Imperial College London

Interrogating pancreatic islet alpha cell calcium dynamics

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This research project is submitted in partial fulfilment of the requirements for the degree of MRes in Clinical Research (Diabetes and Obesity).

Agreed project contributions of supervisor and student to MRes project

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Contribution to:	By Student (in %)	By Supervisor (in %)
Overall project design*	50	50
Determination of Methodology*	0	100
Collection of specimens/material/patient recruitment	80	20
Conducting experiments/ collation of questionnaires etc	100	0
Data analysis	90	10
Write up	100	0
Production of submission	100	0
Problems encountered if any		

Any other comments: Dhruti is an outstanding clinician scientist in the making. She had no lab experience prior to this MRes and took on an extremely demanding project with confidence and precision. She has become independently able to use cutting edge microscopy to extract novel calcium data from pancreatic islets. She has also learned how to image process and produce meaningful statistical analysis. Remarkable learning curve and a particularly strong understanding of the associated literature – which was very new to her given her clinical background.

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MRes Clinical Research

Diabetes and Obesity (2021-22)

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Abstract

Introduction

Type 2 diabetes (T2DM) is the result of relative insulin deficiency and glucagon excess. Whilst insulin-secreting beta cells are well-researched, their neighbouring glucagon-secreting alpha cells are much less poorly understood, particularly how alpha cell calcium dynamics are linked to hormone secretion. This project establishes the use of islets from a novel, transgenic mouse model, PPG-GCaMP6f, to assess alpha cell calcium activity. Understanding how normal alpha cell activity is modulated by glucose and current therapies such as GLP-1 agonists will improve our approach to managing T2DM.

Methods

PPG-GCaMP6f mice express a fluorescent calcium indicator exclusively in alpha cells. Intact islets from these mice were imaged using spinning disk confocal microscopy to delineate alpha cell calcium fluxes over time, at 3mM (G3) and 11mM (G11) glucose concentrations, and following the addition of GLP-1 agonists, exendin-phe (ex-phe) and exendin-asp (ex-asp). Beta cell perifusion experiments previously conducted on islets from INScre-GCaMP6f mice (which express the fluorophore in beta cells only) at 2mM and 12mM glucose, were used for comparison. Calcium peaks were analysed for oscillatory activity, amplitude, frequency, and connectivity.

Results

22/191 (11.5%) of all alpha cells over 4 mice studied were discernibly active, although all demonstrated a positive response to KCl. Comparing G3 to G11, there were no significant differences in the proportion of active alpha cells (7.33% vs 5.76%; p=0.68), in median (IQR) oscillation frequency (0.7/min (0.20-1.20) vs 1/min (0.33-2.70); Mann Whitney U=53; p=0.19), and in median (IQR) peak amplitude (0.21 (0.16-0.25) vs 0.24 (0.18-0.31); Mann Whitney U=796; p=0.11). There was no evidence of overall alpha cell connectivity (R=0.45).

234/244 (95.9%) of studied beta cells were oscillatory at 12mM (G12) and none were active at 2mM (G2) concentrations (p<0.001). The average (±SD) wave frequency at G12 was 0.88/min (±0.58), with an average amplitude of 0.34units (±0.09).

In these experiments, 4/57 (7.01%) alpha cells were oscillatory at G11 in GLP-1 experiments, compared to none at G3. Compared to vehicle, biased GLP-1 agonists were not found to cause significant changes in mean peak frequency (Veh: 0.85/min (0.45) vs ex-phe: 1.8/min

vs ex-asp 0.5/min (0.10); p=0.33) or mean peak amplitude (Veh: 0.22units (0.04) vs ex-phe: 0.17units vs ex-asp: 0.20units (0.02); p=0.30).

Conclusion

PPG-GCaMP6f mouse models can be used to measure alpha cell calcium activity. This study found alpha cells to be active at both high and low glucose concentrations and provided early data to show GLP-1 agonists may directly modulate alpha cell calcium activity. Further work is needed to understand the mechanisms and influencers of glucagon secretion, which will better inform therapeutic developments.

Keywords: alpha cell, calcium, GLP-1 agonists, biased agonism, connectivity

List of Abbreviations

BMI	Body Mass Index (kg/m^2)
BSA	Bovine Serum Albumin
cAMP	Cyclic adenosine monophosphate
Ex-phe	Exendin-phe1
Ex-asp	Exendin-asp3
G2	Glucose concentration of 2mM
G3	Glucose concentration of 3mM
G11	Glucose concentration of 11mM
G12	Glucose concentration of 12mM
GIP	Gastric Inhibitory Polypeptide
GLP-1	Glucagon like peptide-1
GLP-1R	Glucagon like peptide-1 receptor
GPCR	G-protein coupled receptor
GSIS	Glucose stimulated insulin secretion
IGT	Impaired glucose tolerance
INS	Insulin gene
KCL	Potassium chloride
KRBH	Krebs-Ringer-Bicarbonate HEPES buffer
mRNA	Messenger ribonucleic acid
NHS	National Health Service
PKA	Protein kinase A
PPG	Preproglucagon
ROI	Regions of interest
T2DM	Type 2 diabetes mellitus
UK	United Kingdom
VGCC	Voltage-gated calcium channel
VGSC	Voltage-gated sodium channel

Declaration of Contributors

Management of PPG-GCaMP6f mice and islet harvesting were kindly performed by Professor Kevin Murphy's laboratory. Beta cell raw data was available from prior experiments in the Salem laboratory performed by KS. DH conducted all alpha cell experiments and Prism analyses, with assistance from BH for connectivity analysis. The thesis was written by DH with input from VS, KS and AT.

Introduction

Diabetes mellitus

Diabetes mellitus is a chronic, progressive metabolic condition characterised by unmatched insulin demands, resulting in persistent hyperglycaemia. Type 2 diabetes (T2DM) accounts for approximately 90% of all diabetes cases (Diabetes UK, 2022), and is generally considered to result from relative insulin deficiency and insulin resistance. Intensive management is essential to reduce the risk of diabetes-related micro- and macro-vascular complications, which are associated with significant morbidity and mortality rates (International Diabetes Federation, 2021a; National Institute for Health and Care Excellence, 2022b).

The global health burden of diabetes

In 2021, over 530 million adults worldwide were living with diabetes mellitus (International Diabetes Federation, 2022), nearly a 400% increase since 1980 (World Health Organisation, 2021). Diabetes was the ninth commonest cause of global death in 2019 (World Health Organisation, 2020), and is estimated to reduce overall life expectancy by up to 20 years (Leung et al., 2015; Wang and Liu, 2016). Projections suggest global prevalence will reach 643 million in the next 8 years, with low- and middle-income countries being most affected (International Diabetes Federation, 2021b).

The financial implications of this surge in diabetes prevalence are immense. In 2017, the National Health Service (NHS) spent 10% of its £140 billion budget on diabetes (The King's Fund, 2022), of which 80% was spent on diabetes-associated complications (Diabetes UK, 2017) and a further £1 billion on prescriptions for diabetes treatments (BBC Health, 2018). Total spending on diabetes in the UK is predicted to reach £39.8 billion in 2035, with £20.5 billion being projected for indirect costs such as increased social care demands and reduced workforce productivity (Hex *et al.*, 2012).

Rising diabetes rates, particularly T2DM, have been strongly driven by a parallel obesity epidemic. Increased availability and affordability of ultra-processed, low-nutrient food, combined with an increased sedentary lifestyle has created an obesogenic environment (Malik, Willett and Hu, 2013). Since 1975, worldwide obesity rates have tripled and obese individuals are 7-times more likely to develop diabetes (Abdullah *et al.*, 2010), making body weight one of the major (reversible) risk factors for T2DM.

Pathophysiology of type 2 diabetes

A normal homeostatic response to changes in blood glucose requires a functioning negative feedback loop between healthy, insulin-secreting pancreatic beta cells and insulin-sensitive tissues (particularly the liver, skeletal muscle and adipose tissue) (DeFronzo, 1988). Normal beta cells can respond to changes in insulin resistance, matching increases in peripheral resistance with a proportional increase in insulin output to maintain constant blood glucose levels (Bergman, Phillips and Cobelli, 1981; Kahn *et al.*, 1993). Environmental insults, such as obesity, encourage progression towards impaired glucose tolerance by disturbing the balance between insulin demand and insulin production. Beta cells are pushed to secrete more insulin to offset a given level of peripheral insulin resistance and do so by increasing beta cell mass and/or secretory output. Eventually, beta cells that are already secreting at their maximal level struggle to meet demands due to the presence of at least one of: beta cell dysfunction, reduced beta cell mass and high insulin resistance. The resultant chronic hyperglycaemia augments beta cell failure due to its direct glucotoxic effects. Combined with the already reduced peripheral effectiveness of insulin (particularly its inability to suppress lipolysis and hepatic endogenous glucose production), the growing relative insulin deficit results in hyperglycaemia of T2DM thresholds (Unger and Grundy, 1985; DeFronzo, Ferrannini and Simonson, 1989; Stumvoll, Goldstein and van Haeften, 2005).

The tendency to develop T2DM depends on the ability of an individual's beta cells to compensate for any level of insulin resistance; those who struggle to increase beta cell output go on to develop T2DM. Family history of T2DM is a significant risk factor, with studies consistently demonstrating strong heritability of beta cell function (Pimenta, 1995; Sakul *et al.*, 1997; Elbein *et al.*, 1999). Elbein *et al* studied normoglycaemic, obese individuals with a positive family history of T2DM and found that they had significantly reduced beta cell response to insulin resistance compared to obese individuals without a family history of T2DM (Elbein, Wegner and Kahn, 2000). These findings suggest that the adaptability of an individual's beta cells may be genetically determined. The rise of sequencing technology has enabled allele variants to be identified by whole genome sequencing, demonstrating the polygenic nature of T2DM, with commonly implicated genes including those involved with regulating insulin secretion (KCNJ11 and TCF7L2) (Fuchsberger *et al.*, 2016). Even within the T2DM population, variation in patient characteristics, disease progression and responsiveness to pharmacotherapy exist, with distinct clinical clusters being proposed to better individualise management (Ahlqvist *et al.*, 2018).

Beta cell dysfunction in T2DM

In healthy humans, pancreatic hormone secretion is pulsatile. In the case of insulin, this helps minimise hepatic receptor desensitisation and enhance insulin sensitivity (Lang *et al*., 1979;

Komjati, Bratusch-Marrain and Waldhäusl, 1986; Hellman *et al*., 1994). In non-diabetic hyperglycaemia (pre-diabetes) and frank T2DM, insulin pulsatility is disordered and irregular. Pulses are of reduced frequency and amplitude and are temporally disconnected from peaks in glucose (Lang *et al.*, 1981; Polonsky *et al.*, 1988; Gumbiner *et al.*, 1996). The functional impact of altered insulin pulsatility is significant as it changes hepatic gene expression in favour of hepatic insulin resistance, thereby exacerbating the relative insulin deficit (Matveyenko *et al.*, 2012). Abnormalities in insulin secretion and action have been consistently found to precede overt T2DM. Those with impaired glucose tolerance (IGT) and diabetes have impaired first-phase insulin secretion compared to normal subjects (Bergman, Phillips and Cobelli, 1981; Yoneda *et al.*, 1992; Weyer *et al.*, 1999). Even more subtly, disorders in insulin pulsatility are also present in normoglycaemic relatives of individuals with T2DM, who have been found to have disordered insulin oscillations compared to controls (O'Rahilly, Turner and Matthews, 1988). Collectively, these findings suggest that it is not just beta cell failure per se, but the loss of ability to co-ordinate insulin pulsatile insulin secretion that are essential features of the pathogenesis of T2DM. Moreover, loss of insulin pulsatility is a very early feature of the natural history of the disease and may even be causally related to hepatic insulin resistance.

Alpha cell dysfunction in T2DM

Since beta cells exist in close functional proximity to a number of other hormone secreting cells in the pancreatic islet, a focus only on the insulin-secreting beta cells is insufficient for a full understanding of healthy insulin secretion and its failure in diabetes. Pancreatic glucagon-producing alpha cells have been increasingly recognised as a key player in the development of diabetes. Glucagon is a counterregulatory hormone to insulin and acts to increase blood glucose levels, mainly by elevating basal hepatic glucose output following periods of fast. Several studies have observed raised glucagon levels and basal hepatic glucose production in patients with diabetes, which normalised following treatment of diabetes (Sherwin *et al.*, 1976; Liljenquist *et al.*, 1977; Baron *et al.*, 1987). This correlates well with the increased proportion of alpha cells seen in islets of patients with T2DM (Yoon *et al.*, 2003). To demonstrate glucagon's role in diabetes, Lee *et al* destroyed the beta cells of glucagon-receptor knockout mice. Whilst wild type mice became progressively unwell with severe metabolic derangements, they found the knockout mice to have high glucagon levels but normal glucose tolerance (Lee *et al.*, 2011). This suggests that normal glucose homeostasis can be restored if glucagon action is switched off, even in the presence of insulin deficiency. Raskin and Unger demonstrated similar findings in humans by giving individuals with insulin-dependent diabetes a continuous insulin infusion and access to a carbohydrate rich diet. Following administration of somatostatin, a potent inhibitor of glucagon release, plasma glucose and ketone levels lowered significantly (Raskin and Unger, 1978). Gerich *et al* went

a step further and took insulin away from individuals with insulin-dependent diabetes and instead, administered somatostatin. They found glucagon suppression the delayed onset of diabetic ketoacidosis by 8 hours (Gerich *et al.*, 1975), highlighting the significant role glucagon plays in the pathophysiology of diabetes. These studies show that the hyperglycaemia seen in diabetes is likely the result of a bihormonal disruption, characterised by relative insulin deficiency and glucagon excess (Unger and Orci, 1975). Some propose that instead of treating patients for insufficient insulin, more should be done to create diabetes management options that better address the state of glucagon excess (Unger and Cherrington, 2012).

Pancreatic Islet Structure-Function Relationship

Pancreatic islets of Langerhans are clusters of predominantly endocrine cells that coordinate normal hormone secretion. Insulin-secreting beta cells are the most common endocrine cell within a human islet, followed by glucagon-secreting alpha cells, somatostatin-secreting delta cells, pancreatic polypeptide-secreting gamma cells and ghrelin-secreting epsilon cells respectively. Amongst them are capillaries, immune cells, neuronal projections, and a protein-rich extracellular matrix that all contribute to normal islet endocrine function (Walker *et al.*, 2021).

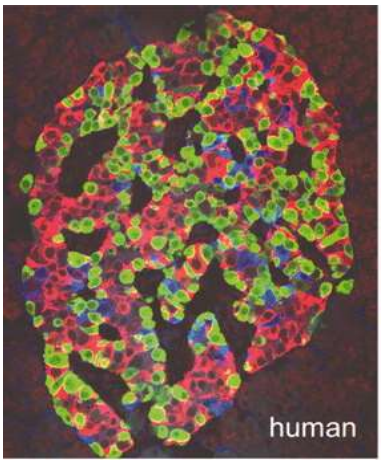

Figure 1: Human pancreatic islet section showing randomly distributed endocrine cells. Insulin is stained in green, glucagon in red and somatostatin in blue. Adapted from: (Cabrera et al., 2006).

The islet cells work as a functional unit, communicating with each other via paracrine signalling and cell-cell connections (Walker *et al.*, 2021; Adams and Blum, 2022). Alpha and beta cells closely interact with each other to determine a glycaemic set point, with glucagon and insulin participating in a negative feedback loop to maintain euglycaemia (Rodriguez-Diaz *et al.*, 2018). Interestingly, alpha cells also potentiate beta cell activity (Noguchi and Huising, 2019).

In the fed state, glucagon enhances glucose-stimulated insulin secretion (GSIS) by acting on beta cells to stimulate cyclic adenosine monophosphate (cAMP) production, ultimately triggering insulin secretion (Gelling *et al.*, 2009; Moede, Leibiger and Berggren, 2020). Beta cells with higher concentrations of glucagon receptors have been found to be associated with increased beta cell function and overall beta cell mass (Gelling *et al.*, 2009), leading to the suggestion that glucagon's insulinotropic effect may be an adaptive response to metabolic stress in attempt to maintain blood glucose levels (Capozzi *et al.*, 2019). In addition to alpha and beta cells regulating each other, delta cells also participate in glucose homeostasis. Delta cells in mice have been noted to have long dendrite-like projections that enable modulation of distant islet cells (Rorsman and Huising, 2018). In high glucose states, they tonically inhibit beta and alpha cells via paracrine mechanisms and gap junctions, releasing somatostatin to suppress glucagon and insulin secretion (Brereton *et al.*, 2015; Briant *et al.*, 2018; Huising *et al.*, 2018). As a result, delta cells are becoming of increasing interest as a potential therapeutic target in T2DM.

T2DM is associated with disruptions to the structure-function relationship of pancreatic islets. States of hyperinsulinaemia, such as obesity or pre-diabetes, are associated with expansions in beta cell volume, and therefore increased secretory capacity (Klöppel *et al*., 1985; Brandhorst *et al*., 2009). However, with the onset of T2DM, affected individuals have an average 40% reduction in beta cell mass and 30% reduction in pancreatic insulin concentration compared to individuals without diabetes (Butler *et al.*, 2003; Rahier *et al.*, 2008). Beta cells also lose their specialised identity during the development of T2DM; some studies show that nearly 40% of pancreatic beta cells from humans with type 2 diabetes were dedifferentiated, with some partially adopting similar features to alpha and delta cells (Talchai *et al.*, 2012; Cinti *et al.*, 2016). In contrast, despite the hyperglucagonaemia of T2DM, alpha cell mass does not appear to change significantly (Henquin and Rahier, 2011), but the alpha cell's unique proliferative ability may explain the stable cell population (Bru-Tari *et al*., 2019). Furthermore, in times of extreme beta cell loss, alpha cells appear to have the ability to reprogramme into beta-cells, with significant quantities of insulin gene mRNA being found in highly purified glucagon-producing alpha cells (Thorel *et al.*, 2010; Blodgett *et al.*, 2015).

Interactions between alpha and beta cells also become ineffective, with disconnected communication between cell types resulting in an abnormal alignment of insulin and glucagon release post-prandially (Menge *et al.*, 2011). The consequences of this mismatch include poor hormone function. Rohrer *et al*'s study on prediabetic individuals highlighted how derangements in insulin pulsatility and secretion caused an inadequate suppression of glucagon after a glucose load (Rohrer *et al.*, 2012). The hyperinsulinaemia of early T2DM also prevents the normal, homeostatic response of glucagon to hypoglycaemia (Banarer,

McGregor and Cryer, 2002). In addition to direct suppression of glucagon via insulin receptors on alpha cells, insulin facilitates somatostatin-mediated suppression of glucagon via sodium-glucose-co-transporter 2 channels (Vergari *et al.*, 2019). Insulin secretion and action are already impaired in T2DM, affecting somatostatin's ability to work effectively. Alpha cells in diabetic islets also develop increased resistance to somatostatin inhibition, with fewer cell-membrane bound somatostatin receptors to facilitate inhibition of glucagon exocytosis (Omar-Hmeadi *et al.*, 2020).

Therefore, islets rely on specific structural foundations to work optimally as a functional unit. The pathogenesis of T2DM includes changes to islet structure, individual cell function and islet paracrine activity.

Calcium dynamics in alpha and beta cells

Beta cells

On a sub-cellular level, a complex network of intracellular processes facilitate normal hormone production and secretion from pancreatic islet cells. Periodic changes in intracellular calcium concentration underlie physiological insulin secretion from beta cells, with glucose concentrations between 6mmol/L and 17mmol/L stimulating oscillatory beta cell calcium activity (Rorsman, Braun and Zhang, 2012). Glucose is taken up from the extracellular environment via GLUT2/1 transporters in the beta cell membrane and undergoes glycolysis to generate ATP. Rising intracellular ATP causes inhibition of ATP-sensitive potassium (K_{ATP}) channels, depolarising the plasma membrane to activate voltage-gated calcium channels (VGCC). Calcium rapidly enters the cell via L-type channels to trigger insulin exocytosis (Klec *et al.*, 2019). To ensure coordinated insulin release, beta cells are connected by gap junctions such as connexin36 to propagate the fast, rhythmic intracellular calcium waves across an islet (Rutter and Hodson, 2013). These cell-cell electrical connections allow beta cells to be more efficient as a unit, with intact islets producing and releasing insulin more efficiently than isolated beta cells in high and low glucose environments (Halban *et al.*, 1982). Furthermore, whilst functionally synchronous, there is increasing evidence to show that the beta cell population is heterogenous, with pacemaker 'hubs' leading islet insulin responses (Johnston *et al.*, 2016a; Salem *et al.*, 2019)

In hyperglycaemia, particularly above 20mmol/L, beta cell calcium activity becomes continuous, correlating with the loss of pulsatile insulin secretion seen in T2DM (Rorsman, Braun and Zhang, 2012). The diabetic state has also been found to be associated with

reduced beta cell synchronisation (Hoang, Hara and Jo, 2016). Connectivity between beta cells have also found to be attenuated in high-fat-diet-fed mice with poor glucose tolerance, with bariatric surgery restoring connectivity and beta-cell function (Akalestou *et al.*, 2021). The electrical decoupling may in part be due to abnormalities disproportionately affecting beta cell hubs. Johnston et al exposed murine islets to pro-inflammatory cytokines associated with glucolipotoxicity and observed reductions in beta cell hub number and connectivity (Johnston *et al.*, 2016b). Targeted ablation of pacemaker cells was found to significantly reduce beta cell calcium activity despite multiple stimulations with glucose (Salem *et al.*, 2019). Therefore, disruptions in beta cell calcium signalling is one manifestation of islet dysfunction, with abnormalities in beta cell hub function possibly playing a key role in T2DM pathophysiology.

