Lay Summary

Our research group is interested in the role of astrocytes in Alzheimer's disease (AD). Astrocytes, the most abundant cell type in the brain, are responsible for a number of essential functions and play a key role in restoring the brain microenvironment in neurodegenerative disease. We have found previously that signalling through the insulin and insulin-like growth factor (IIS) pathway is decreased in astrocytes as Alzheimer's disease develops. This of interest as reduced insulin signalling in AD is supported by other studies which identify type-2 diabetes as a risk factor for AD.

We are now investigating the impact of reduced insulin/IGF-1 signalling in cultured human astrocytes by creating an insulin-resistant state in these cells. We have developed an interest in a group of proteins called the sirtuin proteins as these proteins are involved in regulating energy metabolism. They are also thought to have a protective effect in AD brain.

This project will address which of the sirtuin proteins are expressed in human astrocytes and will determine changes in their expression in insulin-resistant astrocytes. Understanding the roles of these proteins and their response to insulin resistance will further our understanding of the insulin-sirtuin axis and the potential protective properties of sirtuin proteins in the brain.
**Full Project Proposal**

**Background**

Sirtuins are a conserved family of proteins found in all domains of life. The mammalian sirtuin family, a group of histone deacetylases whose activities are dependent on and regulated by NAD$^+$, suppress genome-wide transcription, yet a select set of proteins related to energy metabolism and pro-survival mechanisms are upregulated. To date seven sirtuins have been identified and they are known as sirtuin 1 (SIRT1) through SIRT7 (Michin and Sinclair, 2007). They share significant sequence homology and a conserved catalytic domain and NAD$^+$-binding domain. SIRT1 is the best characterized of the sirtuin family and is considered to be a determining factor in lifespan. SIRT1 is known to inhibit the pro-apoptotic protein p53, reduce activity of the pro-inflammatory protein NFκB and activates PGC-1α which increases glucose levels, insulin sensitivity and mitochondrial biogenesis (Zhang *et al.*, 2007). Our interest in the sirtuin proteins is linked to its role in the regulation of energy metabolism, specifically in astrocytes.

Astrocytes, the most abundant cell type in the brain, are responsible for a number of complex and essential brain functions which include the production and release of neurotrophic factors to promote neuronal survival; regulation of synaptic activity through regulation of potassium ions and synaptic glutamate balance and regulation of cerebral metabolic trafficking between neurons and intracerebral blood vessels via the opposition of astrocyte endfeet with blood vessels (Sofroniew and Vinters, 2010; Garcia Marin *et al.*, 2007; Steele and Robinson, 2010). In addition astrocytes play a key role in restoring brain homeostasis in brain injuries and neurodegenerative disease (Pekny and Nilsson, 2005; Rodriguez *et al.*, 2009).

Our research group has described previously that astrocytes might contribute to the development of brain aging and the development of Alzheimer-type pathology through loss of, or altered, function (Simpson *et al.*, 2010a; Simpson *et al.*, 2010b) and using microarray analysis we have shown that insulin/IGF-1 signalling pathway is downregulated in astrocytes as Alzheimer’s-type pathology develops in ageing brain (Simpson *et al.*, 2011). Downregulation of this particular pathway is of interest as impaired insulin/IGF-1 signaling in the pathogenesis of Alzheimer’s disease is supported by epidemiological studies identifying type-2 diabetes as a risk factor for the disease (Li and Holsher, 2007; Ristow, 2004). We are now investigating the functional impact of impaired insulin/IGF-1 signalling in cultured human primary astrocytes by inducing an insulin resistant state in these cells.

SIRT1 is reported to be involved in both glucose metabolism and insulin secretion and peripherally, at least, insulin resistance is associated with reduced expression of SIRT1 (Sun *et al.*, 2007). Given that insulin signalling in astrocytes is dysregulated with an increasing burden of Alzheimer’s pathology, and that insulin resistance is evident early on in AD brain (Talbot *et al.*, 2012), it is of interest to determine the relationship of sirtuin protein expression (and potentially activation) to insulin signaling.

Furthermore, there is evidence to suggest that SIRT1, at least, has a neuroprotective role in AD (Zhang *et al.*, 2011). This further highlights the need to determine the relationship between insulin signaling and the sirtuin proteins, particularly in astrocytes, since these cells are crucial in maintaining brain homeostasis and are widely thought to facilitate disease progression. The involvement of other sirtuin proteins is less well defined since they have been less extensively studied, however all are known to be expressed in brain and although expression in specific cell types and function are not well defined, cultured mouse astrocytes are known to express all 7 isoforms with SIRT2, SIRT3 and SIRT7 expressed at the highest levels (Xie *et al.*, 2007).
This project will therefore address which of the sirtuin proteins are expressed in cultured human primary astrocytes and will determine changes in their expression in insulin resistant astrocytes. Understanding the roles of these proteins and their involvement/response to insulin resistance will further our understanding of the insulin-sirtuin axis and the potential neuroprotective properties of sirtuin proteins.

Hypothesis: Sirtuin proteins are downregulated in insulin-resistant human astrocytes and this is associated with impaired astrocyte function.

Research Plan
In this proposed study the expression and cellular localisation of sirtuin proteins will initially be determined under normal culturing conditions. Due to time constraints this research project will investigate the expression of sirtuin proteins 1-4. Subsequently an insulin-resistant state will be induced in these cells using a combined insulin-fructose treatment protocol already optimised for these cells in our laboratory and changes in the expression of sirtuin proteins will be investigated at different time-points (both before and after the cells become insulin-resistant). If time-permits changes in the localisation of these proteins will also be determined using confocal microscopy and the InCell Analyzer. Data generated will be linked to already determined changes in astrocyte phenotype and function in insulin-resistant cells.

Objectives
- Determine the pattern of sirtuin protein expression in cultured human primary astrocytes using immunoblotting and immunofluorescence.
- Induce insulin resistance in human astrocytes in these cells and investigate changes in sirtuin protein expression at different time points.
- Investigate changes in the cellular localisation of sirtuin protein(s) in insulin-resistant astrocytes (time permitting).

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Feasibility:
The proposed experiments are feasible in the 6 week period allowed for this project. The cells are already routinely cultured in the host laboratory and the insulin resistance model has been characterised. The antibodies will need optimising but the project has been designed so that should this take less time than planned then the student will investigate the localisation of these proteins in these cells both under control and insulin-resistant cells.

References:
• Xie et al., SIN-1 causes downregulation of sirtuin expression in mouse astrocytes. Faseb J, 2010, 24, Meeting Abstract.