Mechanisms of lipid-induced cell dysfunction in pre-diabetes

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a. Rationale of the study

Lipids are necessary to many complex and various cellular functions ranging from energy providers through β-oxidation, hormonal precursors, protein post-translational modification agents (i.e. palmitoylation) or membrane structural blocks (phospholipids). More recently, with the deorphanization of the free fatty acid receptor (FFAR) family in 2003 [1, 2], **free fatty acids (FFAs) were also identified as signaling molecules**.
Medium- to long-chain fatty acids were shown to target FFAR1 (GPR40) which is expressed in metabolically active tissues, such as pancreatic β-cells potentiating glucose-stimulated insulin secretion (GSIS) [3]. FFAR1 couples to the G-protein subunit Gαq/11 and its signaling cascade activates protein kinase C (PKC) and mobilizes ER Ca^{2+} stores. FFAR1 abrogation protects mice from obesity-induced hyperinsulinemia and glucose intolerance, while its upregulation reduces beta-cell function and insulinemia [3].
FFAs exert dual, time-dependent effects on beta-cell function and viability. The short-term effect is an acute amplification of GSIS from beta-cells [2, 4], while **chronically elevated levels of FFAs promote beta-cell dysfunction** and demise via a process named glucolipotoxicity [5]. The positive effect of lipids on GSIS induced great interest in FFA receptors as therapeutic targets for diabetes. While GPCRs are the most common molecular targets of the modern drug therapy (50% of currently licensed medicines) there are still significant challenges in the delineation of the mechanisms of action of lipids and drugs on these receptors. Many questions are being raised about how GPCR biology can be best exploited for the development of more selective and effective medicines.
Ultimately, **data point to a *ménage à trois* implicating FFAR1, FFA, and glucose**; underlining the importance of the receptor as a regulator for insulin secretion in response to elevated concentrations of glucose and other nutrients such as FFAs.

Diabetes mellitus is the most common metabolic disease and affects more than 350 million people worldwide. The number of diabetic patients is expected to double in the next 15-20 years. **Atherosclerosis is accelerated by diabetes and the metabolic syndrome** and is the cause of a majority of cardiovascular failure, a primary cause of death in diabetic patients. Similarly to beta-cell research, the determination of the chronic effects of FFAs on cardiovascular events according the chemical nature of the lipids, their intracellular metabolism, the time of exposure and the relationship with (hyper)glycemia needs to be investigated in joint studies. Thus, plasma lipidomic measures may enable improved prediction of cardiovascular outcomes in secondary prevention [6].

In the context of obesity associated type 2 diabetes (T2D), glucose and lipids coming from an overload of nutrients and from the release from adipose tissues are chronically present in the blood stream, dramatically affecting β-cell function [7-9]. These effects, referred to as glucolipotoxicity were mainly described for the saturated fatty acid palmitate and involve several mechanisms such as ceramide production and ER stress [10-12]. However, not much is known regarding the chronic effects of other types of FFAs on β-cell metabolism and function. We recently showed that lipotoxic effects were very much dependent on the chemical nature of the lipid itself rather than the lipid class, affecting different steps of the insulin secretion pathway (unpublished data). Moreover, these specific lipotoxic effects were highlighted only in the presence of high chronic glucose: 1) palmitate triggered apoptosis while oleate protected against glucose-induced β-cell death, 2) both palmitate and oleate affected mitochondrial coupling while polyunsaturated fatty acids linoleate and linolenate had no major effects. Interestingly, **under glucotoxic conditions, insulin secretion was preserved with all fatty acids pointing out to alternative mechanisms to the classical pathway of GSIS**.

The chronic exposure of cells to fatty acids also **promotes fat depots in the cytosol under the form of triglycerides**. We recently demonstrated that lipids specifically accumulated according to their chemical nature and that they were also rapidly and differentially mobilized by the cells (unpublished data). Inhibition of lipolysis by orlistat in these lipid-exposed cells showed major impairment of GSIS, thereby stressing the importance of triglyceride mobilization for a sustained β-cell function. To date, the mechanisms by which these mobilized lipids impact insulin secretion remain elusive. The intermediaries coming from triglycerides breakdown through the glycerolipid/FFA cycle such as mono- or di-acyl glycerol (MAG and DAG) have been proposed as intracellular signalling effectors in the potentiation of GSIS by fatty acids [13]. Other possible mechanisms might be the direct action of released FFAs on FFAR1 or remodelling of membranes structure that could facilitate or prevent insulin granules fusion with the cell membrane. Some deep cellular modification such as gene expression modulation by chronic fatty acids may also be involved in the effects of chronic fatty acids on GSIS.

b. Global aims:

In previous work, we identified molecular targets involved in lipid metabolism of the main metabolic stresses tested side-by-side on INS-1E beta-cells [14] and human islets [8, 15]. These stresses included low and high glucose concentrations, saturated and unsaturated fatty acids, and transient oxidative stress. The data have shown that diabetogenic conditions repress FFAR1 gene expression in the insulin-secreting INS-1E cells, while inducing cell death [14]. The next question would be: **can we prevent beta-cell impairment by modulating FFAR1 activity using ligands in a context of glucolipotoxicity**?