Alpha cells

Compared to insulin, much less is certain about the process of glucagon secretion and its regulation. Paracrine hormones (such as somatostatin and insulin), nutritional compounds (such as zinc, amino acids and free fatty acids) and autonomic signals are known to modulate glucagon secretion (Pipeleers *et al.*, 1985; Noguchi and Huising, 2019). However, given the electrical excitability of alpha cells, it is equally possible that intrinsic mechanisms are the predominant regulator of glucagon release. Both alpha and beta cells are thought to contain similar intracellular secretory pathways and components, but one model proposes that their opposing responses to glucose influx are due to differences in K_{ATP} channel activity. Compared to beta cells, alpha cells have lower concentrations of K_{ATP} channels, which at low glucose concentrations are functioning just enough to activate voltage-gated sodium channels (VGSC). Sodium influx further depolarises the plasma membrane, activating VGCC to enable calcium influx to ultimately cause glucagon release. At higher glucose concentrations, however, the K_{ATP} channels close completely, inactivating VGSC. This prevents sufficient membrane depolarisation to allow opening of VGCC and subsequent calcium influx, thereby suppressing glucagon release (Göpel *et al.*, 2000; Gromada, Franklin and Wollheim, 2007; González-Vélez, Piron and Dupont, 2020; Wendt and Eliasson, 2020). Therefore, small electrical changes in the alpha cell plasma membrane can have significant effects on intracellular calcium concentration and glucagon output.

GLP-1 agonists

Given the close and causal association with obesity, T2DM treatment options that also cause weight loss are becoming increasingly desirable. Lifestyle changes, such as diet and exercise, remain as first-line treatment for both T2DM and obesity. However, effective implementation

requires intensive intervention and patient commitment to obtain meaningful and sustained weight loss (Look AHEAD Research group, 2014). Very few pharmacological weight loss options have survived over time, largely due to their adverse cardiovascular or neuropsychiatric side effect profiles (Onakpoya, Heneghan and Aronson, 2016; Müller *et al.*, 2022). Glucagon-like peptide (GLP-1) receptor agonists have been the exception, gaining traction for their positive effects in both obesity and T2DM (Blüher *et al.*, 2022).

GLP-1 boosts insulin secretion in response to ingested nutrients (incretin effect), and is therefore an insulin secretagogue only when circulating sugar is above 4mM. It is a peptide hormone derived from proglucagon and secreted from L cells in the intestine. It is also expressed in neurones in the central nervous system. GLP-1 acts to: increase beta-cell insulin secretion, reduce alpha-cell glucagon secretion, slow gastric emptying, reduce food intake, and reduce inflammation (Tan *et al.*, 2022). Its dual effect on glucagon and insulin drove the development of GLP-1 receptor (GLP-1R) agonists for T2DM, and their use has since extended to patients with obesity (in the absence of diabetes) due to appetite inhibitory and weight loss effects with low risk of hypoglycaemia. Delivered subcutaneously, short-acting (e.g. exenatide, lixisenatide) and long-acting (e.g. liraglutide, semaglutide, dulaglutide) formulations are clinically available, which vary in their pharmacokinetic properties and the extent of their similarities to the native, human GLP-1 molecule (Klen and Dolžan, 2022; National Institute for Health and Care Excellence, 2022a). These agents were revolutionary due their dual ability to improve glycaemic control (Singh *et al.*, 2017) and cause weight loss (Astrup *et al.*, 2009; Davies *et al.*, 2015), with semaglutide being associated with a mean bodyweight reduction of at least 10% (Davies *et al.*, 2021; Wilding *et al.*, 2021). However, adverse effects, particularly gastrointestinal disturbances such as nausea, vomiting and pancreatitis, have been reported (Astrup *et al.*, 2009; Wilding *et al.*, 2021; Shetty *et al.*, 2022), causing GLP-1R agonists to be poorly tolerated in some patients. Furthermore, for reasons poorly understood (and possibly related to functional differences across the population in the GLP-1 receptor), responses to these drugs, particularly the weight loss effects, can be quite variable (Brown *et al.*, 2019).

GLP-1 in alpha and beta cells

The GLP-1R is a G-protein coupled receptor (GPCR). Its transmembrane structure allows for binding of extracellular ligands, with the resultant conformational change to the receptor structure enabling its associated subunits to modulate various intracellular signalling pathways. GPCR signalling is regulated by beta-arrestins, which bind to G-protein subunits to desensitise the receptor and switch off further signalling by promoting receptor internalisation and recycling. Beta-arrestins also facilitate intracellular GPCR signalling, particularly MAP

kinase pathways, acting as scaffolds to connect proteins and enable signal transduction (Laporte and Scott, 2019).

In beta cells, glucose-stimulated insulin production and secretion by GLP-1R occurs via activation of the G_{as} subunit to generate cAMP. cAMP modulates a cascade of signalling pathways to enable GLP-1's incretin effect (see figure 2). Activation of Epac2 inhibits K_{ATP} channel function, increasing intracellular calcium to stimulate insulin secretion. cAMP also activates Pdx1 via protein kinase A (PKA) to promote insulin gene transcription (Marzook, Tomas and Jones, 2021; Mayendraraj, Rosenkilde and Gasbjerg, 2022). Given the cAMP-dependent pathways involved in GLP-1 mediated hormone secretion, intracellular calcium concentration has been utilised as a surrogate measure for beta cell insulin output, with existing studies demonstrating increases in oscillatory activity and intensity of calcium fluorescence following beta-cell exposure to GLP-1 (Holz, Leech and Habener, 1995; Akalestou *et al*., 2021; Bitsi *et al*., 2022).

In addition to its stimulatory effect on insulin, the glucose-lowering actions of GLP-1 may be equally due to its effect on glucagon (Hare *et al.*, 2010). GLP-1's inhibitory effect on glucagon secretion from alpha cells is well-established, with GLP-1 causing reductions in glucagon and post-prandial glucose in *in-vivo* and *in-vitro* studies (Ørskov, Holst and Nielsen, 1988; Fehmann *et al.*, 1995; Kielgast, Holst and Madsbad, 2011). On a cellular level, alpha cells have been noted to express GLP-1R, although at much lower levels than that seen in beta cells, with glucagon secretion being regulated by GPCR-mediated cAMP pathways (de Marinis *et al.*, 2010; Ramracheya *et al.*, 2018). In alpha cell-specific GLP-1R knockout mouse models, GLP-1 failed to inhibit glucagon at high glucose concentrations, suggesting that the low receptor expression may be sufficient for GLP-1 to directly modulate glucagon secretion (Zhang *et al.*, 2019).

Biased GLP-1 agonists

From a clinical perspective, the dual action of GLP-1 agonists on insulin and glucagon secretion are favourable in the management of diabetes. As current GLP-1 agonists are limited by their side effect profile, causing variable patient tolerability and/or clinical responses, improving GLP-1R binding and selective promotion of downstream signalling pathways may help minimise off-target actions whilst maintaining pharmacological efficacy. Using exendin-4, a naturally occurring peptide agonist of the GLP-1R, Jones *et al* generated biased agonists, exendin-phe1 (ex-phe) and exendin-asp3 (ex-asp3) by substituting single amino acids close to its N-terminus. In beta cell studies, ex-phe was found to be a superior insulin secretagogue compared to currently available GLP-1 agonists. Ex-phe also underwent fast recycling, preventing receptor desensitisation and permitting chronic secretion of insulin (Jones *et al.*,

2018). Although these early-phase findings are promising, further data in alpha cells is needed to understand the effect of these biased agonists on glucagon secretion, which can help inform future *in-vivo* studies. A readout of the effects of novel agents in development on relative alpha and beta cell activity would also help screen for improved drugs.

Aims

In this study, I aim to establish a genetically encoded readout for calcium activity in alpha cells of intact (murine) islets. I will use islets from a novel transgenic mouse line, PPG-GCaMPf, where cells expressing the glucagon precursor gene, preproglucagon (ppg), are genetically modified to also express a fluorescent calcium indicator, GCaMP6f. I will establish normal PPG-GcAMP6f alpha cell calcium activity in high and low glucose environments, and subsequently explore how this is changed by biased GLP-1 agonists, ex-phe and ex-asp. Better understanding the interaction between GLP-1R agonists and alpha cells will add to our knowledge of normal islet function whilst also guiding development of more efficacious therapies for diabetes.

I will also compare and contrast this new approach to alpha cell readouts with equivalent studies in my lab of beta cell function. This will aid a fuller understanding of the islet as a functional unit. Therefore, a discrete list of the aims for this project are:

1. To establish low and high glucose calcium responses in alpha cells that express GCaMP6f within intact murine islets.
2. To compare and contrast alpha and beta cell low vs high glucose readouts in intact mouse islets.
3. To measure the effect of GLP-1 analogues on alpha cell GCaMP readouts in this model.
4. To assess whether it is possible to measure a difference in islet alpha cell responses to biased GLP-1 analogues.

Hypothesis

Based on the current literature, I anticipate the PPG-GCaMP6f alpha cells to show high calcium activity at low glucose concentrations and be inhibited at high glucose concentrations. I also anticipate the GLP-1 agonists will attenuate alpha cell calcium flux, with ex-phe having a particularly inhibitory effect.

Methods

Breeding of transgenic mice for reporter islets

All animal work was conducted in compliance with the UK Animals Scientific Procedures Act (1986) and the Imperial College London Animal Welfare ethical guidelines. Animals were kept in controlled conditions (21-23°C, 12:12h light:dark cycle) and in well ventilated cages, with *ad libitum* access to standard chow diet. All animal care was overseen by the Central Biological Services unit at Imperial College.

Transgenic mice (PPG-GCaMP6f) expressing the genetically encoded calcium marker, GCaMP6f, in alpha cells were kindly bred, genotyped and isolated by Professor Kevin Murphy's lab (Imperial College London, PPL Murphy PD75F462C). PPG-GCaMP6f mice were generated by crossing mice expressing CreERT2 recombinase under the regulation of the PPG promoter (Glu-CreERT2) with RCL-GCaMP6f (Ai95-D) mice, which had a lox-stop-lox sequence upstream of GCaMP6. Breeding aimed to generate double heterozygous mice that expressed GCaMP6f in PPG-expressing cells (Glu-CreERT2$^{+/-}$;Ai95D$^{fl/+}$). Tamoxifen induction post-islet isolation cleaved the floxed-stop sequences in PPG-expressing cells to enable selective expression of GCaMP6f fluorescence in alpha cells.

In this project, data from islets expressing GCaMP6f in alpha cells was compared with data collected from previous experiments using a mouse line genetically engineered to express GCaMP6f in beta cells (Salem *et al.*, 2019). In brief, those mice were created by crossing Ins1^{Cre}-expressing mice with animals that expressed GCaMP6f$^{fl/fl}$ fluorescent calcium sensor, downstream of a *LoxP*-flanked STOP cassette (The Jackson Laboratory, stock no. 028865).

Islet isolation

Islets were isolated from normal weight animals which had been fed normal chow and were under 6 months old. Donor mice were culled using cervical dislocation. Pancreata were inflated via the common bile duct with RPMI-1640 medium (R8758, Sigma-Aldrich) containing 1mg/ml collagenase from Clostridium histolyticum (S1745602, Nordmark Biochemicals), and dissected. Following water bath incubation at 37°C for 10 minutes, islets were purified and separated using Histopaque gradient (Histopaque-1119, 11191, Sigma-Aldrich, and Histopaque-1083, 10831, Sigma-Aldrich). Islets were transferred to full islet media consisting of RPMI-1640 supplemented with 10% v/v fetal bovine serum (FBS) (F7524, Sigma-Aldrich) and 1% v/v Penicillin/Streptomycin (P/S) solution (15070-063, Invitrogen). Tamoxifen (4-OHT)

was added to the media induce GCaMP6f fluorescence in cells expressing preproglucagon (ppg). Islets were left to recover for 24 to 48 hours at 37°C in 5% CO_2 prior to imaging.

Alpha cell imaging protocol

On the day of imaging, single islets were mounted in Matrigel (356231, Corning) within a glass-bottom MatTek dish (P35G-1.5-10-C). Once set, they were perifused with Krebs-Ringer Bicarbonate-HEPES (KRBH) buffer (140 mM NaCl, 3.6 mM KCl, 1.5 mM $CaCl_2$, 0.5 mM $MgSO_4$, 0.5 mM NaH_2PO_4, 2 mM $NaHCO_3$, 10 mM HEPES, saturated with 95% O2/5% CO_2; pH 7.4) containing 0.1% w/v BSA and either 3mM (KRBH G3), 6mM (KRBH G6) or 11mM (KRBH G11) glucose concentrations.

Using a Yokogawa CSU22 Nipkow spinning disk microscope coupled with a Zeiss Axiovert M200, islets were visualised with a 60x objective whilst being maintained at 37°C on a heated stage. They were excited at 488nm with 250ms exposure, with images recorded at 0.6s per image (0.6Hz). For variable glucose concentration videos, islets were perifused in KRBH buffer containing either: KRBH G3 +/- 8mM glucose or 20mM KCl or KRBH G11 +/- pure KRBH or 20mM KCl. GLP-1 agonist videos followed a similar protocol, with the addition of either vehicle (KRBH), 100nM ex-asp or 100nM ex-phe. Solutions were manually added to the MatTek dishes at set time points.

The beta cell imaging experiments were performed previously in the Salem lab using the same microscope and imaging parameters. However, the isolated islets were imaged at 1Hz in a perifusion system, starting at 2mM glucose for 2 minutes, followed by 4mM, 6mM, 8mM, 10mM, 12mM and 17mM glucose Krebs solutions for 8 minutes per condition.

Image Analysis

Images/videos were analysed using Image J v1.53n. Regions of interest (ROI) were manually drawn around fluorescent alpha or beta cells on a single cross section. All visible cells were included, whether or not they had oscillating calcium signal. Fluorescent intensity traces over time were normalised to $F_{average}$ and plotted. Peaks determined as representative of biologically significant calcium flux were defined as those with amplitude >15% of baseline as these were consistently associated with a visible change on the videos. Beta cell data from previously performed perifusion experiments were reanalysed using the same criteria as that developed for the alpha cells, to ensure consistency of comparisons.

Calcium time courses and peaks were analysed by amplitude and frequency as well as an assessment of the number of cells that were active (in terms of oscillating calcium signal). Alpha-cell connectivity was determined as described previously by (Salem *et al.*, 2019) as applied to beta cells. Briefly, $[Ca^{2+}]_I$ traces extracted from cellular ROIs were subjected to Pearson R correlation analyses, with all cell-cell pairs in in the islet cross section represented in a heat map of R values.

Statistics

Statistical significance between proportions of oscillatory cells were assessed using Fisher's exact test. Differences in alpha cell peak frequency and amplitude between multiple conditions were compared using Mann Whitney U and Kruskal Wallis tests since all datasets were not normally distributed. Pearson R analyses was used for connectivity analysis. Analyses were performed using Microsoft Excel, GraphPad Prism (GraphPad Software v.8.0) and MATLAB (Mathworks). Values are plotted as mean ± SEM or median (IQR) unless otherwise stated, and statistical significance was defined as $p<0.05$.

Results

3. 1 Islets from PPG-GcAMP mice display differential alpha cell activity at low and high glucose levels.

Aim 1 of this project was to establish whether islets from mice bred to express the fluorophore GcAMP6f in their alpha cells (under the PPG promoter) could be utilised to study calcium dynamics over different glucose levels.

3.1.1 Not all alpha cells in a given islet have discernible calcium activity.

A total of 21 islets from 4 donors were studied. Not all alpha cells were active within an islet using this readout. Overall, in all experiments at all glucose levels (accounting for the fact that all depolarised with KCl i.e., were physiologically active), only 22 of 191 (11.52%) alpha cells were discernibly active.

Figure 2 shows a representative trace for an experiment in one islet. Part A shows a cross-sectional image of that islet with all of the alpha cell ROIs depicted. Part B shows the calcium traces for each ROI over the course of the experiment, with the timepoints for the switch from low to high glucose and the addition of (positive control) KCl indicated by dashed lines. All of the traces for these experiments (for each islet) are provided in Appendix 1.

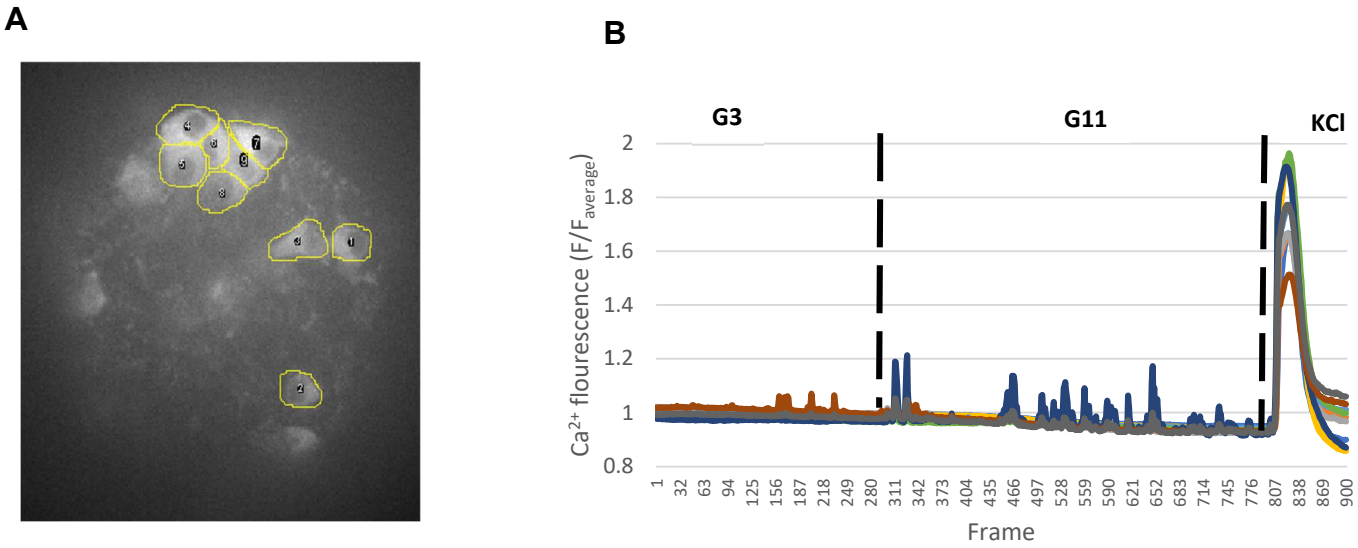

*Figure 2: (**A**) A snapshot of an islet cross-section, with individual alpha cell regions of interest (ROIs) outlined in yellow. (**B**) Normalised alpha cell ROI calcium traces of a single islet across an entire imaging session (9 minutes), with each coloured line corresponding to an ROI in (**A**). Peaks represent periods of increased calcium fluorescence i.e. increased alpha cell activity. Islets were imaged in 3mM (G3) glucose for 3mins, followed by 11mM (G11) glucose for 5mins and KCL for 1min.*

3.1.2 There is no difference in proportion of alpha cells with discernible activity at G3 or G11

Looking at the proportion of alpha cell activity by glucose level over all experiments, 14 out of 191 (7.33%) ROIS were active at G3, compared with 11 out of 191 (5.76%) ROIs at G11 (p=0.68). 3 of 191 (1.57%) ROIs were active at both G3 and G11.

3.1.3 The frequency of alpha cell oscillations is similar in G3 and G11

The median number of peaks in calcium activity at G3 and G11 were 0.7 (0.20-1.20) and 1.0 (0.33-2.70), respectively (Mann Whitney U=67; p=0.60). As shown in Figure 3, there was no statistical difference between the median (IQR) peak frequency over time in G3 and G11 environments (0.7/min (0.20-1.20) vs 1/min (0.33-2.70); Mann Whitney U=53; p=0.19). There was also no statistical difference in median (IQR) amplitude between high and low glucose environments (G3: 0.21 (0.16-0.25) vs G11: 0.24 (0.18-0.31); Mann Whitney U=796; p=0.11).

There were 3 cells identified which were spiking both at G3 and G11. There were no statistical differences in their mean (±SD) peak amplitude (G3: 0.29 ±0.09 vs G11: 0.33 ±0.13; p=0.70) or peak frequency (G3: 0.53/min ±0.58 vs G11: 1.3/min ±1.2; p=0.46).

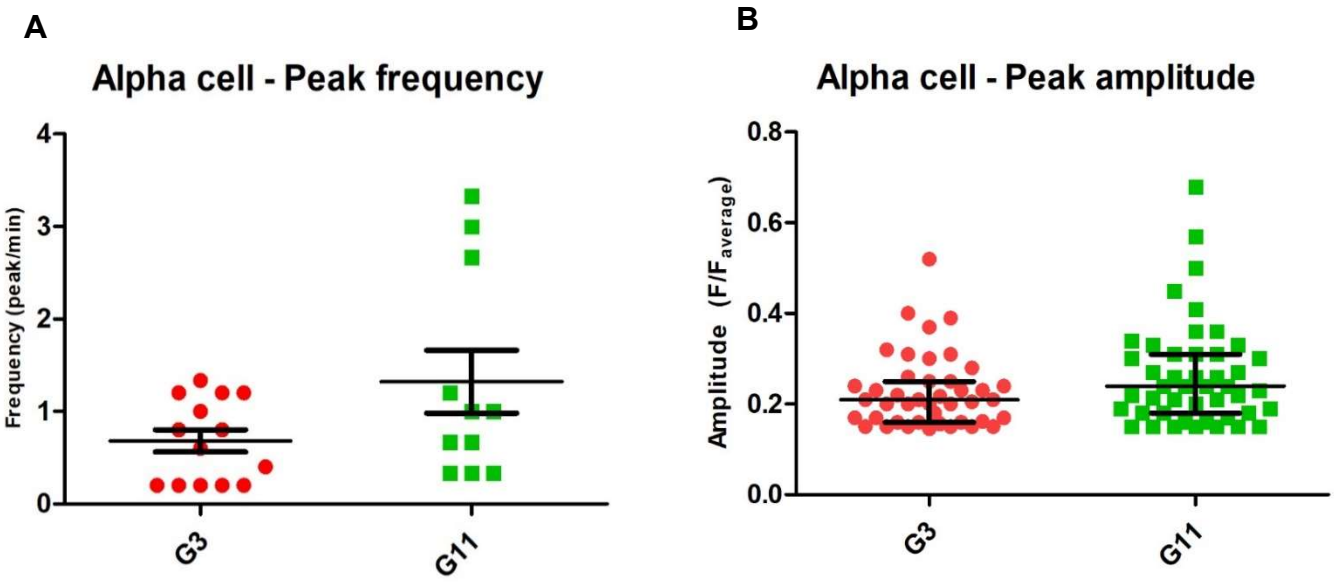

*Figure 3: No significant differences were found in median peak frequencies (**A**) or peak amplitude (**B**) between low (3mM; G3) and high glucose (11mM; G11) in all oscillating alpha cells. Black lines demonstrate median (IQR). Statistical tests: Mann Whitney U.*

3.1.4 There is no evidence of connectivity across alpha cells in a given islet

Beta cell functional connectivity is a well-established concept, with electrical coupling between cells (augmented by paracrine and nervous inputs) underpinning pulsatile insulin secretion. Thus, average R values between beta cell calcium traces in islet cross sections at high glucose are typically >0.9 (Hodson *et al.*, 2013; Johnston *et al.*, 2016b; Salem *et al.*, 2019). It remains unknown whether similar connectivity patterns exist between alpha cells. A Pearson-R based correlation analysis was performed on a single islet cross-section, chosen for the most alpha cells that were visibly responding (Figures 4). Alpha cells showed poor connectivity over the entire experiment (R=0.45; Figure 5). Whilst the number of oscillating alpha cells were low, they appeared to be more connected at G11 (R=0.74) compared to G3 (R=0.46). However, the synchronised oscillatory alpha cells at G11 were in close proximity to each other so this is unlikely to be a pan-islet phenomenon.

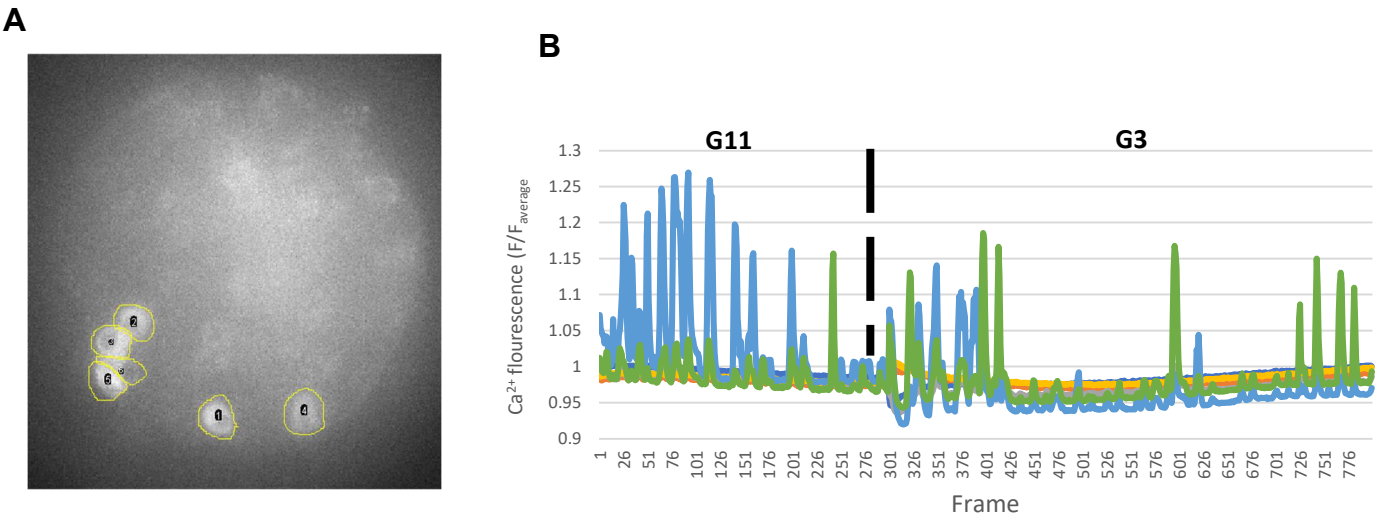

*Figure 4. (**A**) A snapshot of an islet cross-section, with individual alpha cell regions of interest (ROIs) outlined in yellow. The ROIs shown below were used for connectivity analysis. (**B**) Normalised alpha cell ROI calcium traces of a single islet over time at 11mM (G11) and 3mM (G3) glucose concentrations. Each coloured line corresponding to its ROI in Figure (**A**). Peaks represent periods of increased calcium fluorescence i.e. alpha cell activity.*

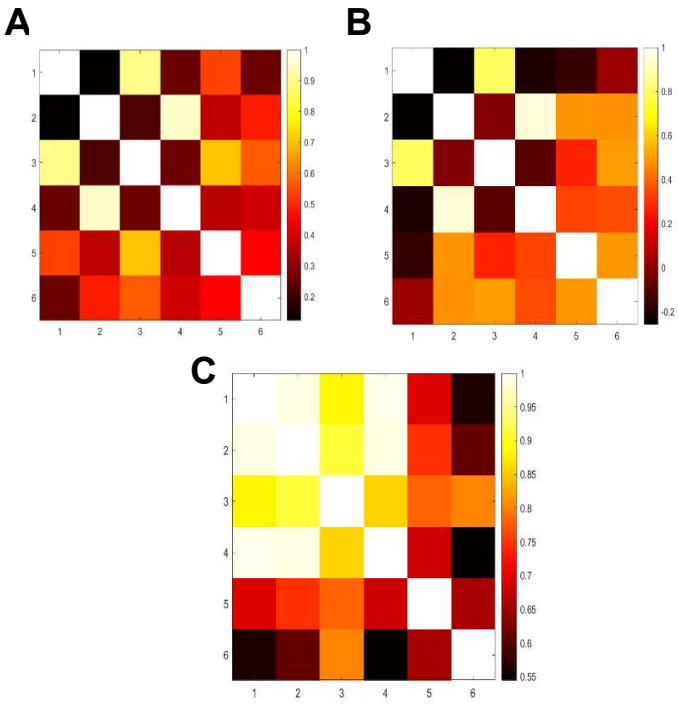

*Figure 5: Connectivity heat maps for each alpha cell pair from the islet depicted in Fig. 4. There is poor connectivity between cell pairs across the entire experiment (**A**) and between all cell pairs at G3 (**B**). There is high connectivity between cell pairs at G11 (**C**). Black boxes indicate strong negative correlation between a cell-pair and white boxes, very high positive correlation (hence the diagonal white line which is the autocorrelation line of 1.0). Statistical tests: Pearson R.*

3. 2 Islets from INS-1-GcAMP mice display differential beta cell activity at low and high glucose levels, which is very different to that observed in alpha cells

Aim 2 of this project was to compare alpha cell calcium readouts in intact islets with those obtained from a similar beta cell (calcium fluorophore expressing) model. In Figure 6a, a cross section of an islet expressing GCaMP6f in its beta cells is depicted, with the associated ROIs used for single cell analysis. Figure 6b shows an equivalent readout of the activity of each of these beta cells and low and high glucose.

The majority (95.9%) of beta cells in these experiments (234 of 244 ROIs over 15 islets derived from n=3 mice) became active at high glucose, whilst none were active at low glucose. The number of oscillating cells in beta cell experiments was significantly greater than that seen in alpha cell experiments ($p<0.001$). Beta cell activity occurred in waves, consisting of multiple, regular spikes. At high glucose, the average frequency of waves was 0.88/min (±0.15), with an average amplitude of 0.34 units (±0.02).

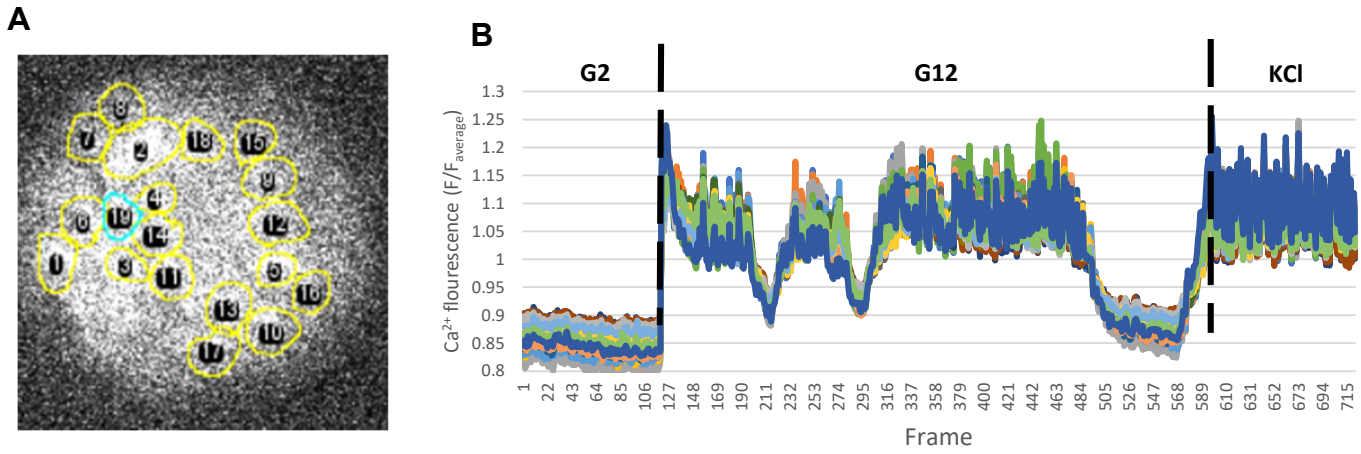

*Figure 6. (**A**) A snapshot of an islet cross-section, with individual beta cell regions of interest (ROIs) outlined in yellow. (**B**) Normalised beta cell ROI calcium traces of a single islet, with each coloured line corresponding to its ROI in (**A**). Peaks represent periods of increased calcium fluorescence i.e. increased beta cell activity. Islets were imaged in 2mM (G2) glucose for 2mins, followed by 12mM (G12) glucose for 8mins and KCL for 1min.*

3.3 The role of GLP-1 agonists on alpha cell activity in high and low glucose could not be determined

The effect of GLP-1 biased agonists, exendin-phe1 (ex-phe) and exendin-asp3 (ex-asp), on alpha cell calcium activity at high and low glucose were interrogated.

In these experiments, no oscillating alpha cells were seen during low glucose conditions despite the addition of GLP-1 agonists and an increase in glucose concentration (n=2 with a total of 45 ROIs across all conditions).

In islets exposed to a high glucose environment, 4 (7.01%) individual alpha cells showed oscillatory activity (n=2 with a total of 57 ROIs across all conditions; see Appendix 2). Compared to vehicle, biased GLP-1 agonists were not found to cause significant changes in mean peak frequency (Veh: 0.85/min (0.45) vs ex-phe: 1.8/min vs ex-asp 0.5/min (0.1); p=0.33) or mean peak amplitude (Veh: 0.22units (0.04) vs ex-phe: 0.17units vs ex-asp: 0.20units (0.02); p=0.30) (see Figure 7a & 7b).

Due to the low proportion of oscillatory alpha cells across all conditions in these experiments, further experimental repeats would be required to uncover the true effect of GLP-1 agonists on alpha cell calcium activity.

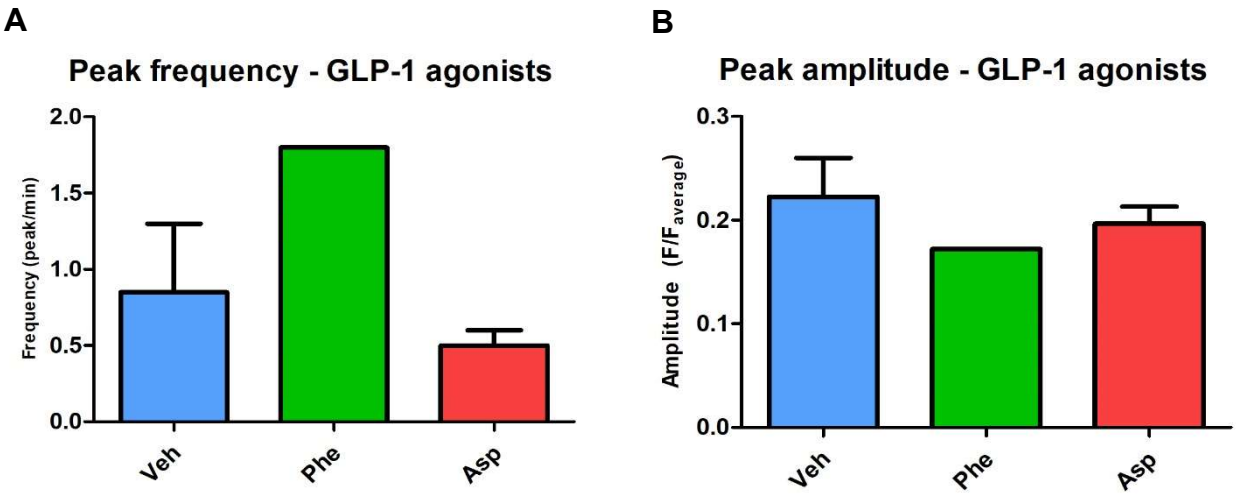

*Figure 7: No significant differences were found in mean peak frequencies (**A**) or peak amplitude (**B**) between vehicle (veh), exendin-phe (phe) and exendin-asp (asp) at high glucose in all oscillating alpha cells. Black lines demonstrate S.E.M. Statistical tests: Kruskal-Wallis.*

Discussion

This study is the first to investigate normal alpha cell calcium dynamics and explore how GLP-1 agonists modulated alpha cell calcium activity using a novel PPG-GCaMP6f mouse line. In general, there were low proportions of alpha cells showing significant calcium pulsations. Neither glucose concentration nor GLP-1 agonists induced statistically significant alterations in calcium wave characteristics and no evidence of alpha cell connectivity was found. However, interesting patterns were found that may provide further insight into alpha cell activity and better inform ongoing studies.

The characteristics of alpha cell calcium activity are distinctly different to that seen in beta cells. As seen in this study, beta cells consistently respond to high glucose with wave-like oscillations (Rorsman, Braun and Zhang, 2012; Johnston *et al.*, 2016b). Rather than sustained periods of oscillatory waves, alpha cells had bursts of calcium activity. In fluorescent dye calcium imaging experiments, alpha cell calcium oscillations were found to be spontaneous and irregular, with oscillations varying in duration, shape, and amplitude (Quoix *et al.*, 2009; González-Vélez *et al.*, 2012; González-Vélez, Piron and Dupont, 2020; Kellard *et al.*, 2020). Kellard *et al* conducted electrophysiological studies alongside calcium imaging in GCaMP3 mouse islets and found a strong correlation between changes in intracellular calcium and membrane potential oscillations (Kellard *et al.*, 2020). Further work is needed to determine if these electrical changes in alpha cells correlate with measurable changes in glucagon secretion.

Unlike the predictable and consistent beta cell activity, only a small proportion of alpha cells demonstrated significant changes in calcium activity, but all had a positive response to KCl. Given glucagon's actions, this study hypothesised that low glucose environments would stimulate alpha cells and increasing glucose concentrations would inhibit them. There is certainly evidence to support this, with groups noting reductions in calcium oscillation frequency and amplitude in high glucose environments (Quesada *et al.*, 2006; González-Vélez, Piron and Dupont, 2020). However, this data suggests alpha cell calcium activity is stimulated by both hypo- and hyperglycaemia. Others have also found high and low glucose concentrations to induce similar proportions of alpha cell calcium oscillations of similar average peak frequencies (le Marchand and Piston, 2010). Increased glucagon secretion from normal murine islets have also been noted at high glucose concentrations, with calcium imaging using Fluo4 fluorescent dye showing associated increases in calcium oscillation frequency (le Marchand and Piston, 2010). The hyperglycaemia-induced rise in glucagon output is amplified in T2DM. Glucose's inhibitory effect was seen to be lost in T2DM islets from human donors, with high glucose conditions resulting in increased glucagon production. They

were also found to secrete less glucagon at lower glucose concentrations, contributing to the fasting hyperglycaemia seen in T2DM (Walker *et al.*, 2011; Zhang *et al.*, 2013).

Glucagon secretion in alpha cells is still poorly understood, with one theory suggesting that high glucose concentrations induce closure of all K_{ATP} channels, subsequently inactivating voltage-gated sodium and calcium channels to suppress calcium activity and glucagon secretion (Göpel *et al.*, 2000; Franklin *et al.*, 2005a). This hypothesis is supported, to some extent, by le Marchand and Piston's study, who investigated the effect of glucose on intracellular calcium concentration and glucagon output. They confirmed that K_{ATP} channels were integral to maintaining a set membrane potential at low glucose levels, thereby facilitating influx of calcium via L-type calcium channels to stimulate glucagon-granule exocytosis. Stimulation of glucagon secretion by both arginine and KCl was attenuated by high glucose but this was not accompanied by reductions in intracellular calcium concentration (le Marchand and Piston, 2012). This mismatch between intracellular calcium readouts and glucagon secretion may help explain why this study observed calcium oscillations at high glucose. It is possible that glucoses' suppressive effects do not solely occur via a K_{ATP}-calcium channel pathway, raising the question as to whether measuring intracellular calcium alone is sufficient in understanding alpha cell activity at high glucose concentrations.

Some groups have used cAMP as an alternative measure for alpha cell activity, with high glucose concentrations causing clear reductions in oscillatory cAMP activity and glucagon secretion (Tian *et al.*, 2011; Elliott, Ustione and Piston, 2015). However, as alpha cell cAMP and calcium activity are poorly correlated, the underlying mechanisms may be different. There is a suggestion that cAMP-PKA signalling pathways may underlie glucagon suppression at high glucose (Elliott, Ustione and Piston, 2015), which supports the argument that glucose-mediated glucagon suppression is regulated by paracrine factors, particularly somatostatin and insulin. Increased insulin secretion has been found to accompany reductions in glucagon secretion (le Marchand and Piston, 2012), and inhibition of the insulin receptor causes increased alpha cell cAMP activity (Elliott, Ustione and Piston, 2015). Similarly, somatostatin inhibits glucagon secretion and is associated with reductions in the frequency of alpha cell cAMP oscillations (Elliott, Ustione and Piston, 2015). Therefore, the extent to which glucose inhibits glucagon directly and indirectly, and the possible underlying mechanisms continues to require further interrogation.

Connectivity in beta cells is a well-established phenomenon, with its disruption in T2DM and obesity (Salem *et al.*, 2019; Akalestou *et al.*, 2021) highlighting the importance of cellular coordination in insulin secretion. In contrast, this study found alpha cells to be poorly connected. Whilst the data suggests oscillating alpha cells in an individual islet were more

connected at high glucose concentrations, only the traces of cells in close contact with each other appeared coordinated. This is likely due to junctional cell-cell connections between alpha cells that are essential for efficient glucagon secretion, as evidenced by reaggregation of isolated alpha cells causing reduced basal glucagon secretion (Gromada, Franklin and Wollheim, 2007). Junctional connections are unlikely to be the complete story given not all oscillating cells in contact with an adjacent oscillating cell had coordinated calcium traces. Furthermore, activity patterns at any glucose concentration varied between islets of the same mouse and even between alpha cells of the same islet. This corroborated the findings of previous studies who found calcium activity to be asynchronous, suggesting heterogeneity of the alpha cell population (Quesada *et al.*, 2006; Noguchi *et al.*, 2021). Due to the absence of intra- and inter-islet coordination, the dominance of non-oscillatory cells and the overall irregularity in calcium activity, it may not be appropriate to apply connectivity to alpha cells. Furthermore, recent data sheds light on the possibility that coordinated glucagon release from alpha cells may be under beta cell control. Ren *et al* found alpha cell calcium peaks to occur approximately 20 seconds after beta cell calcium peaks, with glucose concentrations of 10mM causing synchronisation of all oscillatory alpha and beta cells (Ren *et al.*, 2022). However, this does not account for this study's finding of poor alpha cell connectivity at low glucose concentrations, an environment where beta cells are quiet. The cellular mechanisms behind the pulsatile glucagon secretion seen *in-vivo* (Menge *et al.*, 2011), therefore, remains unknown.

GLP-1 agonists are a growing group of anti-diabetic therapies which work by increasing insulin secretion and suppressing glucagon in hyperglycaemia, with this dual effect being thought to underlie their high efficacy (Hare *et al.*, 2010). Despite its significant glucose-lowering effect, monotherapy with GLP-1 agonists infer a low risk of hypoglycaemia (Trujillo, Nuffer and Smith, 2021) due to its ability to also stimulate glucagon in low glucose environments (Zhang *et al.*, 2019). In this study, however, alpha cells were only noted to have significant oscillatory activity during GLP-1 agonist experiments conducted at high glucose, a pattern that has been similarly observed by Moens *et al* in their cAMP experiments on purified alpha cells (Moens *et al.*, 1996). In terms of biased agonists, whilst no statistical differences were seen in peak amplitude or frequency, the trend of ex-phe traces suggest that it may influence alpha cell activity more than ex-asp. Correlation of these pulses with glucagon output would provide some preliminary information about the overall physiological consequences of these GLP-1 agonists.

The GLP-1R intracellular signalling pathways in alpha cells is uncertain and it is unlikely to be identical to that seen in beta cells given GLP-1's bidirectional action on glucagon. Some groups suggest GLP-1 directly act on alpha cells via GLP-1 receptors, initiating cAMP-PKA

dependent pathways to inhibit N-, L- and P/Q-type calcium channels, ultimately suppressing glucagon exocytosis (de Marinis *et al.*, 2010; Ramracheya *et al.*, 2018). The distribution of GLP-1R on alpha cells is much lower than that in beta cells (Heller, Kieffer and Habener, 1997; Tornehave *et al.*, 2008), and could explain the low calcium pulsations seen at high glucose concentrations. However, the small receptor number is likely sufficient for normal physiological function, as demonstrated by the failure of GLP-1 to inhibit glucagon in alpha-cell specific GLP-1R knockout mice (Zhang *et al.*, 2019). In addition, alpha cells in both mice and humans have been noted to produce their own GLP-1, with larger quantities noted in T2DM islets (Fava, Dong and Wu, 2016; Campbell *et al.*, 2020). Alongside the alpha cell hyperplasia seen in T2DM, increased production of alpha-cell derived GLP-1 may be an attempt to generate more GLP-1 to promote beta cell regeneration (Lee *et al.*, 2018). The low receptor distribution may be an adaptation to prevent excessive autologous GLP-1R stimulation and/or GLP-1R desensitisation, given the role of alpha cells in times of metabolic stress. Nonetheless, if truly functionally similar and efficacious to that produced by intestinal L-cells, alpha cells could become a therapeutic target, where agents aim to coax alpha cells to produce more GLP-1.

There is also evidence to strengthen the hypothesis that GLP-1 exerts its effect on alpha cells via paracrine mechanisms. De Heer *et al* showed that somatostatin receptor antagonists eliminated GLP-1's suppressive effects on glucagon, suggesting delta cells may mediate inhibition of alpha cells by GLP-1 (de Heer *et al.*, 2008). As glucagon's counter-regulatory hormone, insulin also directly inhibits glucagon secretion. Given that GLP-1 augments insulin production and secretion, it is possible that GLP-1 acts via insulin to indirectly affect alpha cell output (Franklin *et al.*, 2005b; Unger and Orci, 2010). This theory, however, struggles to explain how GLP-1 retains its glucagon suppressive effects in individuals with type 1 diabetes who do not have any beta cells (Kielgast *et al.*, 2010).

There were limitations to this study. Firstly, the n number is small, amplified by the small number of oscillating alpha cells. However, in the limited project period, this study has established a new mouse model and robust experimental protocol to enable completion of the dataset. Secondly, only one Z axis per islet was imageable at any one time, limiting the number of alpha cells included and preventing junctional relationships to be fully interrogated. Keeping to one plane of view helped ensure multiple images were collected in a short period of time, enabling short bursts of calcium activity to be captured. More sophisticated imaging set ups would be needed to retain the speed of image acquisition whilst obtaining multiple Z axis views. Finally, due to the genetic fluorescence, only alpha cells were imaged. Whilst it may be logistically challenging, investigating the simultaneous beta and delta cell activity would give a better picture of the paracrine influences exerted on alpha cells.

The underlying mechanisms of alpha cell glucagon secretion and GLP-1 modulation continue to require further research, including the need to determine the optimal surrogate marker for alpha cell electrical activity. Although essential to determine normal alpha cell activity, experiments would need to be repeated in T2DM islets, particularly as intracellular signalling is thought to be altered by diabetic conditions (Nakashima *et al.*, 2018). Given the promising outcomes associated with the dual GLP-1/GIP agonist, tirzepatide, in T2DM patients (Frias *et al.*, 2018), investigating the effect of gastric inhibitory polypeptide (GIP) and tirzepatide on alpha cell activity would help provide a more holistic picture of incretin-mediated glucagon suppression. Furthermore, repeating alpha cell experiments in human islets and pancreatic tissue would help validate theories derived from animal models. This would ensure applicability to clinical medicine and would help guide future new T2DM therapies, including islet-replacement endeavours.

In conclusion, this project was able to validate a novel transgenic mouse line to investigate alpha cell calcium dynamics. Alpha cells are equally active at high and low glucose concentrations and do not demonstrate significant pan-islet or inter-islet connectivity. Alpha cells may be more receptive to the effects of GLP-1 agonists at high glucose, which could be exploited therapeutically, but further research is needed to understand the mechanisms underlying alpha cell glucagon secretion.

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References

Abdullah, A. *et al.* (2010) “The magnitude of association between overweight and obesity and the risk of diabetes: A meta-analysis of prospective cohort studies,” *Diabetes Research and Clinical Practice*, 89(3), pp. 309–319. Available at: https://doi.org/10.1016/j.diabres.2010.04.012.

Adams, M.T. and Blum, B. (2022) “Determinants and dynamics of pancreatic islet architecture,” *Islets*, 14(1), pp. 82–100. Available at: https://doi.org/10.1080/19382014.2022.2030649.

Ahlqvist, E. *et al.* (2018) “Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables,” *The Lancet Diabetes & Endocrinology*, 6(5), pp. 361–369. Available at: https://doi.org/10.1016/S2213-8587(18)30051-2.

Akalestou, E. *et al.* (2021) “Intravital imaging of islet Ca2+ dynamics reveals enhanced β cell connectivity after bariatric surgery in mice,” *Nature Communications*, 12(1), p. 5165. Available at: https://doi.org/10.1038/s41467-021-25423-8.

Astrup, A. *et al.* (2009) “Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study,” *The Lancet*, 374(9701), pp. 1606–1616. Available at: https://doi.org/10.1016/S0140-6736(09)61375-1.

Banarer, S., McGregor, V.P. and Cryer, P.E. (2002) “Intraislet Hyperinsulinemia Prevents the Glucagon Response to Hypoglycemia Despite an Intact Autonomic Response,” *Diabetes*, 51(4), pp. 958–965. Available at: https://doi.org/10.2337/diabetes.51.4.958.

Baron, A.D. *et al.* (1987) “Role of Hyperglucagonemia in Maintenance of Increased Rates of Hepatic Glucose Output in Type II Diabetics,” *Diabetes*, 36(3), pp. 274–283. Available at: https://doi.org/10.2337/diab.36.3.274.

BBC Health (2018) *Diabetes prescriptions now cost NHS £1bn, figures show*. Available at: https://www.bbc.co.uk/news/health-46139595 (Accessed: April 23, 2022).

Bergman, R.N., Phillips, L.S. and Cobelli, C. (1981) “Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose.,” *Journal of Clinical Investigation*, 68(6), pp. 1456–1467. Available at: https://doi.org/10.1172/JCI110398.

Bitsi, S. *et al.* (2022) “Divergent acute versus prolonged in vivo GLP-1R responses in β-arrestin 2-deleted primary beta cells,” *Biorxiv* [Preprint].

Blodgett, D.M. *et al.* (2015) “Novel Observations From Next-Generation RNA Sequencing of Highly Purified Human Adult and Fetal Islet Cell Subsets,” *Diabetes*, 64(9), pp. 3172–3181. Available at: https://doi.org/10.2337/db15-0039.

Blüher, M. *et al.* (2022) “Managing weight and glycaemic targets in people with type 2 diabetes—How far have we come?,” *Endocrinology, Diabetes & Metabolism*, 5(3). Available at: https://doi.org/10.1002/edm2.330.

Brereton, M.F. *et al.* (2015) “Alpha-, Delta- and PP-cells: Are They the Architectural Cornerstones of Islet Structure and Co-ordination?,” *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*, 63(8), pp. 575–91. Available at: https://doi.org/10.1369/0022155415583535.

Briant, L.J.B. *et al.* (2018) “δ-cells and β-cells are electrically coupled and regulate α-cell activity via somatostatin,” *The Journal of Physiology*, 596(2), pp. 197–215. Available at: https://doi.org/10.1113/JP274581.

Brown, E. *et al.* (2019) “Weight loss variability with SGLT2 inhibitors and GLP-1 receptor agonists in type 2 diabetes mellitus and obesity: Mechanistic possibilities,” *Obesity Reviews*, 20(6), pp. 816–828. Available at: https://doi.org/10.1111/obr.12841.

Bru-Tari, E. *et al.* (2019) “Pancreatic alpha-cell mass in the early-onset and advanced stage of a mouse model of experimental autoimmune diabetes,” *Scientific Reports*, 9(1), p. 9515. Available at: https://doi.org/10.1038/s41598-019-45853-1.

Butler, A.E. *et al.* (2003) “β-Cell Deficit and Increased β-Cell Apoptosis in Humans With Type 2 Diabetes,” *Diabetes*, 52(1), pp. 102–110. Available at: https://doi.org/10.2337/diabetes.52.1.102.

Cabrera, O. *et al.* (2006) “The unique cytoarchitecture of human pancreatic islets has implications for islet cell function,” *PNAS*, 103. Available at: https://doi.org/10.1073/pnas.0510790103.

Campbell, S.A. *et al.* (2020) “Human islets contain a subpopulation of glucagon-like peptide-1 secreting α cells that is increased in type 2 diabetes,” *Molecular Metabolism*, 39, p. 101014. Available at: https://doi.org/10.1016/j.molmet.2020.101014.

Capozzi, M.E. *et al.* (2019) “Glucagon lowers glycemia when β cells are active,” *JCI Insight*, 4(16). Available at: https://doi.org/10.1172/jci.insight.129954.

Cinti, F. *et al.* (2016) “Evidence of β-Cell Dedifferentiation in Human Type 2 Diabetes,” *The Journal of Clinical Endocrinology & Metabolism*, 101(3), pp. 1044–1054. Available at: https://doi.org/10.1210/jc.2015-2860.

Davies, M. *et al.* (2021) “Semaglutide 2·4 mg once a week in adults with overweight or obesity, and type 2 diabetes (STEP 2): a randomised, double-blind, double-dummy, placebo-controlled, phase 3 trial,” *The Lancet*, 397(10278), pp. 971–984. Available at: https://doi.org/10.1016/S0140-6736(21)00213-0.

Davies, M.J. *et al.* (2015) “Efficacy of Liraglutide for Weight Loss Among Patients With Type 2 Diabetes,” *JAMA*, 314(7), p. 687. Available at: https://doi.org/10.1001/jama.2015.9676.

DeFronzo, R.A. (1988) “The Triumvirate: β-Cell, Muscle, Liver: A Collusion Responsible for NIDDM,” *Diabetes*, 37(6), pp. 667–687. Available at: https://doi.org/10.2337/diab.37.6.667.

DeFronzo, R.A., Ferrannini, E. and Simonson, D.C. (1989) “Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: Contributions of excessive hepatic glucose production and impaired tissue glucose uptake,” *Metabolism*, 38(4), pp. 387–395. Available at: https://doi.org/10.1016/0026-0495(89)90129-7.

Diabetes UK (2017) *The Cost of Diabetes Report*. Available at: https://www.diabetes.org.uk/resources-s3/2017-11/diabetes%20uk%20cost%20of%20diabetes%20report.pdf (Accessed: April 23, 2022).

Diabetes UK (2022) *Type 2 diabetes*. Available at: https://www.diabetes.org.uk/diabetes-the-basics/types-of-diabetes/type-2 (Accessed: April 23, 2022).

Elbein, S.C. *et al.* (1999) “Heritability of Pancreatic β-Cell Function among Nondiabetic Members of Caucasian Familial Type 2 Diabetic Kindreds,” *The Journal of Clinical Endocrinology & Metabolism*, 84(4), pp. 1398–1403. Available at: https://doi.org/10.1210/jcem.84.4.5604.

Elbein, S.C., Wegner, K. and Kahn, S.E. (2000) “Reduced beta-cell compensation to the insulin resistance associated with obesity in members of caucasian familial type 2 diabetic kindreds.,” *Diabetes Care*, 23(2), pp. 221–227. Available at: https://doi.org/10.2337/diacare.23.2.221.

Elliott, A.D., Ustione, A. and Piston, D.W. (2015) “Somatostatin and insulin mediate glucose-inhibited glucagon secretion in the pancreatic α-cell by lowering cAMP,” *American Journal of Physiology-Endocrinology and Metabolism*, 308(2), pp. E130–E143. Available at: https://doi.org/10.1152/ajpendo.00344.2014.

Fava, G.E., Dong, E.W. and Wu, H. (2016) “Intra-islet glucagon-like peptide 1,” *Journal of Diabetes and its Complications*, 30(8), pp. 1651–1658. Available at: https://doi.org/10.1016/j.jdiacomp.2016.05.016.

Fehmann, H.-C. *et al.* (1995) “The Effects of Glucagon-like Peptide-I (GLP-I) on Hormone Secretion from Isolated Human Pancreatic Islets,” *Pancreas*, 11(2), p. 196. Available at: https://doi.org/10.1097/00006676-199508000-00014.

Franklin, I. *et al.* (2005a) “β-Cell Secretory Products Activate α-Cell ATP-Dependent Potassium Channels to Inhibit Glucagon Release,” *Diabetes*, 54(6), pp. 1808–1815. Available at: https://doi.org/10.2337/diabetes.54.6.1808.

Franklin, I. *et al.* (2005b) “β-Cell Secretory Products Activate α-Cell ATP-Dependent Potassium Channels to Inhibit Glucagon Release,” *Diabetes*, 54(6), pp. 1808–1815. Available at: https://doi.org/10.2337/diabetes.54.6.1808.

Frias, J.P. *et al.* (2018) “Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor agonist, in patients with type 2 diabetes: a randomised, placebo-controlled and active comparator-controlled phase 2 trial,” *The Lancet*, 392(10160), pp. 2180–2193. Available at: https://doi.org/10.1016/S0140-6736(18)32260-8.

Fuchsberger, C. *et al.* (2016) “The genetic architecture of type 2 diabetes,” *Nature*, 536(7614), pp. 41–47. Available at: https://doi.org/10.1038/nature18642.

Gelling, R.W. *et al.* (2009) “Pancreatic β-cell overexpression of the glucagon receptor gene results in enhanced β-cell function and mass,” *American Journal of Physiology-Endocrinology and Metabolism*, 297(3), pp. E695–E707. Available at: https://doi.org/10.1152/ajpendo.00082.2009.

Gerich, J.E. *et al.* (1975) “Prevention of Human Diabetic Ketoacidosis by Somatostatin,” *New England Journal of Medicine*, 292(19), pp. 985–989. Available at: https://doi.org/10.1056/NEJM197505082921901.

González-Vélez, V. *et al.* (2012) “Model for Glucagon Secretion by Pancreatic α-Cells,” *PLoS ONE*, 7(3), p. e32282. Available at: https://doi.org/10.1371/journal.pone.0032282.

González-Vélez, V., Piron, A. and Dupont, G. (2020) “Calcium Oscillations in Pancreatic α-cells Rely on Noise and ATP-Driven Changes in Membrane Electrical Activity,” *Frontiers in Physiology*, 11. Available at: https://doi.org/10.3389/fphys.2020.602844.

Göpel, S.O. *et al.* (2000) “Regulation of glucagon release in mouse α-cells by K ATP channels and inactivation of TTX-sensitive Na+ channels,” *The Journal of Physiology*, 528(3), pp. 509–520. Available at: https://doi.org/10.1111/j.1469-7793.2000.00509.x.

Gromada, J., Franklin, I. and Wollheim, C.B. (2007) “α-Cells of the Endocrine Pancreas: 35 Years of Research but the Enigma Remains,” *Endocrine Reviews*, 28(1), pp. 84–116. Available at: https://doi.org/10.1210/er.2006-0007.

Gumbiner, B. *et al.* (1996) “Abnormalities of insulin pulsatility and glucose oscillations during meals in obese noninsulin-dependent diabetic patients: effects of weight reduction.,” *The Journal of Clinical Endocrinology & Metabolism*, 81(6), pp. 2061–2068. Available at: https://doi.org/10.1210/jcem.81.6.8964829.

Halban, P.A. *et al.* (1982) “The Possible Importance of Contact between Pancreatic Islet Cells for the Control of Insulin Release*,” *Endocrinology*, 111(1), pp. 86–94. Available at: https://doi.org/10.1210/endo-111-1-86.

Hare, K.J. *et al.* (2010) “The Glucagonostatic and Insulinotropic Effects of Glucagon-Like Peptide 1 Contribute Equally to Its Glucose-Lowering Action,” *Diabetes*, 59(7), pp. 1765–1770. Available at: https://doi.org/10.2337/db09-1414.

de Heer, J. *et al.* (2008) “Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas,” *Diabetologia*, 51(12), pp. 2263–2270. Available at: https://doi.org/10.1007/s00125-008-1149-y.

Heller, R.S., Kieffer, T.J. and Habener, J.F. (1997) “Insulinotropic Glucagon-Like Peptide I Receptor Expression in Glucagon-Producing α-Cells of the Rat Endocrine Pancreas,” *Diabetes*, 46(5), pp. 785–791. Available at: https://doi.org/10.2337/diab.46.5.785.

Henquin, J.C. and Rahier, J. (2011) “Pancreatic alpha cell mass in European subjects with type 2 diabetes,” *Diabetologia*, 54(7), pp. 1720–1725. Available at: https://doi.org/10.1007/s00125-011-2118-4.

Hex, N. *et al.* (2012) “Estimating the current and future costs of Type 1 and Type 2 diabetes in the UK, including direct health costs and indirect societal and productivity costs,” *Diabetic Medicine*, 29(7), pp. 855–862. Available at: https://doi.org/10.1111/j.1464-5491.2012.03698.x.

Hoang, D.-T., Hara, M. and Jo, J. (2016) “Design Principles of Pancreatic Islets: Glucose-Dependent Coordination of Hormone Pulses,” *PLOS ONE*, 11(4), p. e0152446. Available at: https://doi.org/10.1371/journal.pone.0152446.

Hodson, D.J. *et al.* (2013) “Lipotoxicity disrupts incretin-regulated human β cell connectivity,” *Journal of Clinical Investigation*, 123(10), pp. 4182–4194. Available at: https://doi.org/10.1172/JCI68459.

Huising, M.O. *et al.* (2018) “The Difference δ-Cells Make in Glucose Control,” *Physiology*, 33(6), pp. 403–411. Available at: https://doi.org/10.1152/physiol.00029.2018.

