Project Title: The relationship between Statins, the NLRP3 Inflammasome and Type 2 Diabetes control

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Level: PhD

Background to the project:

Cardiovascular disease (CVD) is a major complication of Type 2 Diabetes Mellitus (T2DM). CVD is treated with lipid-lowering statins and many people with T2DM are therefore receiving statin therapy. Statins increase T2DM risk and poor glycaemic control. In addition, statins also alter beta cell function and increase insulin resistance, suggesting a potential diabetogenic effect for the drug class. Fluvastatin caused peripheral insulin resistance in the adipose tissue of obese mice, which was found to be NLRP3-dependent.

The NLRP3 inflammasome triggers the secretion of IL-1β, which initiates protective inflammatory responses. IL-1β works synergistically with other inflammatory cytokines to promote beta cell apoptosis and diabetes development.

Monocytes derived from newly identified T2DM patients display elevated NLRP3 expression, whilst insulin resistance was positively associated with incident diabetes during rosuvastatin therapy in healthy individuals. A statin-activated NLRP3-dependent mechanism of insulin resistance has been confirmed in animals, and this proposal will investigate this association in humans, which has not yet been established.

We hypothesise that statin regulation of glycaemia is under the influence of NLRP3 and associated regulatory proteins. Therefore, NLRP3 (and associated regulatory protein) expression may be useful to predict the optimal statin prescription for those with, or at risk of, T2DM.

Objectives of the research project:

This studentship will address the clinical need to identify ways of determining the optimal statin prescription for those at risk of developing T2DM, or for those already diagnosed with T2DM.

Statins reduce cholesterol and have been very successful in the management of cardiovascular disease (CVD). Statins also alter the immune response, independent of their low density lipoprotein (LDL) lowering properties. However, recent reports have implicated statins in the risk of developing Type 2 Diabetes Mellitus (T2DM). Furthermore, statins were shown to impair insulin signalling in a mouse model of T2DM via their immunomodulatory effects. The immune altering abilities of statins appear largely dependent on statin type and dose. Some statins exert pro-inflammatory effects, whilst others exert anti-inflammatory effects. The NLRP3 inflammasome has been identified as a key regulator of statin-mediated alterations of immune function. Indeed, the reported impairments in insulin signalling in a mouse model of T2DM are a direct result of NLRP3 activation. Statins have also been associated with poor response to sulphonylureas, a front line therapy for T2DM. Sulphonylureas act by blocking potassium efflux (leading eventually to insulin exocytosis from the beta cell), which is a major mechanism for NLRP3 activation.
The proposed project will retrospectively investigate the relationship between statin usage and diabetes onset and glycaemic control using a large database of pre-collected data, and will then investigate the role of the NLRP3 inflammasome in statin-regulation of diabetes control using in vitro models and clinical samples already available from rolling studies of patients with T2DM and CVD.

Our **aims and objectives** are outlined below:

**Aim 1: Determine the relationship between statin usage, diabetes onset and glycaemic control.**

**Objective 1.1:** To investigate the correlation between statin usage and the onset of T2DM in a large database of patients with T2DM.

**Objective 1.2:** To investigate the effect of specific statin types/doses on glycaemic control in patients with T2DM.

**Objective 1.3:** To investigate the relationship between statin usage and sulphonylurea response in patients with T2DM.

**Aim 2: Determine the role of the NLRP3 inflammasome in statin-regulation of diabetes control.**

**Objective 2.1:** To investigate if NLRP3 plays a role in statin regulation of glycaemic control.

**Objective 2.2:** To explore the mechanism by which NLRP3 mediates statin regulation of glycaemic control and specifically, sulphonylurea response.

**Methods to be used:**

**Aim 1: Determine the relationship between statin usage, diabetes onset and diabetes control.**

The DIAMOND database will be accessed to collect data on patients (n=100,000) with T2DM and this will be validated in other cohorts including the regional warehouse primary care records (http://www.hscbusiness.hscni.net/services/2454.htm) and the clinical studies data request database (https://clinicalstudydatarequest.com). A case-control analysis will be conducted to investigate statin usage and onset of T2DM. Separate analyses will investigate the type, dose and duration of statin usage and their associations with T2DM onset and glycaemic control via multi-group comparisons. Linear regression analysis will assess the dose response for specific statins. To investigate statin effect on sulphonylurea response, multi-group comparative analyses will be carried out on specific sulphonylureas/statin combinations with using HbA1C time to first insulin administration as markers of sulphonylurea response.

**Aim 2: Determine the role of the NLRP3 inflammasome in statin-regulation of diabetes control.**

This pilot clinical observational study will utilize samples from current rolling studies of T2DM (ORECNI number: 14/NI/1123), Cardiovascular Disease (ORECNI 14/NI/0068) and control (ORECNI number 14/NI/0068) cohorts. The cohorts to be used for analysis will comprise T2DM patients using statins (n=70), T2DM patients not using statins (n=70), CVD patients using statins (n=70) and Control participants (n=70). Plasma, serum, whole blood, DNA and RNA have been stored for all subjects. NLRP3 and associated proteins (IL-1β and caspase 1) will be measured using western blotting, ELISA and qPCR. Analyses will explore the relationship between statin type/dose, sulphonylurea use, NLRP3 activation and glycaemic control (HbA1c), via correlation analysis, linear regression, logistic regression and ANOVA. NLRP3 mRNA expression in our CVD cohort is significantly increased compared to controls (p=0.02).

Pancreatic beta cell lines (BRIN-BD11 and MIN6) and THP1 macrophages will be used to investigate the mechanisms of statin induced NLRP3 activation *in vitro*, and to determine if specific statins compete with sulphonylureas resulting in altered insulin secretion. To condition THP1 cell culture media, THP1 macrophages will be cultured in the presence of 25 mM glucose for 48 h. Preliminary work has shown that this upregulates NLRP3 mRNA expression. NLRP3 activation will be measured as described above. ELISA will measure sulphonylurea-induced insulin secretion from beta cell lines. Selected experiments will be confirmed in primary islets isolated from NLRP3 KO mice in collaboration with our collaborator Professor Visha Dixit (GenTech, San Francisco), who has confirmed his support for this project.
Skills required of applicant:

A primary degree in an area cognisant with Biomedical Sciences/ Bioinformatics. Experience of undertaking a laboratory-based research project and/or analyses of large datasets, as well as the desire to learn novel, cutting edge techniques. An ability to solve biological problems, critically analyse data and present the results of research in written and oral form. Evidence of ability to work both independently and as a member of a team. Evidence of good organisational skills. Ability and willingness to travel.

References: