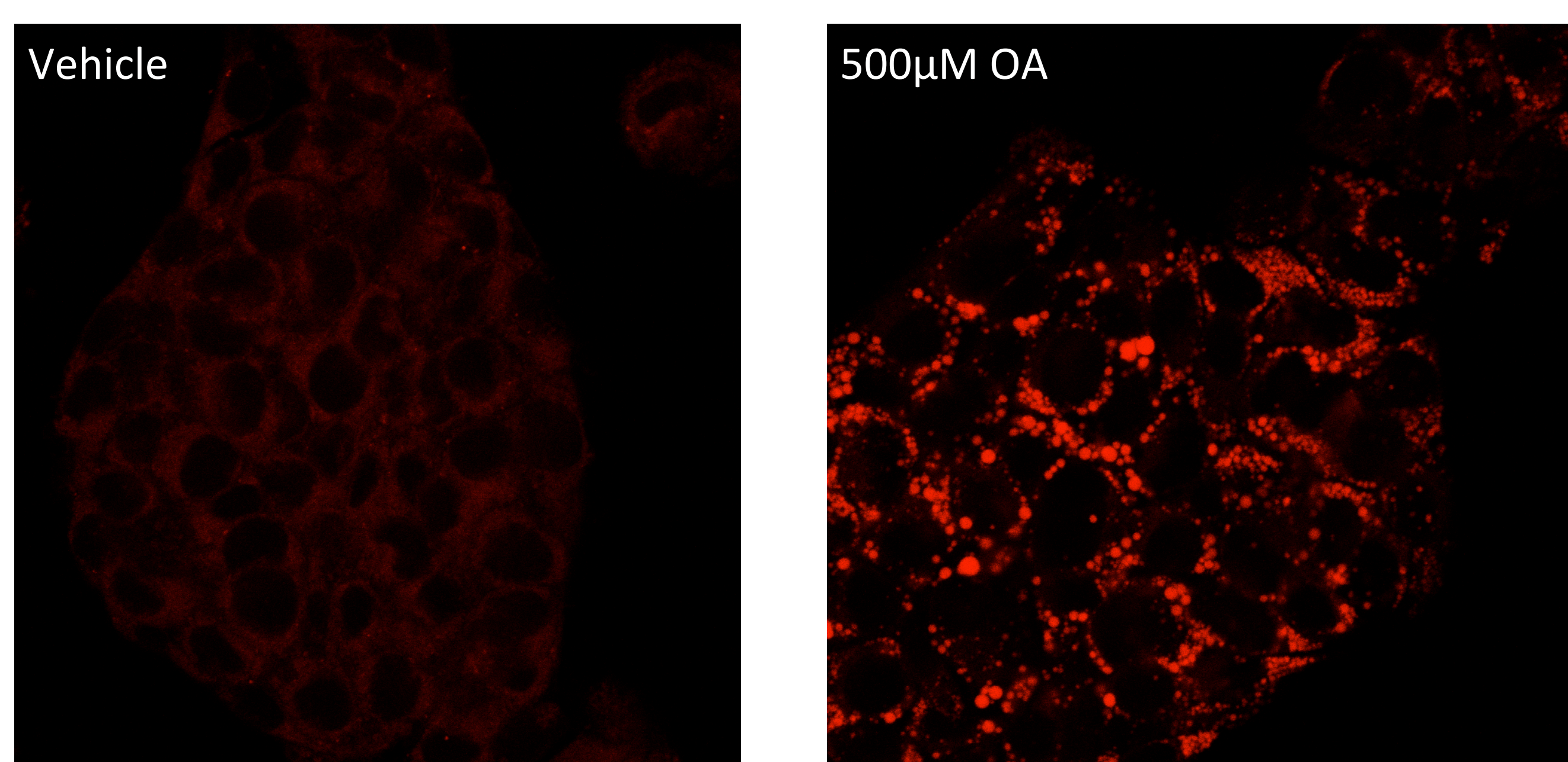
HepG2 and HuH7 cells were cultured in Dulbecco's modified Eagle's media containing low [5mM] or high [25mM] glucose and 10% foetal bovine serum. Cells were then treated with physiological serum levels of PA or OA [0-500µM] in serum-free media for 24 hours.