International Diabetes Federation (2021a) *IDF Diabetes Atlas, 10th edition*. Available at: https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021.pdf (Accessed: April 23, 2022).

International Diabetes Federation (2021b) *IDF Diabetes Atlas, 10th edition*. Available at: https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021.pdf (Accessed: April 23, 2022).

International Diabetes Federation (2022) *Diabetes around the world in 2021*. Available at: https://diabetesatlas.org/#:~:text=Diabetes%20around%20the%20world%20in%202021%3A,%2D%20and%20middle%2Dincome%20countries. (Accessed: April 23, 2022).

Johnston, N.R. *et al.* (2016a) “Beta Cell Hubs Dictate Pancreatic Islet Responses to Glucose,” *Cell Metabolism*, 24(3), pp. 389–401. Available at: https://doi.org/10.1016/j.cmet.2016.06.020.

Johnston, N.R. *et al.* (2016b) “Beta Cell Hubs Dictate Pancreatic Islet Responses to Glucose,” *Cell Metabolism*, 24(3), pp. 389–401. Available at: https://doi.org/10.1016/j.cmet.2016.06.020.

Jones, B. *et al.* (2018) “Targeting GLP-1 receptor trafficking to improve agonist efficacy,” *Nature Communications*, 9(1), p. 1602. Available at: https://doi.org/10.1038/s41467-018-03941-2.

Kahn, S.E. *et al.* (1993) “Quantification of the Relationship Between Insulin Sensitivity and β-Cell Function in Human Subjects: Evidence for a Hyperbolic Function,” *Diabetes*, 42(11), pp. 1663–1672. Available at: https://doi.org/10.2337/diab.42.11.1663.

Kellard, J.A. *et al.* (2020) “Reduced somatostatin signalling leads to hypersecretion of glucagon in mice fed a high-fat diet,” *Molecular Metabolism*, 40, p. 101021. Available at: https://doi.org/10.1016/j.molmet.2020.101021.

Kielgast, U. *et al.* (2010) “Effect of Glucagon-Like Peptide-1 on α- and β-Cell Function in C-Peptide-Negative Type 1 Diabetic Patients,” *The Journal of Clinical Endocrinology & Metabolism*, 95(5), pp. 2492–2496. Available at: https://doi.org/10.1210/jc.2009-2440.

Kielgast, U., Holst, J.J. and Madsbad, S. (2011) “Antidiabetic Actions of Endogenous and Exogenous GLP-1 in Type 1 Diabetic Patients With and Without Residual β-Cell Function,” *Diabetes*, 60(5), pp. 1599–1607. Available at: https://doi.org/10.2337/db10-1790.

Klec, C. *et al.* (2019) “Calcium Signaling in ß-cell Physiology and Pathology: A Revisit,” *International Journal of Molecular Sciences*, 20(24), p. 6110. Available at: https://doi.org/10.3390/ijms20246110.

Klen, J. and Dolžan, V. (2022) “Glucagon-like Peptide-1 Receptor Agonists in the Management of Type 2 Diabetes Mellitus and Obesity: The Impact of Pharmacological Properties and Genetic Factors,” *International Journal of Molecular Sciences*, 23(7), p. 3451. Available at: https://doi.org/10.3390/ijms23073451.

Klöppel, G. *et al.* (1985) “Islet Pathology and the Pathogenesis of Type 1 and Type 2 Diabetes mellitus Revisited,” *Pathology and Immunopathology Research*, 4(2), pp. 110–125. Available at: https://doi.org/10.1159/000156969.

KOMJATI, M., BRATUSCH-MARRAIN, P. and WALDHÄUSL, W. (1986) “Superior Efficacy of Pulsatile vs Continuous Hormone Exposure on Hepatic Glucose Production in-vitro,” *Endocrinology*, 118(1), pp. 312–319. Available at: https://doi.org/10.1210/endo-118-1-312.

Lang, D.A. *et al.* (1979) “Cyclic Oscillations of Basal Plasma Glucose and Insulin Concentrations in Human Beings,” *New England Journal of Medicine*, 301(19), pp. 1023–1027. Available at: https://doi.org/10.1056/NEJM197911083011903.

Lang, D.A. *et al.* (1981) “Brief, Irregular Oscillations of Basal Plasma Insulin and Glucose Concentrations in Diabetic Man,” *Diabetes*, 30(5), pp. 435–439. Available at: https://doi.org/10.2337/diab.30.5.435.

Laporte, S.A. and Scott, M.G.H. (2019) “β-Arrestins: Multitask Scaffolds Orchestrating the Where and When in Cell Signalling,” in, pp. 9–55. Available at: https://doi.org/10.1007/978-1-4939-9158-7_2.

Lee, Y. *et al.* (2011) “Glucagon Receptor Knockout Prevents Insulin-Deficient Type 1 Diabetes in Mice,” *Diabetes*, 60(2), pp. 391–397. Available at: https://doi.org/10.2337/db10-0426.

Lee, Y.-S. *et al.* (2018) “Glucagon-Like Peptide 1 Increases β-Cell Regeneration by Promoting α- to β-Cell Transdifferentiation,” *Diabetes*, 67(12), pp. 2601–2614. Available at: https://doi.org/10.2337/db18-0155.

Leung, M.-Y.M. *et al.* (2015) “Life Years Lost and Lifetime Health Care Expenditures Associated With Diabetes in the U.S., National Health Interview Survey, 1997–2000,” *Diabetes Care*, 38(3), pp. 460–468. Available at: https://doi.org/10.2337/dc14-1453.

Liljenquist, J.E. *et al.* (1977) “Evidence for an important role of glucagon in the regulation of hepatic glucose production in normal man.,” *Journal of Clinical Investigation*, 59(2), pp. 369–374. Available at: https://doi.org/10.1172/JCI108649.

Look AHEAD Research group (2014) “Eight-year weight losses with an intensive lifestyle intervention: The look AHEAD study,” *Obesity*, 22(1), pp. 5–13. Available at: https://doi.org/10.1002/oby.20662.

Malik, V.S., Willett, W.C. and Hu, F.B. (2013) “Global obesity: trends, risk factors and policy implications,” *Nature Reviews Endocrinology*, 9(1), pp. 13–27. Available at: https://doi.org/10.1038/nrendo.2012.199.

le Marchand, S.J. and Piston, D.W. (2010) “Glucose Suppression of Glucagon Secretion,” *Journal of Biological Chemistry*, 285(19), pp. 14389–14398. Available at: https://doi.org/10.1074/jbc.M109.069195.

le Marchand, S.J. and Piston, D.W. (2012) “Glucose Decouples Intracellular Ca2+ Activity from Glucagon Secretion in Mouse Pancreatic Islet Alpha-Cells,” *PLoS ONE*, 7(10), p. e47084. Available at: https://doi.org/10.1371/journal.pone.0047084.

de Marinis, Y.Z. *et al.* (2010) “GLP-1 Inhibits and Adrenaline Stimulates Glucagon Release by Differential Modulation of N- and L-Type Ca2+ Channel-Dependent Exocytosis,” *Cell Metabolism*, 11(6), pp. 543–553. Available at: https://doi.org/10.1016/j.cmet.2010.04.007.

Marzook, A., Tomas, A. and Jones, B. (2021) “The Interplay of Glucagon-Like Peptide-1 Receptor Trafficking and Signalling in Pancreatic Beta Cells,” *Frontiers in Endocrinology*, 12. Available at: https://doi.org/10.3389/fendo.2021.678055.

Matveyenko, A. v. *et al.* (2012) “Pulsatile Portal Vein Insulin Delivery Enhances Hepatic Insulin Action and Signaling,” *Diabetes*, 61(9), pp. 2269–2279. Available at: https://doi.org/10.2337/db11-1462.

Mayendraraj, A., Rosenkilde, M.M. and Gasbjerg, L.S. (2022) “GLP-1 and GIP receptor signaling in beta cells – A review of receptor interactions and co-stimulation,” *Peptides*, 151, p. 170749. Available at: https://doi.org/10.1016/j.peptides.2022.170749.

Menge, B.A. *et al.* (2011) “Loss of Inverse Relationship Between Pulsatile Insulin and Glucagon Secretion in Patients With Type 2 Diabetes,” *Diabetes*, 60(8), pp. 2160–2168. Available at: https://doi.org/10.2337/db11-0251.

Moede, T., Leibiger, I.B. and Berggren, P.-O. (2020) “Alpha cell regulation of beta cell function,” *Diabetologia*, 63(10), pp. 2064–2075. Available at: https://doi.org/10.1007/s00125-020-05196-3.

Moens, K. *et al.* (1996) “Expression and Functional Activity of Glucagon, Glucagon-Like Peptide I, and Glucose-Dependent Insulinotropic Peptide Receptors in Rat Pancreatic Islet Cells,” *Diabetes*, 45(2), pp. 257–261. Available at: https://doi.org/10.2337/diab.45.2.257.

Müller, T.D. *et al.* (2022) “Anti-obesity drug discovery: advances and challenges,” *Nature Reviews Drug Discovery*, 21(3), pp. 201–223. Available at: https://doi.org/10.1038/s41573-021-00337-8.

Nakashima, K. *et al.* (2018) “Pancreatic alpha cells in diabetic rats express active GLP-1 receptor: Endosomal co-localization of GLP-1/GLP-1R complex functioning through intra-islet paracrine mechanism,” *Scientific Reports*, 8(1), p. 3725. Available at: https://doi.org/10.1038/s41598-018-21751-w.

National Institute for Health and Care Excellence (2022a) *Diabetes - type 2: GLP-1 receptor agonists*. Available at: https://cks.nice.org.uk/topics/diabetes-type-2/prescribing-information/glp-1-receptor-agonists/ (Accessed: May 25, 2022).

National Institute for Health and Care Excellence (2022b) *Diabetes - type 2: What are the complications?* Available at: https://cks.nice.org.uk/topics/diabetes-type-2/background-information/complications/ (Accessed: April 28, 2022).

Noguchi, G.M. *et al.* (2021) “Functional Heterogeneity Among Pancreatic Alpha Cells,” *Journal of the Endocrine Society*, 5(Supplement_1), pp. A320–A321. Available at: https://doi.org/10.1210/jendso/bvab048.654.

Noguchi, G.M. and Huising, M.O. (2019) “Integrating the inputs that shape pancreatic islet hormone release,” *Nature Metabolism*, 1(12), pp. 1189–1201. Available at: https://doi.org/10.1038/s42255-019-0148-2.

Omar-Hmeadi, M. *et al.* (2020) “Paracrine control of α-cell glucagon exocytosis is compromised in human type-2 diabetes,” *Nature Communications*, 11(1), p. 1896. Available at: https://doi.org/10.1038/s41467-020-15717-8.

Onakpoya, I.J., Heneghan, C.J. and Aronson, J.K. (2016) “Post-marketing withdrawal of anti-obesity medicinal products because of adverse drug reactions: a systematic review,” *BMC Medicine*, 14(1), p. 191. Available at: https://doi.org/10.1186/s12916-016-0735-y.

O’Rahilly, S., Turner, R.C. and Matthews, D.R. (1988) “Impaired Pulsatile Secretion of Insulin in Relatives of Patients with Non-Insulin-Dependent Diabetes,” *New England Journal of Medicine*, 318(19), pp. 1225–1230. Available at: https://doi.org/10.1056/NEJM198805123181902.

Ørskov, C., Holst, J.J. and Nielsen, O. v. (1988) “Effect of Truncated Glucagon-Like Peptide-1 [Proglucagon-(78–107) amide] on Endocrine Secretion from Pig Pancreas, Antrum, and Nonantral Stomach,” *Endocrinology*, 123(4), pp. 2009–2013. Available at: https://doi.org/10.1210/endo-123-4-2009.

Pimenta, W. (1995) “Pancreatic Beta-Cell Dysfunction as the Primary Genetic Lesion in NIDDM,” *JAMA*, 273(23), p. 1855. Available at: https://doi.org/10.1001/jama.1995.03520470063033.

Pipeleers, D.G. *et al.* (1985) “Interplay of Nutrients and Hormones in the Regulation of Glucagon Release*,” *Endocrinology*, 117(3), pp. 817–823. Available at: https://doi.org/10.1210/endo-117-3-817.

Polonsky, K.S. *et al.* (1988) “Abnormal Patterns of Insulin Secretion in Non-Insulin-Dependent Diabetes Mellitus,” *New England Journal of Medicine*, 318(19), pp. 1231–1239. Available at: https://doi.org/10.1056/NEJM198805123181903.

Quesada, I. *et al.* (2006) “Glucose Induces Opposite Intracellular Ca2+ Concentration Oscillatory Patterns in Identified α- and β-Cells Within Intact Human Islets of Langerhans,” *Diabetes*, 55(9), pp. 2463–2469. Available at: https://doi.org/10.2337/db06-0272.

Quoix, N. *et al.* (2009) “Glucose and Pharmacological Modulators of ATP-Sensitive K+ Channels Control [Ca2+]c by Different Mechanisms in Isolated Mouse α-Cells,” *Diabetes*, 58(2), pp. 412–421. Available at: https://doi.org/10.2337/db07-1298.

Rahier, J. *et al.* (2008) “Pancreatic β-cell mass in European subjects with type 2 diabetes,” *Diabetes, Obesity and Metabolism*, 10, pp. 32–42. Available at: https://doi.org/10.1111/j.1463-1326.2008.00969.x.

Ramracheya, R. *et al.* (2018) “GLP-1 suppresses glucagon secretion in human pancreatic alpha-cells by inhibition of P/Q-type Ca2+ channels,” *Physiological Reports*, 6(17), p. e13852. Available at: https://doi.org/10.14814/phy2.13852.

Raskin, P. and Unger, R.H. (1978) “Hyperglucagonemia and Its Suppression,” *New England Journal of Medicine*, 299(9), pp. 433–436. Available at: https://doi.org/10.1056/NEJM197808312990901.

Rodriguez-Diaz, R. *et al.* (2018) “Paracrine Interactions within the Pancreatic Islet Determine the Glycemic Set Point,” *Cell Metabolism*, 27(3), pp. 549-558.e4. Available at: https://doi.org/10.1016/j.cmet.2018.01.015.

Rohrer, S. *et al.* (2012) “Impaired Crosstalk between Pulsatile Insulin and Glucagon Secretion in Prediabetic Individuals,” *The Journal of Clinical Endocrinology & Metabolism*, 97(5), pp. E791–E795. Available at: https://doi.org/10.1210/jc.2011-3439.

Rorsman, P., Braun, M. and Zhang, Q. (2012) “Regulation of calcium in pancreatic α- and β-cells in health and disease,” *Cell Calcium*, 51(3–4), pp. 300–308. Available at: https://doi.org/10.1016/j.ceca.2011.11.006.

Rorsman, P. and Huising, M.O. (2018) “The somatostatin-secreting pancreatic δ-cell in health and disease,” *Nature Reviews Endocrinology*, 14(7), pp. 404–414. Available at: https://doi.org/10.1038/s41574-018-0020-6.

Rutter, G.A. and Hodson, D.J. (2013) “Minireview: Intraislet Regulation of Insulin Secretion in Humans,” *Molecular Endocrinology*, 27(12), pp. 1984–1995. Available at: https://doi.org/10.1210/me.2013-1278.

Sakul, H. *et al.* (1997) “Familiality of physical and metabolic characteristics that predict the development of non-insulin-dependent diabetes mellitus in Pima Indians.,” *American journal of human genetics*, 60(3), pp. 651–6.

Salem, V. *et al.* (2019) “Leader β-cells coordinate Ca2+ dynamics across pancreatic islets in vivo,” *Nature Metabolism*, 1(6), pp. 615–629. Available at: https://doi.org/10.1038/s42255-019-0075-2.

Sherwin, R.S. *et al.* (1976) “Hyperglucagonemia and Blood Glucose Regulation in Normal, Obese and Diabetic Subjects,” *New England Journal of Medicine*, 294(9), pp. 455–461. Available at: https://doi.org/10.1056/NEJM197602262940901.

Shetty, R. *et al.* (2022) “Adverse drug reactions of GLP-1 agonists: A systematic review of case reports,” *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 16(3), p. 102427. Available at: https://doi.org/10.1016/j.dsx.2022.102427.

Singh, S. *et al.* (2017) “Glucagon-like peptide-1 receptor agonists compared with basal insulins for the treatment of type 2 diabetes mellitus: a systematic review and meta-analysis,” *Diabetes, Obesity and Metabolism*, 19(2), pp. 228–238. Available at: https://doi.org/10.1111/dom.12805.

Stumvoll, M., Goldstein, B.J. and van Haeften, T.W. (2005) “Type 2 diabetes: principles of pathogenesis and therapy,” *The Lancet*, 365(9467), pp. 1333–1346. Available at: https://doi.org/10.1016/S0140-6736(05)61032-X.

Talchai, C. *et al.* (2012) “Pancreatic β Cell Dedifferentiation as a Mechanism of Diabetic β Cell Failure,” *Cell*, 150(6), pp. 1223–1234. Available at: https://doi.org/10.1016/j.cell.2012.07.029.

Tan, Q. *et al.* (2022) “Recent Advances in Incretin-Based Pharmacotherapies for the Treatment of Obesity and Diabetes,” *Frontiers in Endocrinology*, 13. Available at: https://doi.org/10.3389/fendo.2022.838410.

The King’s Fund (2022) *The NHS budget and how it has changed*. Available at: https://www.kingsfund.org.uk/projects/nhs-in-a-nutshell/nhs-budget (Accessed: April 23, 2022).

Thorel, F. *et al.* (2010) “Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss,” *Nature*, 464(7292), pp. 1149–1154. Available at: https://doi.org/10.1038/nature08894.

Tian, G. *et al.* (2011) “Glucose- and Hormone-Induced cAMP Oscillations in α- and β-Cells Within Intact Pancreatic Islets,” *Diabetes*, 60(5), pp. 1535–1543. Available at: https://doi.org/10.2337/db10-1087.

Tornehave, D. *et al.* (2008) “Expression of the GLP-1 Receptor in Mouse, Rat, and Human Pancreas,” *Journal of Histochemistry & Cytochemistry*, 56(9), pp. 841–851. Available at: https://doi.org/10.1369/jhc.2008.951319.

Trujillo, J.M., Nuffer, W. and Smith, B.A. (2021) “GLP-1 receptor agonists: an updated review of head-to-head clinical studies,” *Therapeutic Advances in Endocrinology and Metabolism*, 12, p. 204201882199732. Available at: https://doi.org/10.1177/2042018821997320.

Unger, R. and Orci, L. (1975) “The Essential Role of Glucagon in the Pathogenesis of Diabetes Mellitus,” *The Lancet*, 305(7897), pp. 14–16. Available at: https://doi.org/10.1016/S0140-6736(75)92375-2.

Unger, R.H. and Cherrington, A.D. (2012) “Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover,” *Journal of Clinical Investigation*, 122(1), pp. 4–12. Available at: https://doi.org/10.1172/JCI60016.

Unger, R.H. and Grundy, S. (1985) “Hyperglycaemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes,” *Diabetologia*, 28(3), pp. 119–121. Available at: https://doi.org/10.1007/BF00273856.

Unger, R.H. and Orci, L. (2010) “Paracrinology of islets and the paracrinopathy of diabetes,” *Proceedings of the National Academy of Sciences*, 107(37), pp. 16009–16012. Available at: https://doi.org/10.1073/pnas.1006639107.

Vergari, E. *et al.* (2019) “Insulin inhibits glucagon release by SGLT2-induced stimulation of somatostatin secretion,” *Nature Communications*, 10(1), p. 139. Available at: https://doi.org/10.1038/s41467-018-08193-8.

Walker, J.N. *et al.* (2011) “Regulation of glucagon secretion by glucose: paracrine, intrinsic or both?,” *Diabetes, Obesity and Metabolism*, 13, pp. 95–105. Available at: https://doi.org/10.1111/j.1463-1326.2011.01450.x.

Walker, J.T. *et al.* (2021) “The Human Islet: Mini-Organ With Mega-Impact,” *Endocrine Reviews*, 42(5), pp. 605–657. Available at: https://doi.org/10.1210/endrev/bnab010.

Wang, Z. and Liu, M. (2016) “Life years lost associated with diabetes: An individually matched cohort study using the U.S. National Health Interview Survey data,” *Diabetes Research and Clinical Practice*, 118, pp. 69–76. Available at: https://doi.org/10.1016/j.diabres.2016.06.015.

Wendt, A. and Eliasson, L. (2020) “Pancreatic α-cells – The unsung heroes in islet function,” *Seminars in Cell & Developmental Biology*, 103, pp. 41–50. Available at: https://doi.org/10.1016/j.semcdb.2020.01.006.

Weyer, C. *et al.* (1999) “The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus,” *Journal of Clinical Investigation*, 104(6), pp. 787–794. Available at: https://doi.org/10.1172/JCI7231.