We will characterize expression of FFAR1 in healthy and unhealthy conditions (in particular glucolipotoxicity) in order to validate FFAR1 ligands in INS-1E beta-cells and human pancreatic islets. To study the function of cells in the presence of various FFAs and hit FFAR1 compounds, we will apply semi high throughput readouts underlying events of the signaling cascade, leading to GSIS according to well-established protocols [14, 16, 17]. We will measure insulin secretion in naïve INS-1E beta-cells [18] and modified INS-1E beta-cells [19] by luminescence-based secretion assay in order to quantify glucose-responses in cells exposed or not to FFAs and concomitantly treated with FFAR1 ligands. We will test a) the natural ligands: linoleic acid, linolenic acid, arachidonic acid, palmitic acid, DHA, oleic acid b) the agonists : TAK-875, AMG 837, GW9508 c) the antagonist: GW1100.

We will also investigate the mechanisms by which chronic lipids preserve insulin secretion under glucotoxic conditions. The role of the glycerolipid/FFA cycle will be emphasized as well as the putative mechanistic differences that may be observed with the nature of the fatty acid chronically applied to the cell environment.

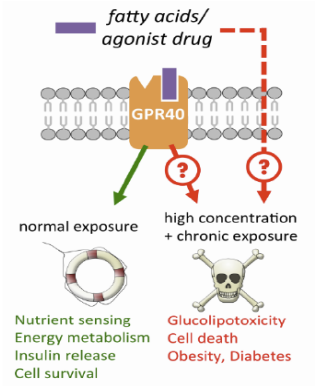
c. Experimental design

The models: Naïve or modified INS-1E beta-cells or human islets will be cultured for 3-6 days in regular media at different glucose concentrations (low, standard, high) in the presence or absence of FFAs complexed to BSA in order to control the free fraction of FFAs as detailed previously [8, 14, 20]. At the end of this chronic exposure to diabetogenic milieus, expression of FFAR1 will be assessed by qRT-PCR, cell function will be tested by

measuring secretory responses to glucose, viability will be determined by MTT and cleaved-CASPASE 3 immunoblotting. Intracellular lipid mobilization will be assessed using the Bodipy probe. As the phenotypic assessment is already established (unpublished results), the same procedures and measurements will be conducted on cells cultured in the presence of FFAR1 ligands.

Insulin secretion assay: We will quantify GSIS from the cells in the presence or absence of chronic FFAs load by a newly developed luminescence-based assay. In brief, modified INS-1E ß-cells expressing a Gaussia luciferase in place of the insulin c-peptide [19] will be used to assess kinetics of insulin secretion after the chronic lipids/agonists exposure. Luminescence will be monitored for 40 min using a thermostated plate-reader (Fluostar) as the cells will be exposed to G2.5, G8.3 and G15, followed by the addition of KCl (40 mM final). These assays will validate the effect of the compounds in potentiating and improving beta-cell function.

d. Importance of the proposal:



About a decade ago, FFAR1 agonists have attracted much attention for potential use in diabetes management, mainly through identification of TAK-875 and the associated milestone publication [2]. Although very promising, the development of TAK-875 was terminated due to liver toxicity issues, leaving the field poorly investigated. In this study, we will revisit lipid signalling and FFAR1 function in beta-cells through the potential implication of these FFA receptors in the glucolipotoxicity response. Indeed, the beneficial or detrimental contribution of chronic exposure to FFA on GSIS remains unclear. Modulating FFAR1 activity, positively or negatively, might help preserving beta-cell function and survival when exposed to diabetogenic conditions.

e. Facilities available:

In the Department of Cell Physiology and Metabolism in the Geneva University Medical Center building, the group of Prof. Pierre Maechler is allocated with three laboratory units (46 + 18 + 25 m2) plus attached offices (12 + 23m2), as well as free access to shared facilities. The Department is completely equipped with modern and well maintained materials and machines (cell culture facility, P2 laboratory, centrifuges, counters, shared confocal microscope, animal facilities, perfusion setups, spectro- and fluoremeter, etc). All this equipment is accessible for the applicant (Lucie Oberhauser). The University Medical Center benefits from a high quality environment and equipment with modern core facilities, including transgenesis, bioimaging, genomic, proteomic, phenotyping of the small animal and others (www.medecine.unige.ch/organisation/services/index.php).

f. Budget

Salary: One PhD student (Lucie Oberhauser) for 3 months. The applicant started her thesis in March 2015. The present application is intended to cover 3 months of support for her project partially supported by CTI grant and by Fondation Romande du Diabète grant. Practically, the present budget would cover part of the salary over the final period of the thesis project.

Sub-total: 15’292,75.- CHF (including the 20% social charges)

Supplies:
Cell culture: 4’500
Biochemical reagents and consumables: 7’500
Antibodies for immunoblotting: 2’500

Sub-total: 14’500.- CHF

Grand total: 29’792.75.- CHF

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