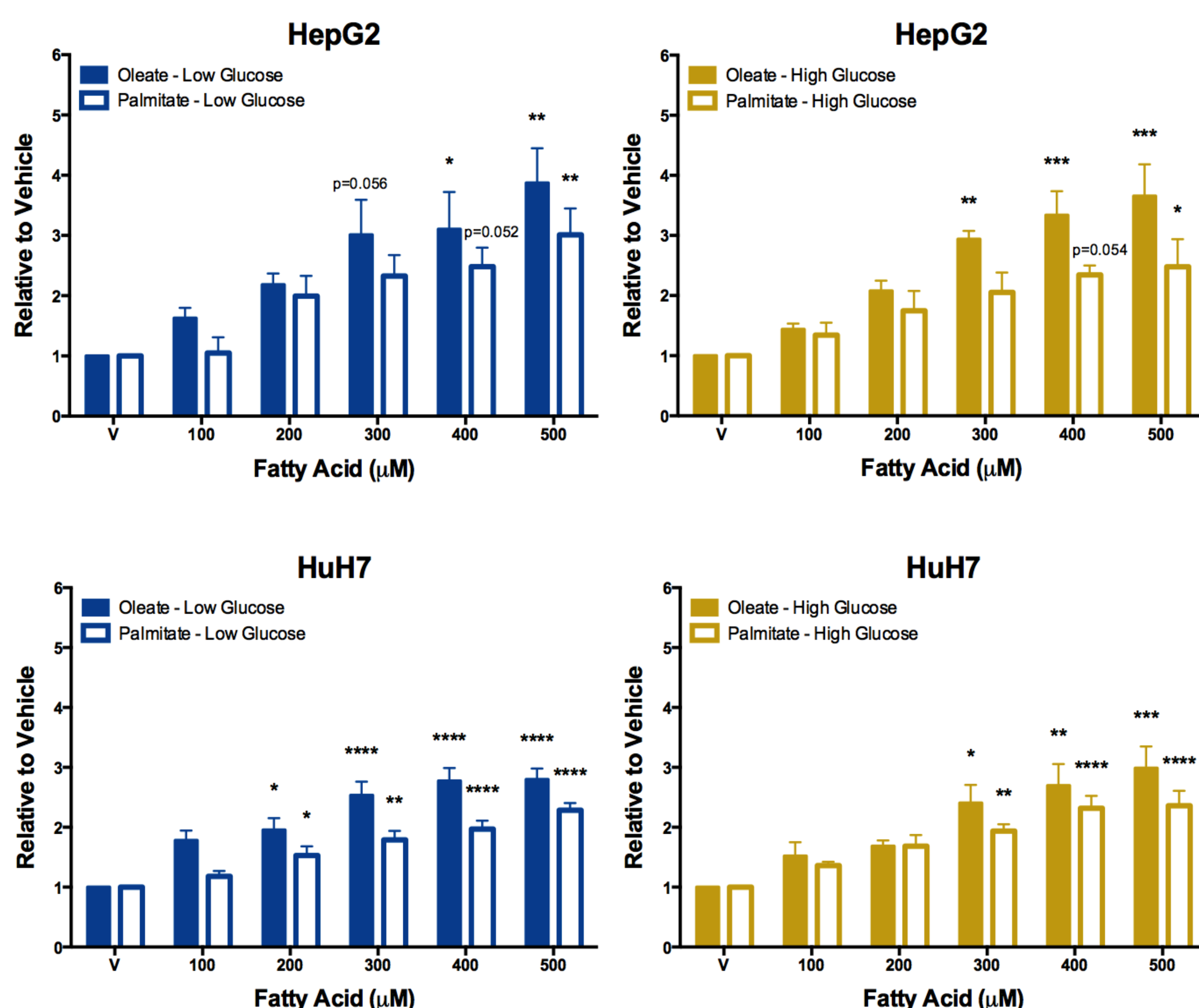
## INTRACELLULAR LIPID

LipidTOX™ staining and confocal microscopy was used to visualise lipid accumulation in HepG2 cells. Nile Red staining was used to quantify intracellular lipid in both HepG2 and HuH7 cells.