Wilding, J.P.H. *et al.* (2021) “Once-Weekly Semaglutide in Adults with Overweight or Obesity,” *New England Journal of Medicine*, 384(11), pp. 989–1002. Available at: https://doi.org/10.1056/NEJMoa2032183.

World Health Organisation (2020) *The top 10 causes of death*. Available at: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death (Accessed: April 23, 2022).

World Health Organisation (2021) *Diabetes*. Available at: https://www.who.int/news-room/fact-sheets/detail/diabetes (Accessed: April 23, 2022).

Yoneda, H. *et al.* (1992) “Analysis of Early-Phase Insulin Responses in Nonobese Subjects With Mild Glucose Intolerance,” *Diabetes Care*, 15(11), pp. 1517–1521. Available at: https://doi.org/10.2337/diacare.15.11.1517.

Yoon, K.H. *et al.* (2003) “Selective β-Cell Loss and α-Cell Expansion in Patients with Type 2 Diabetes Mellitus in Korea,” *The Journal of Clinical Endocrinology & Metabolism*, 88(5), pp. 2300–2308. Available at: https://doi.org/10.1210/jc.2002-020735.

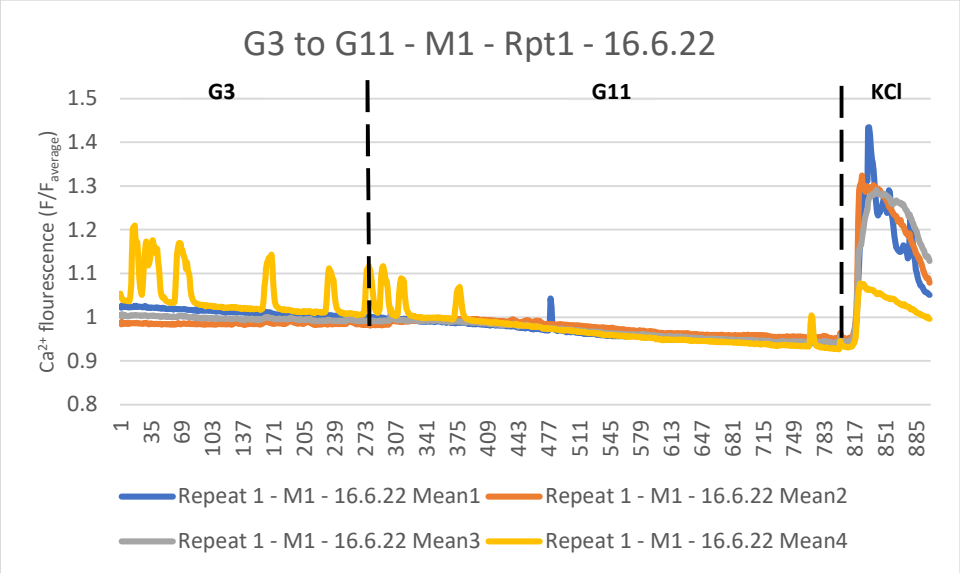
Zhang, Q. *et al.* (2013) “Role of KATP Channels in Glucose-Regulated Glucagon Secretion and Impaired Counterregulation in Type 2 Diabetes,” *Cell Metabolism*, 18(6), pp. 871–882. Available at: https://doi.org/10.1016/j.cmet.2013.10.014.

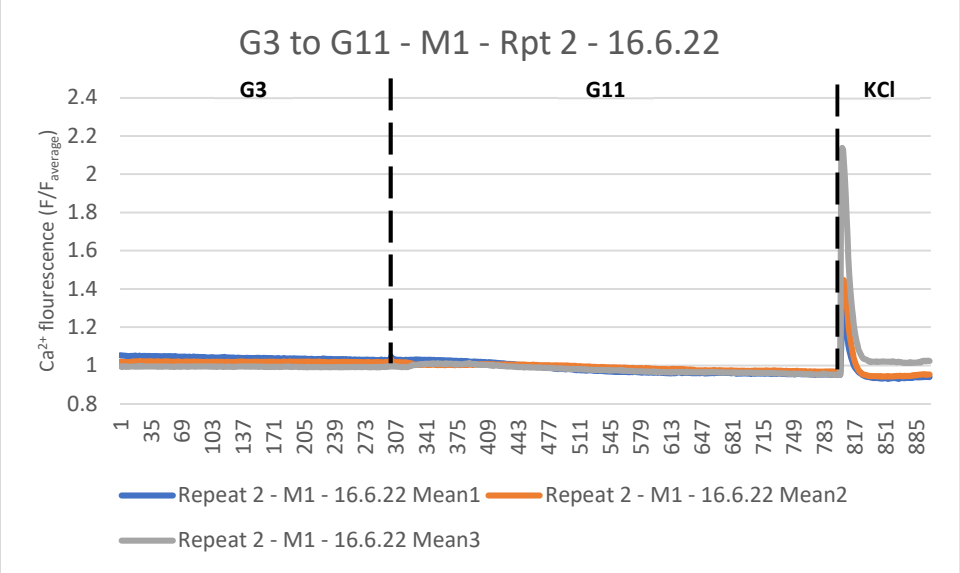
Zhang, Y. *et al.* (2019) “GLP-1 Receptor in Pancreatic α-Cells Regulates Glucagon Secretion in a Glucose-Dependent Bidirectional Manner,” *Diabetes*, 68(1), pp. 34–44. Available at: https://doi.org/10.2337/db18-0317.

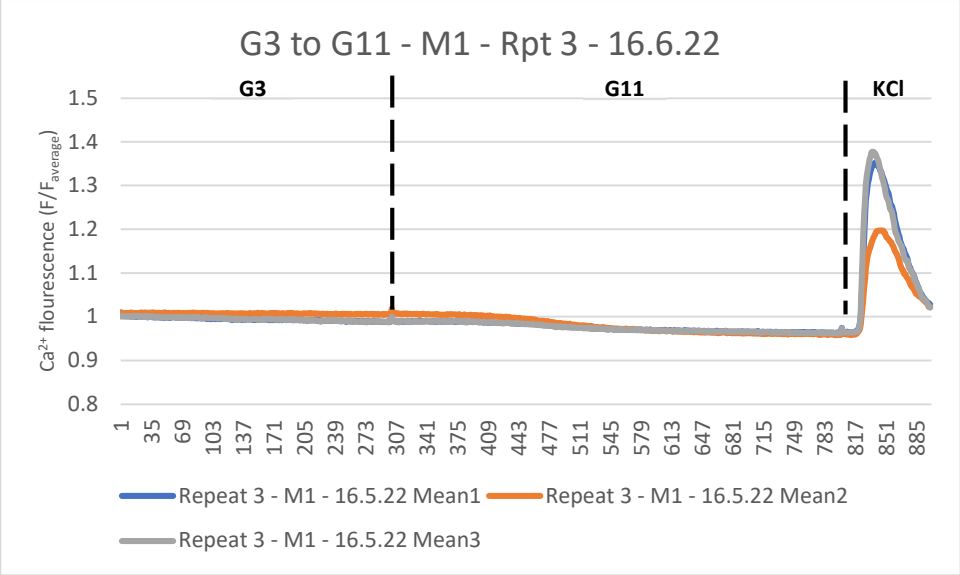
Appendix 1

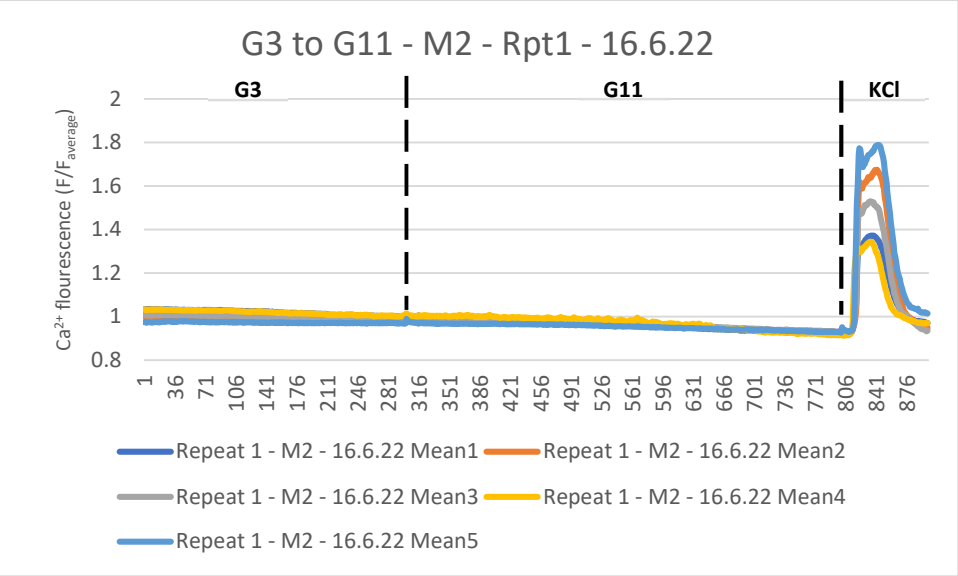
All ROI traces for all alpha cell experiments conducted in varying glucose concentrations. Each graph represents one video (=one islet).

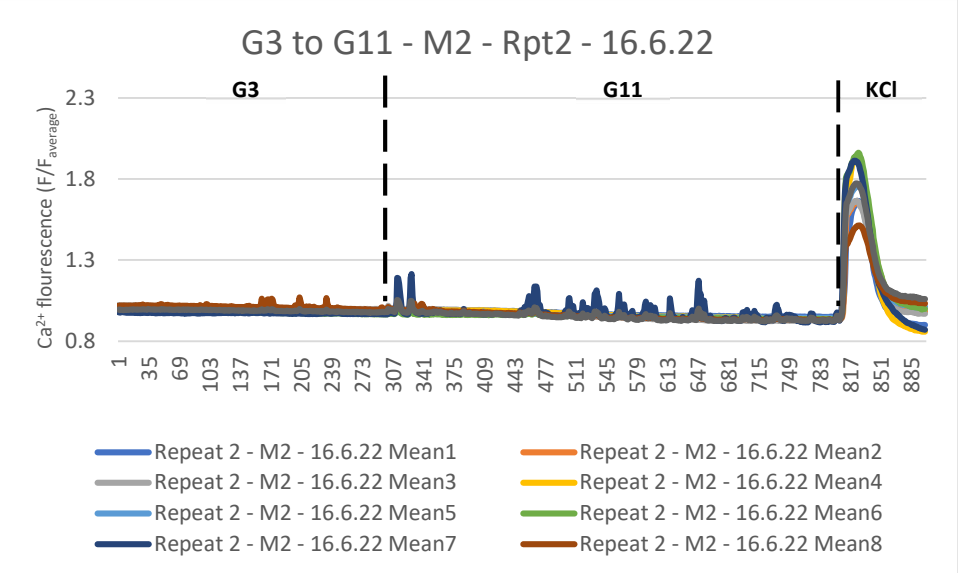
G3 to G11

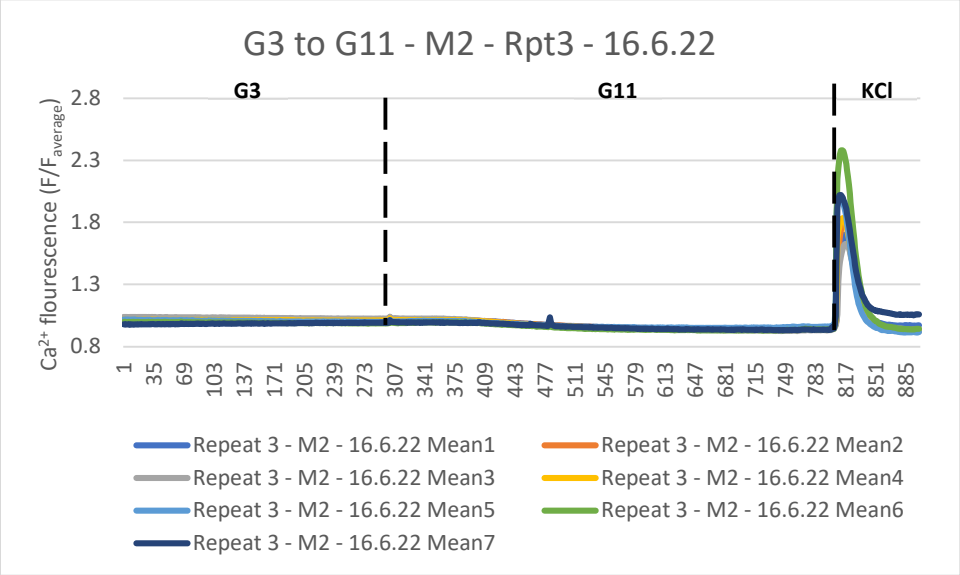


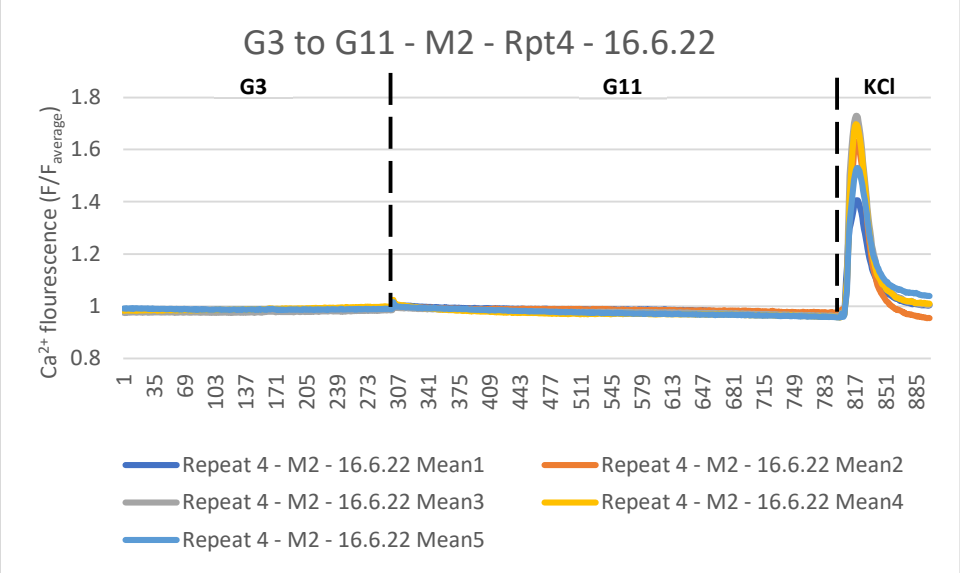


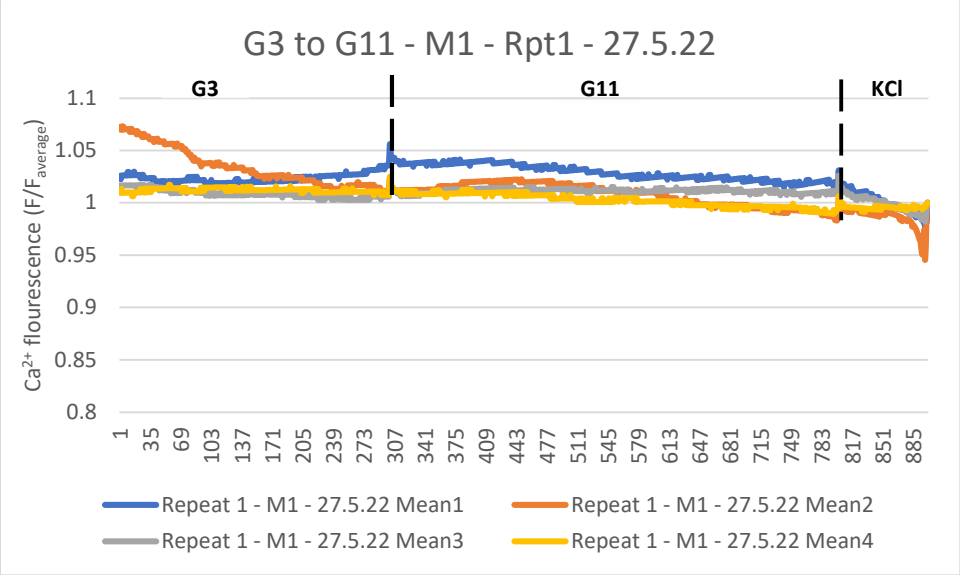

G3 to G11 - M1 - Rpt 3 - 16.6.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
1 35 69 103 137 171 205 239 273 307 341 375 409 443 477 511 545 579 613 647 681 715 749 783 817 851 885
Repeat 3 - M1 - 16.5.22 Mean1
Repeat 3 - M1 - 16.5.22 Mean2
Repeat 3 - M1 - 16.5.22 Mean3

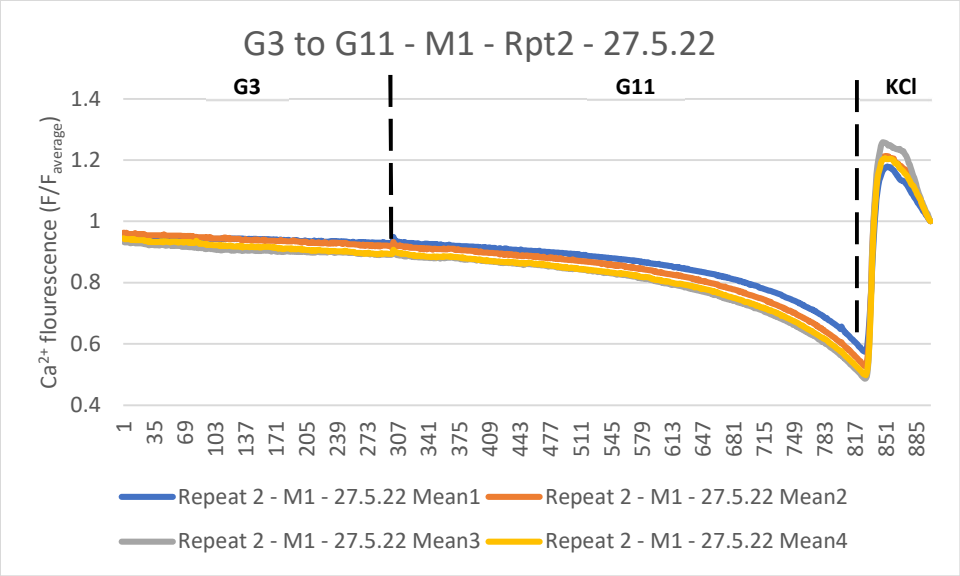

G3 to G11 - M2 - Rpt1 - 16.6.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
2
1.8
1.6
1.4
1.2
1
0.8
1 36 71 106 141 176 211 246 281 316 351 386 421 456 491 526 561 596 631 666 701 736 771 806 841 876
Repeat 1 - M2 - 16.6.22 Mean1
Repeat 1 - M2 - 16.6.22 Mean2
Repeat 1 - M2 - 16.6.22 Mean3
Repeat 1 - M2 - 16.6.22 Mean4
Repeat 1 - M2 - 16.6.22 Mean5

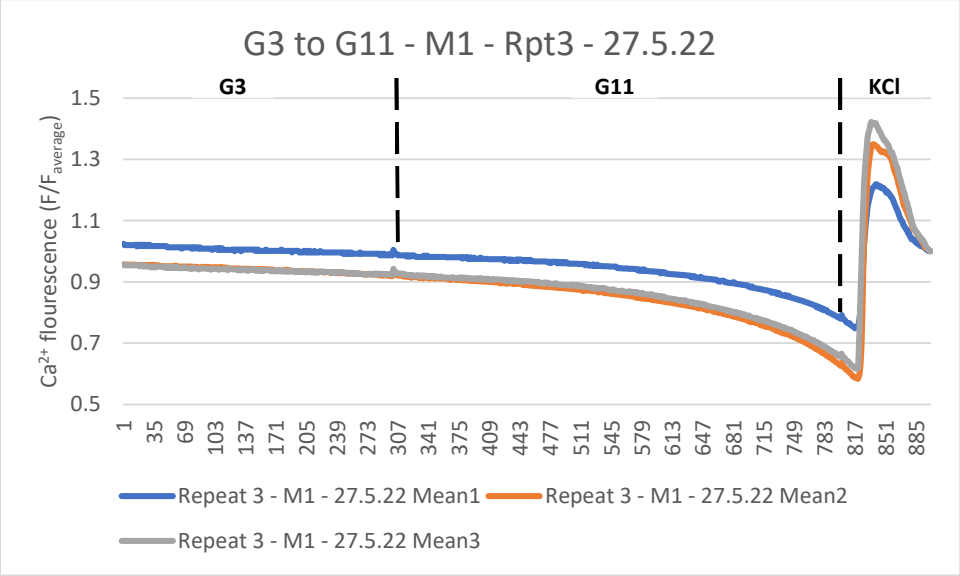

G3 to G11 - M2 - Rpt2 - 16.6.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
2.3
1.8
1.3
0.8
1 35 69 103 137 171 205 239 273 307 341 375 409 443 477 511 545 579 613 647 681 715 749 783 817 851 885
Repeat 2 - M2 - 16.6.22 Mean1
Repeat 2 - M2 - 16.6.22 Mean2
Repeat 2 - M2 - 16.6.22 Mean3
Repeat 2 - M2 - 16.6.22 Mean4
Repeat 2 - M2 - 16.6.22 Mean5
Repeat 2 - M2 - 16.6.22 Mean6
Repeat 2 - M2 - 16.6.22 Mean7
Repeat 2 - M2 - 16.6.22 Mean8

