**TITLE:** Fluctuating hyperglycaemia has the same effect as sustained hyperglycaemia in an in vitro model of diabetic retinopathy

**DESIGN OF STUDY:**

Laboratory based optimisation study using human retinal endothelial cells (hREC).

**PURPOSE:**

To investigate advances in treatment of DR, in vitro studies should be completed to evaluate efficacy of proposed interventions. To achieve this, a cell culture model mirroring the cellular changes seen in DR would be useful. We have previously developed a model using sustained hyperglycaemia and 2% oxygen conditions to mimic DR using hREC, that has been found to effectively induce the oxidative stress and cell barrier changes found in DR. To further optimise the existing model of DR, this study investigated whether fluctuating hyperglycaemia was more effective at inducing the pathological changes found in DR than sustained hyperglycaemia.

**METHODS:**

hREC were cultured under 2% and 20% oxygen and either sustained healthy glucose (5.5mM), a sustained hyperglycaemia (33mM), or fluctuations between these two values with 33mM administered during the day and 5.5mM administered to cells overnight. Cells were kept in experimental conditions for 72H after which analysis of metabolic activity, immunofluorescence antibody staining of angiogenic and oxidative stress response and tight junction-associated protein expression, cell counts and senescence associated ß-galactosidase (SAß-G) staining were undertaken. Transendothelial electrical resistance (TEER) measurements were assessed as an indicator of cell membrane integrity.

**RESULTS:**

It was observed that hREC cultured under 2% oxygen and treated with fluctuating hyperglycaemia demonstrated increased presence of oxidative stress markers (Ang2, SOD1, SOD2, HIF-1α and VEGF-R2) in comparison to their sustained hyperglycaemia counterparts. However, no significant differences in metabolic activity and cell count within the 72H timeframe suggest this did not lead to hREC loss. Cell-cell membrane integrity was maintained across all experimental conditions, with no significant differences in TEER measurements or immunofluorescence VE-cad, CD31, Cx43 or ZO-1 expression.

**CONCLUSIONS:**

Based on this study, the current conditions of sustained hyperglycaemia and 2% oxygen can continue to be used to model DR in hREC. However, as DR is the sequelae of a chronic disease, 72H may not be a long enough time frame to induce changes. This study should be repeated with a longer culture period.

WC: 332/350 (excluding title)