### LipidTOX



### Nile Red

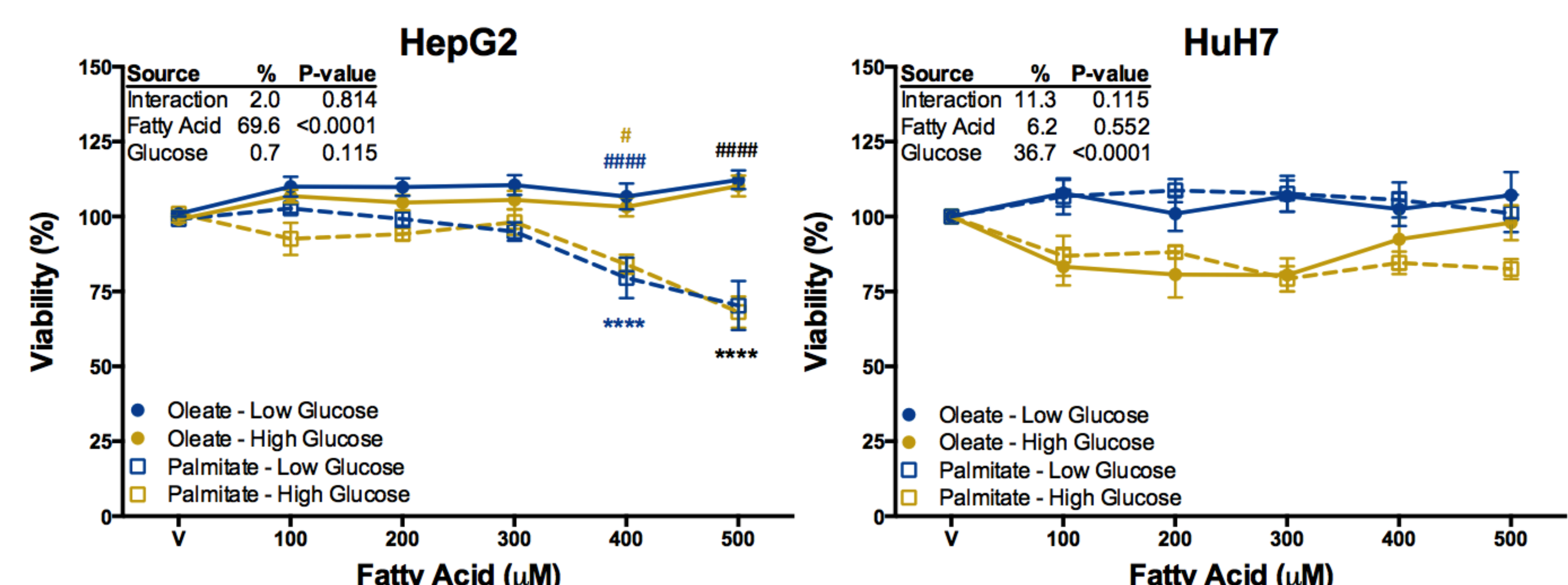


Values are expressed as mean  $\pm$  SEM; HepG2 (n=3), PA-treated HuH7 (n=6), OA-treated HuH7 (n=5). One-way ANOVA with Tukey's test post hoc. \*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*  $P<0.001$ , \*\*\*\*  $P<0.0001$  compared to vehicle.