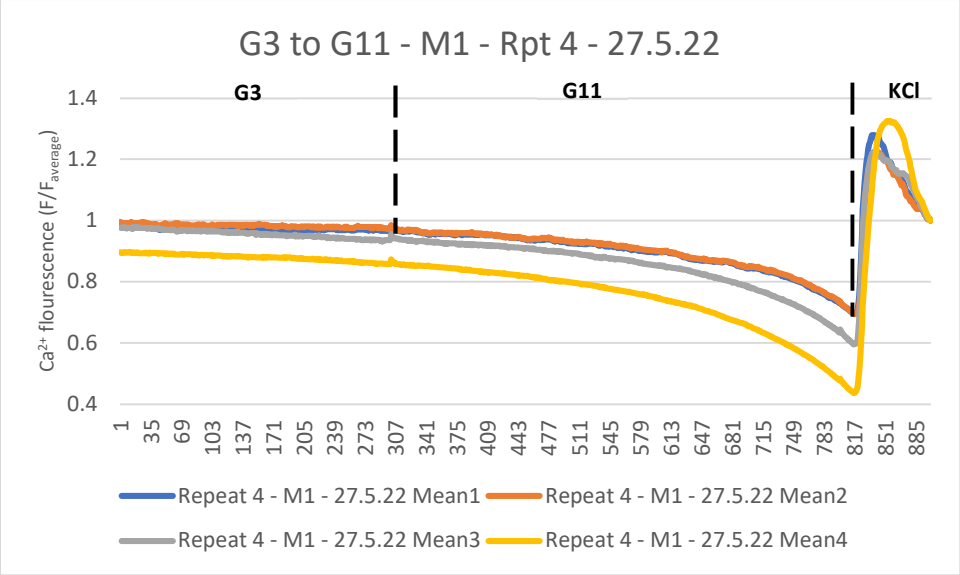

G3 to G11 - M2 - Rpt3 - 16.6.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
2.8
2.3
1.8
1.3
0.8
1 35 69 103 137 171 205 239 273 307 341 375 409 443 477 511 545 579 613 647 681 715 749 783 817 851 885
Repeat 3 - M2 - 16.6.22 Mean1
Repeat 3 - M2 - 16.6.22 Mean2
Repeat 3 - M2 - 16.6.22 Mean3
Repeat 3 - M2 - 16.6.22 Mean4
Repeat 3 - M2 - 16.6.22 Mean5
Repeat 3 - M2 - 16.6.22 Mean6
Repeat 3 - M2 - 16.6.22 Mean7

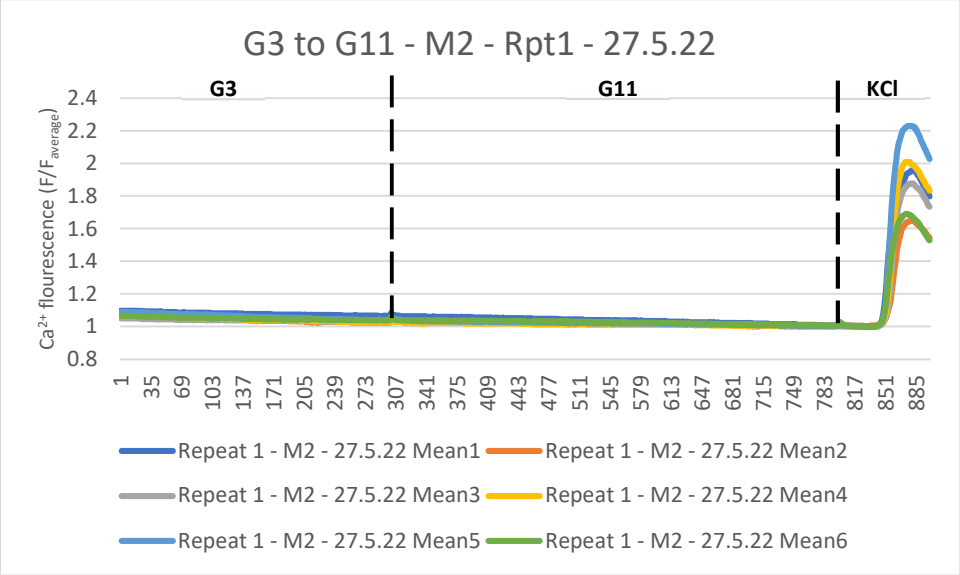

G3 to G11 - M2 - Rpt4 - 16.6.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
1.8
1.6
1.4
1.2
1
0.8
1 35 69 103 137 171 205 239 273 307 341 375 409 443 477 511 545 579 613 647 681 715 749 783 817 851 885
Repeat 4 - M2 - 16.6.22 Mean1
Repeat 4 - M2 - 16.6.22 Mean2
Repeat 4 - M2 - 16.6.22 Mean3
Repeat 4 - M2 - 16.6.22 Mean4
Repeat 4 - M2 - 16.6.22 Mean5

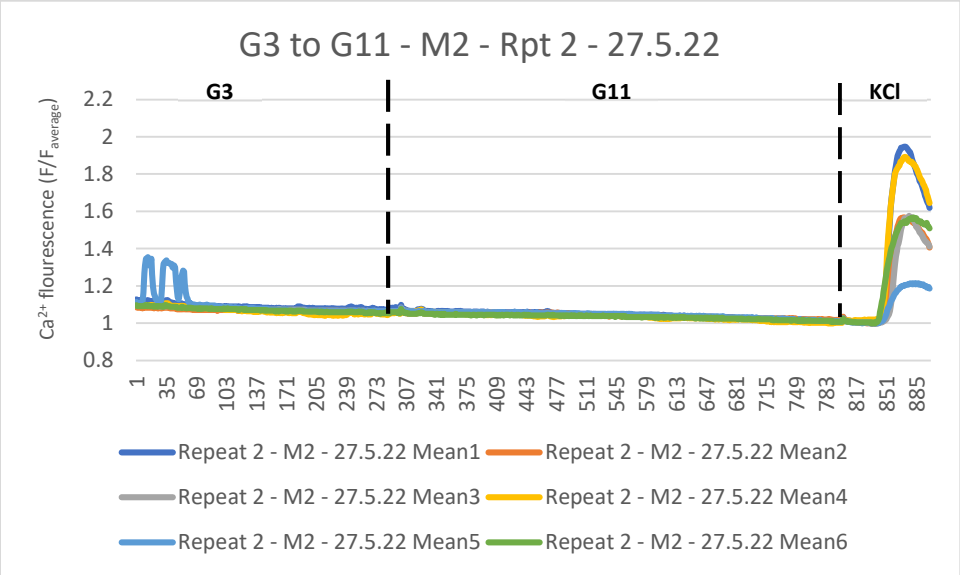

G3 to G11 - M1 - Rpt1 - 27.5.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
Repeat 1 - M1 - 27.5.22 Mean1
Repeat 1 - M1 - 27.5.22 Mean2
Repeat 1 - M1 - 27.5.22 Mean3
Repeat 1 - M1 - 27.5.22 Mean4

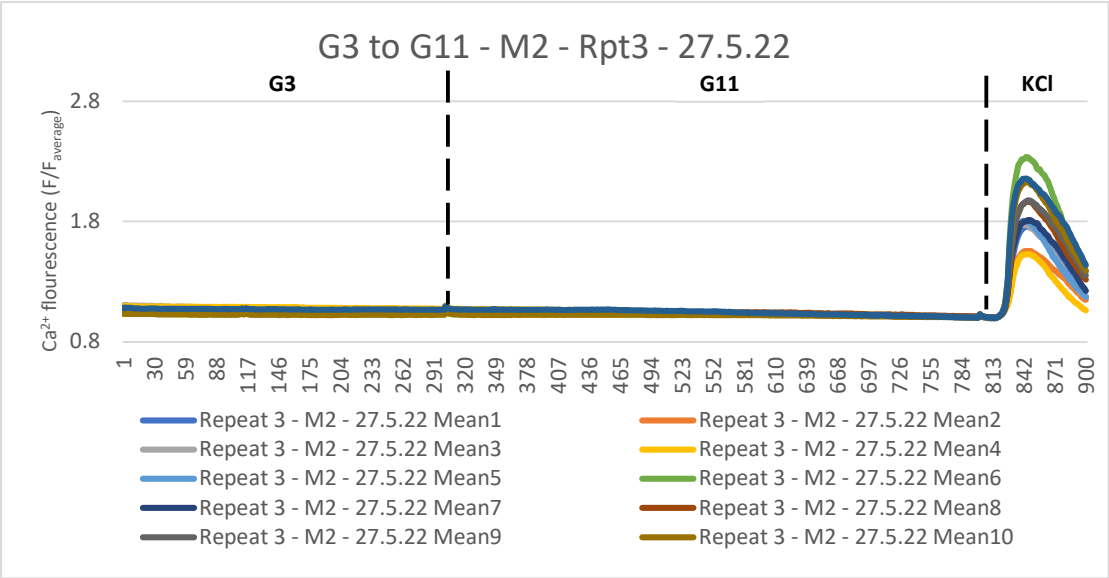

G3 to G11 - M1 - Rpt2 - 27.5.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
Repeat 2 - M1 - 27.5.22 Mean1
Repeat 2 - M1 - 27.5.22 Mean2
Repeat 2 - M1 - 27.5.22 Mean3
Repeat 2 - M1 - 27.5.22 Mean4

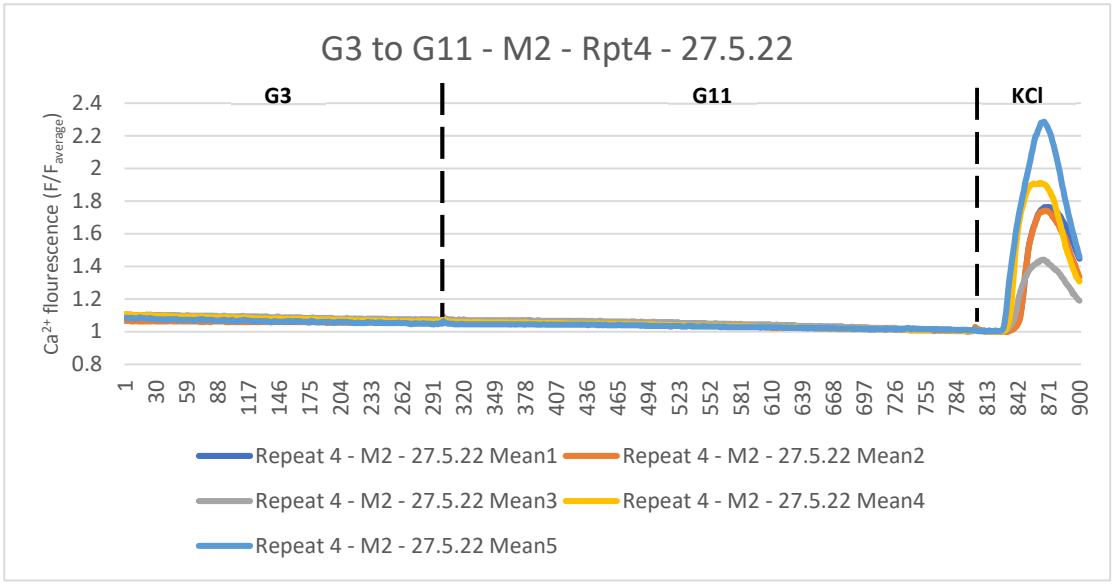

G3 to G11 - M1 - Rpt3 - 27.5.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
Repeat 3 - M1 - 27.5.22 Mean1
Repeat 3 - M1 - 27.5.22 Mean2
Repeat 3 - M1 - 27.5.22 Mean3


G3 to G11 - M1 - Rpt 4 - 27.5.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
Repeat 4 - M1 - 27.5.22 Mean1
Repeat 4 - M1 - 27.5.22 Mean2
Repeat 4 - M1 - 27.5.22 Mean3
Repeat 4 - M1 - 27.5.22 Mean4

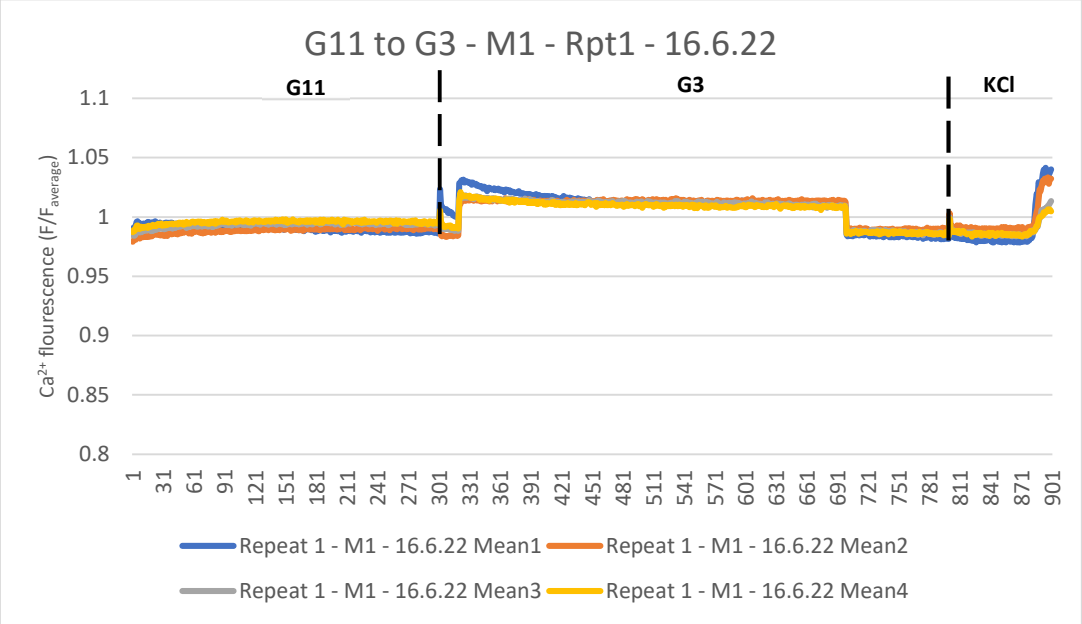

G3 to G11 - M2 - Rpt1 - 27.5.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
Repeat 1 - M2 - 27.5.22 Mean1
Repeat 1 - M2 - 27.5.22 Mean2
Repeat 1 - M2 - 27.5.22 Mean3
Repeat 1 - M2 - 27.5.22 Mean4
Repeat 1 - M2 - 27.5.22 Mean5
Repeat 1 - M2 - 27.5.22 Mean6

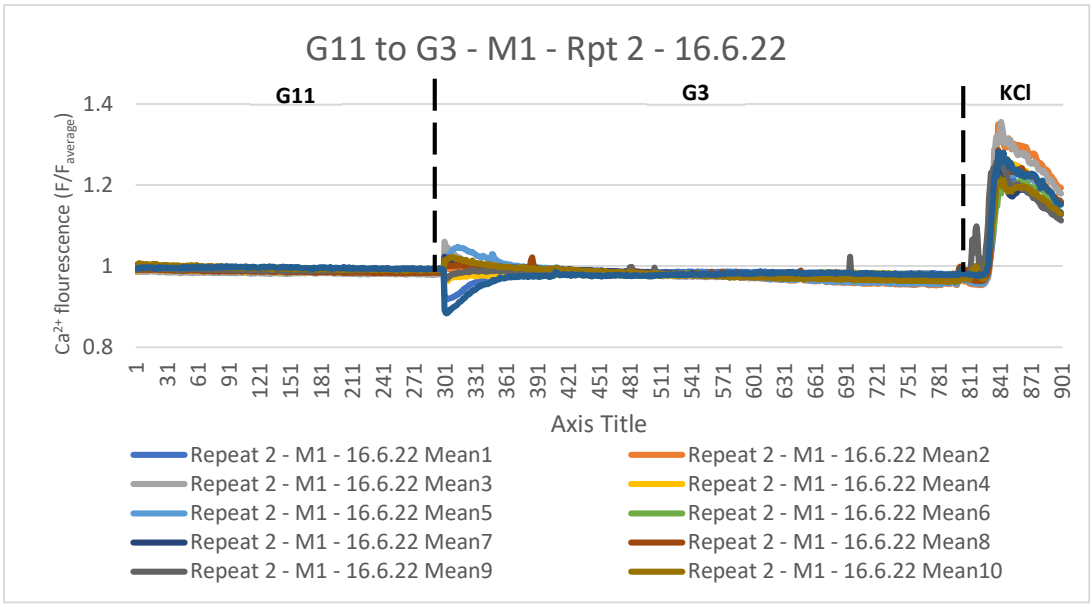

G3 to G11 - M2 - Rpt 2 - 27.5.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
Repeat 2 - M2 - 27.5.22 Mean1
Repeat 2 - M2 - 27.5.22 Mean2
Repeat 2 - M2 - 27.5.22 Mean3
Repeat 2 - M2 - 27.5.22 Mean4
Repeat 2 - M2 - 27.5.22 Mean5
Repeat 2 - M2 - 27.5.22 Mean6

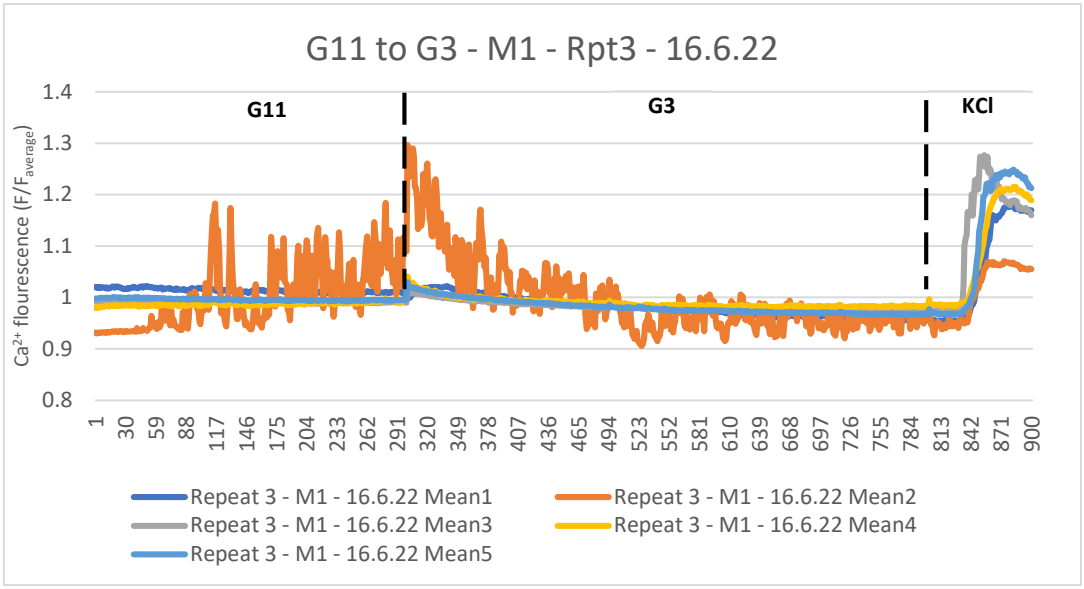

G3 to G11 - M2 - Rpt3 - 27.5.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
2.8
1.8
0.8
1
30
59
88
117
146
175
204
233
262
291
320
349
378
407
436
465
494
523
552
581
610
639
668
697
726
755
784
813
842
871
900
Repeat 3 - M2 - 27.5.22 Mean1
Repeat 3 - M2 - 27.5.22 Mean2
Repeat 3 - M2 - 27.5.22 Mean3
Repeat 3 - M2 - 27.5.22 Mean4
Repeat 3 - M2 - 27.5.22 Mean5
Repeat 3 - M2 - 27.5.22 Mean6
Repeat 3 - M2 - 27.5.22 Mean7
Repeat 3 - M2 - 27.5.22 Mean8
Repeat 3 - M2 - 27.5.22 Mean9
Repeat 3 - M2 - 27.5.22 Mean10