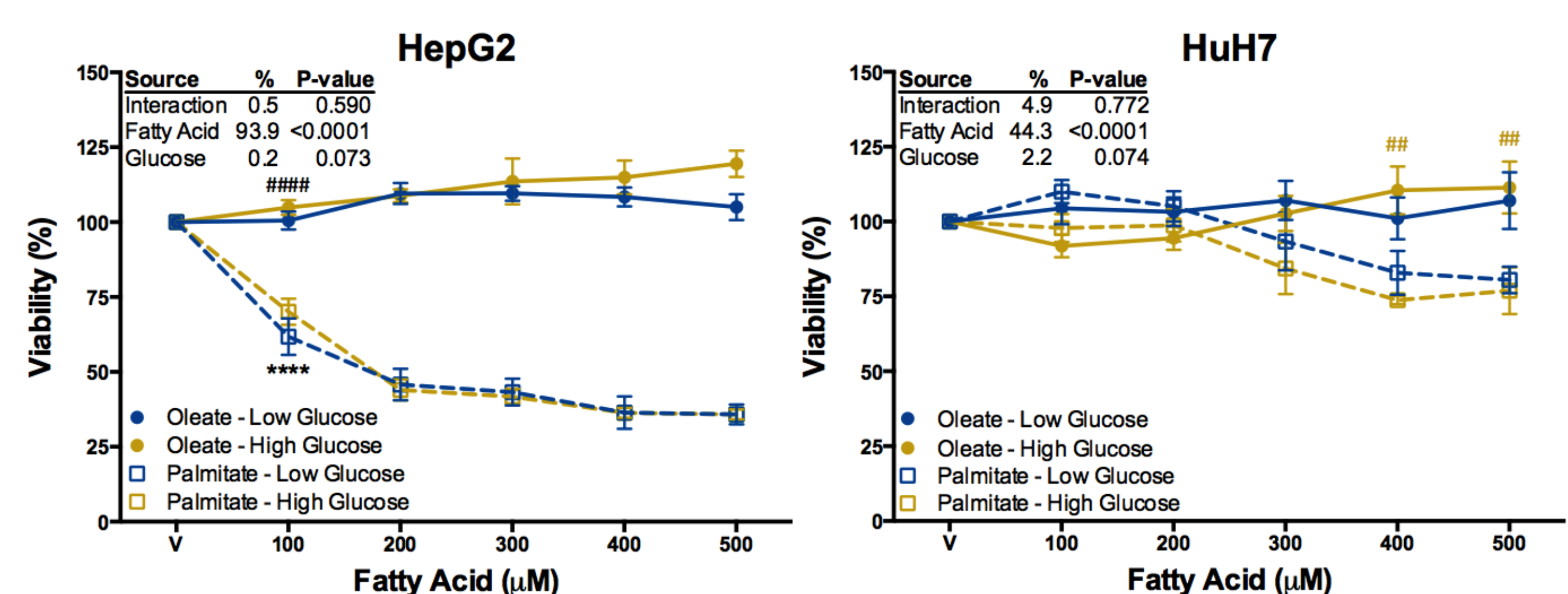
## CELL VIABILITY

Lactate dehydrogenase (LDH) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays were both performed to assess cell viability in both HepG2 and HuH7 cells.

### LDH



### MTT



Values are expressed relative to vehicle as mean  $\pm$  SEM; HepG2 (n=5), HuH7 (n=4). Two-way ANOVA with Tukey's test post hoc. \*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*  $P<0.001$ , \*\*\*\*  $P<0.0001$  compared to vehicle; #  $P<0.05$ , ##  $P<0.01$ , ###  $P<0.001$ , ####  $P<0.0001$  between OA and PA in blue (low glucose), yellow (high glucose) or black (both).

## CONCLUSION

- The magnitude of intracellular lipid accumulation varied between cell lines in response to fatty acid treatment.
- The fatty acid effect on cell viability was dramatically different between cell lines, with results also influenced by assay.
- Glucose had no effect on the response to fatty acid treatment in either cell line.
- These models of hepatocyte lipid loading will be used to facilitate an iterative systems biology approach of *in silico* model reconstruction, hypothesis generation and experimentation.