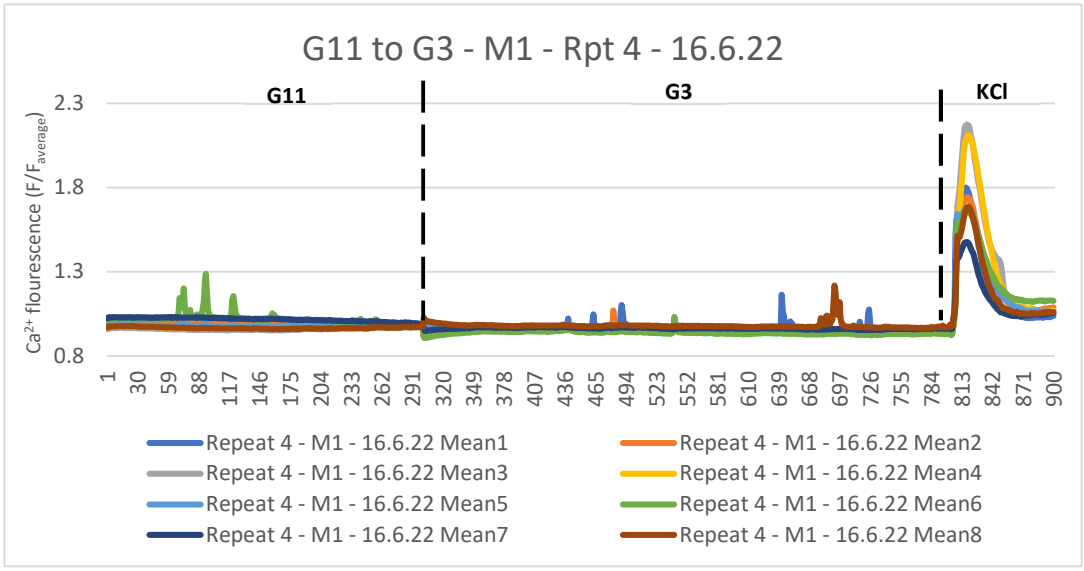

G3 to G11 - M2 - Rpt4 - 27.5.22
G3
G11
KCl
Ca2+ flourescence (F/Faverage)
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
1
30
59
88
117
146
175
204
233
262
291
320
349
378
407
436
465
494
523
552
581
610
639
668
697
726
755
784
813
842
871
900
Repeat 4 - M2 - 27.5.22 Mean1
Repeat 4 - M2 - 27.5.22 Mean2
Repeat 4 - M2 - 27.5.22 Mean3
Repeat 4 - M2 - 27.5.22 Mean4
Repeat 4 - M2 - 27.5.22 Mean5

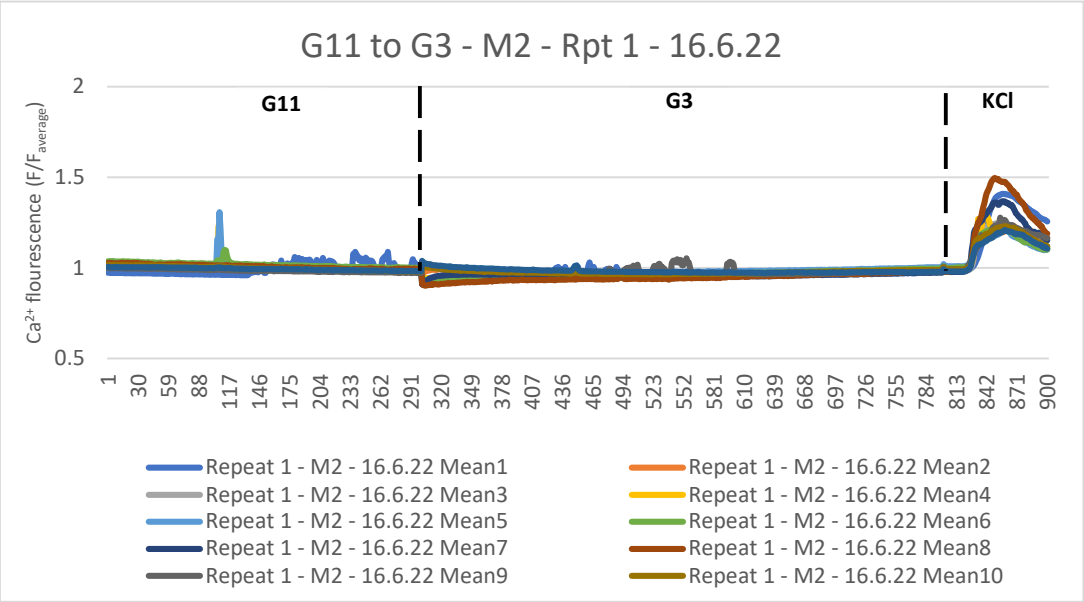
G11 to G3

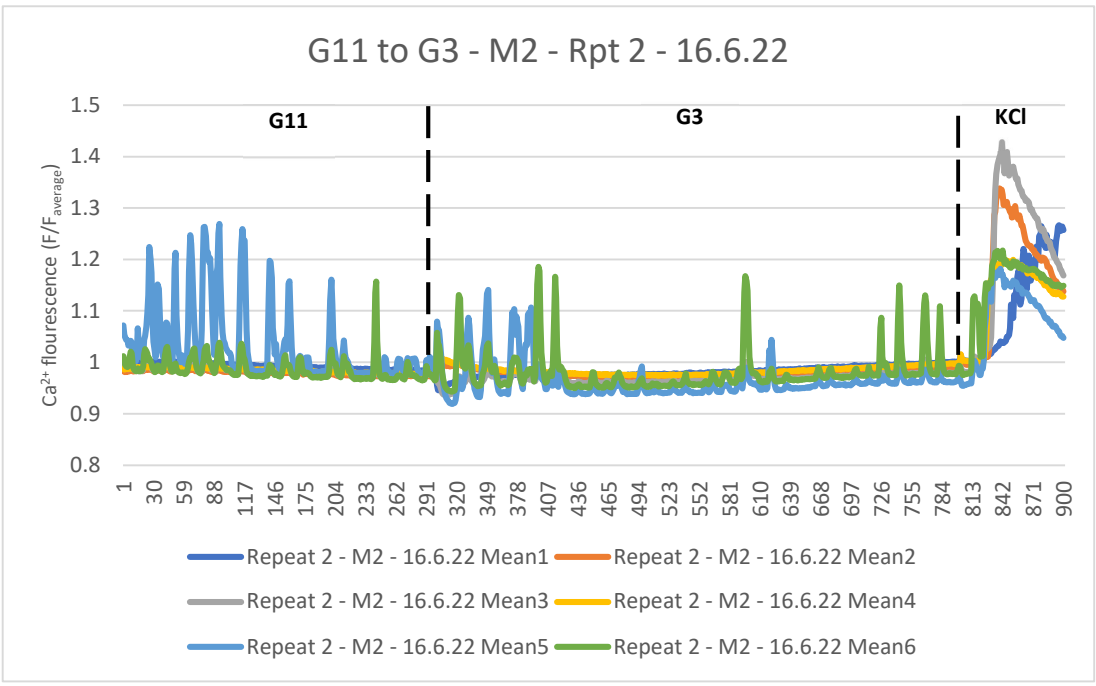

G11 to G3 - M1 - Rpt1 - 16.6.22
G11
G3
KCl
1.1
1.05
1
0.95
0.9
0.85
0.8
Ca2+ flourescence (F/Faverage)
1 31 61 91 121 151 181 211 241 271 301 331 361 391 421 451 481 511 541 571 601 631 661 691 721 751 781 811 841 871 901
Repeat 1 - M1 - 16.6.22 Mean1
Repeat 1 - M1 - 16.6.22 Mean2
Repeat 1 - M1 - 16.6.22 Mean3
Repeat 1 - M1 - 16.6.22 Mean4

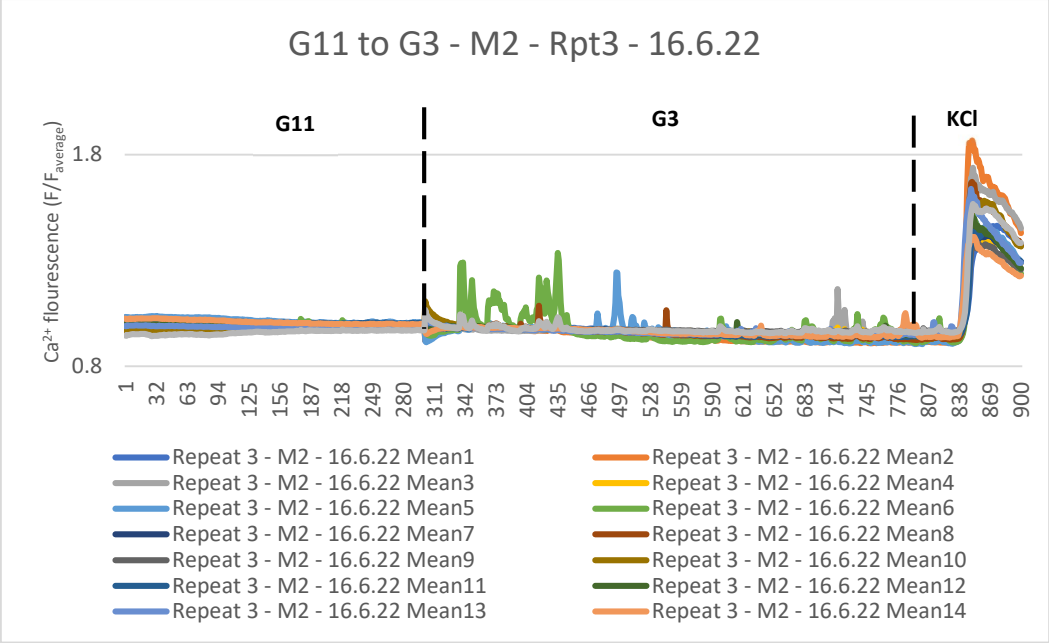

G11 to G3 - M1 - Rpt 2 - 16.6.22
G11
G3
KCl
1.4
1.2
1
0.8
Ca2+ flourescence (F/Faverage)
1 31 61 91 121 151 181 211 241 271 301 331 361 391 421 451 481 511 541 571 601 631 661 691 721 751 781 811 841 871 901
Axis Title
Repeat 2 - M1 - 16.6.22 Mean1
Repeat 2 - M1 - 16.6.22 Mean2
Repeat 2 - M1 - 16.6.22 Mean3
Repeat 2 - M1 - 16.6.22 Mean4
Repeat 2 - M1 - 16.6.22 Mean5
Repeat 2 - M1 - 16.6.22 Mean6
Repeat 2 - M1 - 16.6.22 Mean7
Repeat 2 - M1 - 16.6.22 Mean8
Repeat 2 - M1 - 16.6.22 Mean9
Repeat 2 - M1 - 16.6.22 Mean10


G11 to G3 - M1 - Rpt3 - 16.6.22
G11
G3
KCl
Ca2+ flourescence (F/Faverage)
1.4
1.3
1.2
1.1
1
0.9
0.8
1 30 59 88 117 146 175 204 233 262 291 320 349 378 407 436 465 494 523 552 581 610 639 668 697 726 755 784 813 842 871 900
Repeat 3 - M1 - 16.6.22 Mean1
Repeat 3 - M1 - 16.6.22 Mean2
Repeat 3 - M1 - 16.6.22 Mean3
Repeat 3 - M1 - 16.6.22 Mean4
Repeat 3 - M1 - 16.6.22 Mean5


G11 to G3 - M1 - Rpt 4 - 16.6.22
G11
G3
KCl
Ca2+ flourescence (F/Faverage)
2.3
1.8
1.3
0.8
1 30 59 88 117 146 175 204 233 262 291 320 349 378 407 436 465 494 523 552 581 610 639 668 697 726 755 784 813 842 871 900
Repeat 4 - M1 - 16.6.22 Mean1
Repeat 4 - M1 - 16.6.22 Mean2
Repeat 4 - M1 - 16.6.22 Mean3
Repeat 4 - M1 - 16.6.22 Mean4
Repeat 4 - M1 - 16.6.22 Mean5
Repeat 4 - M1 - 16.6.22 Mean6
Repeat 4 - M1 - 16.6.22 Mean7
Repeat 4 - M1 - 16.6.22 Mean8

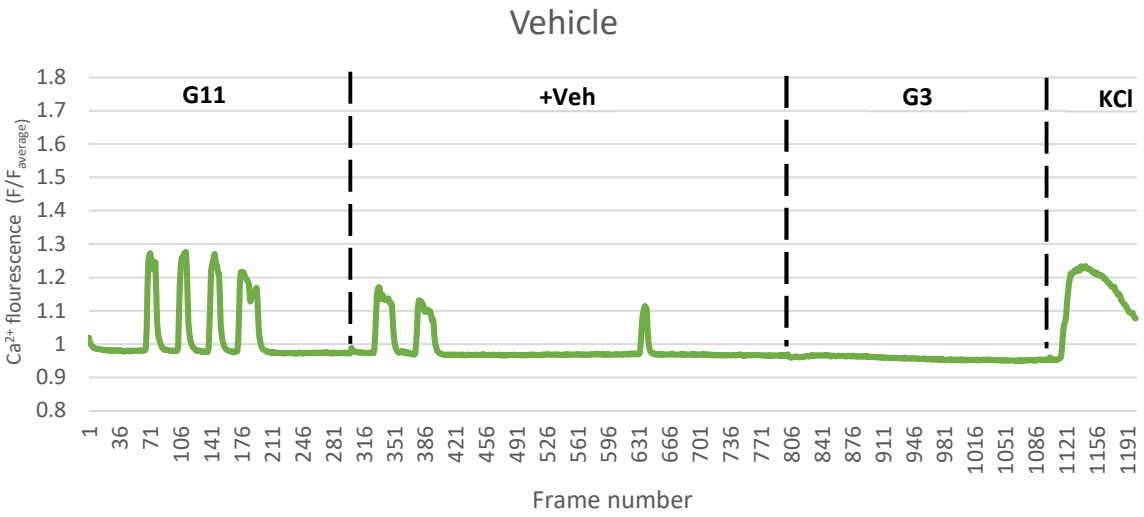

G11 to G3 - M2 - Rpt 1 - 16.6.22
G11
G3
KCl
Ca2+ flourescence (F/Faverage)
2
1.5
1
0.5
1 30 59 88 117 146 175 204 233 262 291 320 349 378 407 436 465 494 523 552 581 610 639 668 697 726 755 784 813 842 871 900
Repeat 1 - M2 - 16.6.22 Mean1
Repeat 1 - M2 - 16.6.22 Mean2
Repeat 1 - M2 - 16.6.22 Mean3
Repeat 1 - M2 - 16.6.22 Mean4
Repeat 1 - M2 - 16.6.22 Mean5
Repeat 1 - M2 - 16.6.22 Mean6
Repeat 1 - M2 - 16.6.22 Mean7
Repeat 1 - M2 - 16.6.22 Mean8
Repeat 1 - M2 - 16.6.22 Mean9
Repeat 1 - M2 - 16.6.22 Mean10

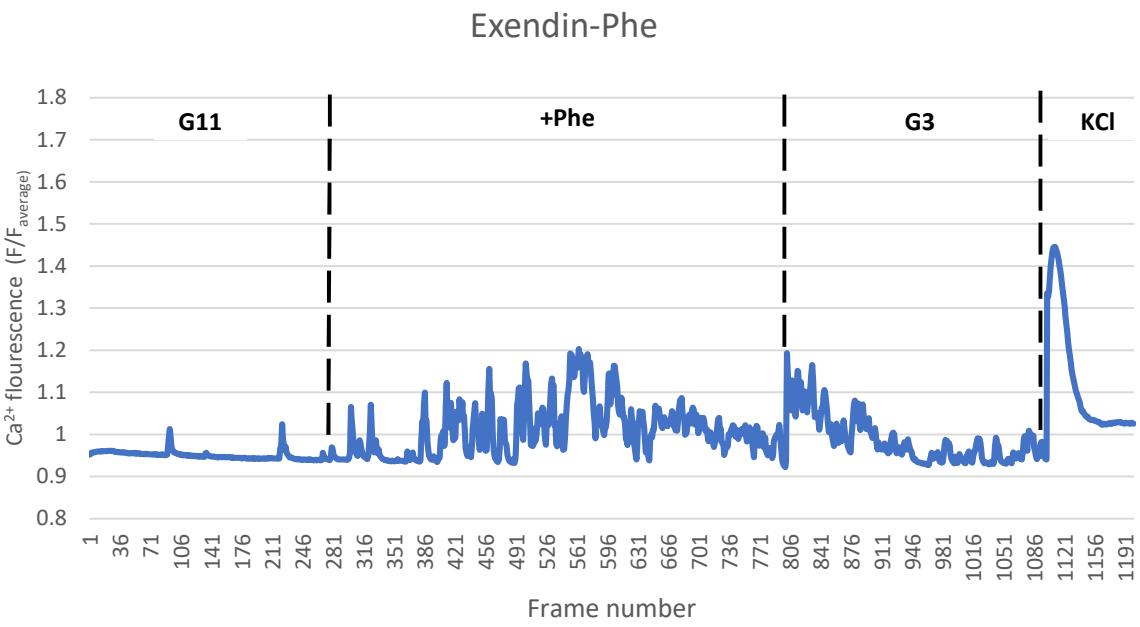

G11 to G3 - M2 - Rpt 2 - 16.6.22
G11
G3
KCl
Ca2+ flourescence (F/Faverage)
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
1 30 59 88 117 146 175 204 233 262 291 320 349 378 407 436 465 494 523 552 581 610 639 668 697 726 755 784 813 842 871 900
Repeat 2 - M2 - 16.6.22 Mean1
Repeat 2 - M2 - 16.6.22 Mean2
Repeat 2 - M2 - 16.6.22 Mean3
Repeat 2 - M2 - 16.6.22 Mean4
Repeat 2 - M2 - 16.6.22 Mean5
Repeat 2 - M2 - 16.6.22 Mean6

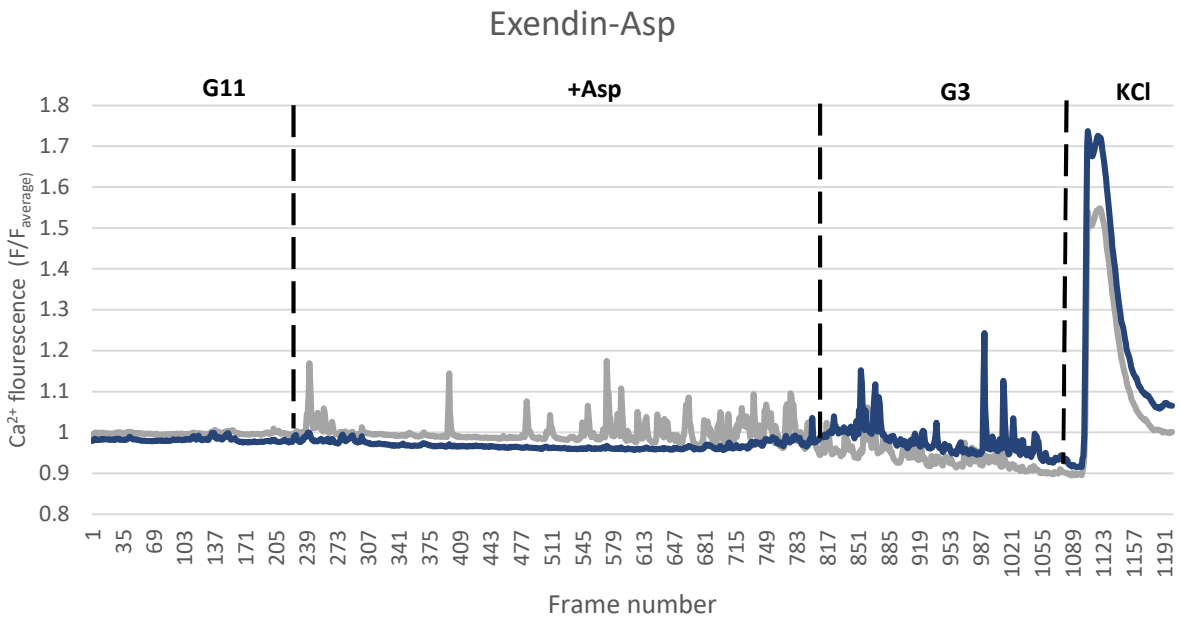


Appendix 2

Alpha cell experiments starting in high glucose, followed by addition of GLP-1 agonists (exendin-phe and exendin-asp) and reduction of glucose to low glucose state. The graphs below depict only the oscillating alpha cells (4 ROIs over n=2 mice) from all conducted GLP-1 experiments.




Exendin-Phe
G11
+Phe
G3
KCl
Ca2+ flourescence (F/Faverage)
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
1
36
71
106
141
176
211
246
281
316
351
386
421
456
491
526
561
596
631
666
701
736
771
806
841
876
911
946
981
1016
1051
1086
1121
1156
1191
Frame number


Exendin-Asp
G11
+Asp
G3
KCl
Ca2+ flourescence (F/Faverage)
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
1
35
69
103
137
171
205
239
273
307
341
375
409
443
477
511
545
579
613
647
681
715
749
783
817
851
885
919
953
987
1021
1055
1089
1123
1157
1191
Frame number