To determine the effect of endogenous GLP-1 secretion on islet function in people with and without Type 2 Diabetes

Adrian Vella MD

IRB #: 20-003993

NCT: 04466618

V3 1/10/2022

Abstract

Post-translational processing of proglucagon in enteroendocrine cells yields a highly conserved peptide hormone, Glucagon-Like Peptide-1 (GLP-1)[1,2]. Although GLP-1-based therapy is now used to treat type 2 diabetes (T2DM) and obesity[3], important questions about its physiological role remain[4]. It has a $t_{1/2}$ ~ 1 minute in the circulation due to rapid degradation by a ubiquitous peptidase – Di-Peptidyl Peptidase-4 (DPP-4)[5] and indeed, a significant proportion of endogenous GLP-1 is degraded close to the site of production[6]. Competitive antagonism of the GLP-1 receptor (GLP1R) with exendin-9,39 has little effect on postprandial insulin secretion – unless post-prandial GLP-1 concentrations are markedly elevated[7]. These conditions are typically only fulfilled after Roux-en-Y Gastric Bypass (RYGB)[8] and, to a lesser extent sleeve gastrectomy[9]. Nevertheless, data from our lab[10,11] and others[12] could suggest a physiologic role for GLP-1 secretion beyond the postprandial state.

Accumulating evidence suggests that in rodents[13,14] and humans[15] GLP-1 is synthesized within islets and may act locally in a paracrine fashion. Indeed, mice with genetic loss of intra-islet GLP-1 exhibit decreased insulin secretion and impaired response to metabolic stressors[14]. 'Pancreatic' GLP-1 may contribute to the effects of DPP-4 inhibitors in rodents[16,17] and humans. Antagonism of GLP1R with exendin-9,39 during fasting impairs the islet cell response to an I.V. glucose challenge[11,12]. Islet GLP-1 content is increased in T2DM and in islets from non-diabetic humans exposed to hyperglycemia and Free Fatty Acids (FFA). These observations imply that paracrine GLP-1 secretion supports islet function in the presence of glucolipotoxicity[13]. We will examine the role of endogenous GLP-1 secretion in people with and without T2DM and during β-cell stress induced by FFA elevation.

I. Hypothesis and Specific Aims

Defects in α-cell[18] and β-cell function[19] are central to the pathogenesis of T2DM and to the progression from prediabetes through to T2DM[20]. There is accumulating evidence that α-cells can produce GLP-1 which in pharmacologic doses has potent anti-diabetic effects[4]. It has been hypothesized that this 'pancreatic' GLP-1 acts in a paracrine fashion to support islet function – at least early in the evolution of prediabetes to diabetes[13]. Indeed, the enzymatic machinery necessary for the production of GLP-1 from proglucagon is expressed in response to inflammation and caloric excess[14,21]. Islet GLP-1 content is increased by T2DM in humans. Moreover, our preliminary data, as well as recent genetic data[22,23] suggest that even in the absence of an oral challenge, GLP-1 receptor signaling can alter islet function. This may identify a therapeutic pathway to prevent or reverse islet dysfunction in T2DM.

Intra-islet expression of GLP-1 seems to be enhanced in T2DM[13]. Certainly, in rodent models and human islets (**Fig.2,3**) exposure to substrate excess or inflammation increases the presence of GLP-1 within the islet[14,24]. Perhaps as islet cells dedifferentiate or transdifferentiate[25,26] in T2DM, GLP-1 production increases. Whether this is an adaptive or maladaptive response is uncertain. We will compare the effects of GLP1R blockade in people with and without T2DM (**Fig.5**)**.** An acute rise in FFA decreases β-cell function (as quantified by Disposition Index) in humans[27]. In addition, fasting and nadir post-prandial glucagon concentrations are increased by FFA elevation[18]. It is unknown if 'pancreatic' GLP-1 helps dampen these deleterious effects on islet function and if this applies equally to α-cell and β-cell function (**Fig.4**). By comparing the effect of FFA elevation on islet function in the presence and absence of GLP-1R blockade in people without T2DM, the experiments will help determine the role of GLP-1 in protecting islet function during metabolic stress (as recreated by FFA elevation).

We will therefore address the following specific aim: -

Specific Aim: Determine the effect of endogenous GLP-1 secretion on islet function in people with T2DM.

1° Hypothesis: GLP1R blockade with exendin-9,39 raises fasting glucagon secretion in people with T2DM

2° Hypotheses: (i) GLP1R blockade with exendin-9,39 impairs α-cell suppression by hyperglycemia to a greater extent in people with T2DM (ii) GLP1R blockade with exendin-9,39 impairs β-cell responses to hyperglycemia to a lesser extent in people with T2DM (iii) FFA elevation exacerbates the effects of GLP1R blockade with exendin-9,39 on β-cell function

II. Background and Significance

Infusion of exendin-9,39 to block GLP1R during an intravenous glucose challenge (that mimics the systemic appearance of a meal) impairs β-cell function in non-diabetic humans with little effect on glucagon secretion (**Fig.1**)[11]. Similar effects are observed in cadaveric islets from people without T2DM (**Fig.3**). Conversely, in people with T2DM (**Fig.1**) or in islets isolated from diabetic humans (**Fig.2**), there is no effect of exendin-9,39 on β-cell function, perhaps due to the degree of β-cell dysfunction. However, in T2DM, exendin-9,39 raises fasting glucagon and impairs its suppression by intravenous glucose *in vivo* (**Fig.1**). **Our preliminary data also suggests that GLP-1 contributes to islet function outside the postprandial period; the effects differ in T2DM compared to non-diabetic subjects (Fig.1)**. In (slight) contrast to our findings Salehi et al. observed an effect of exendin-9,39 on β-cell function in T2DM. This may be due to the small sample size of our preliminary data or to differences in baseline β-cell function of each cohort[12].

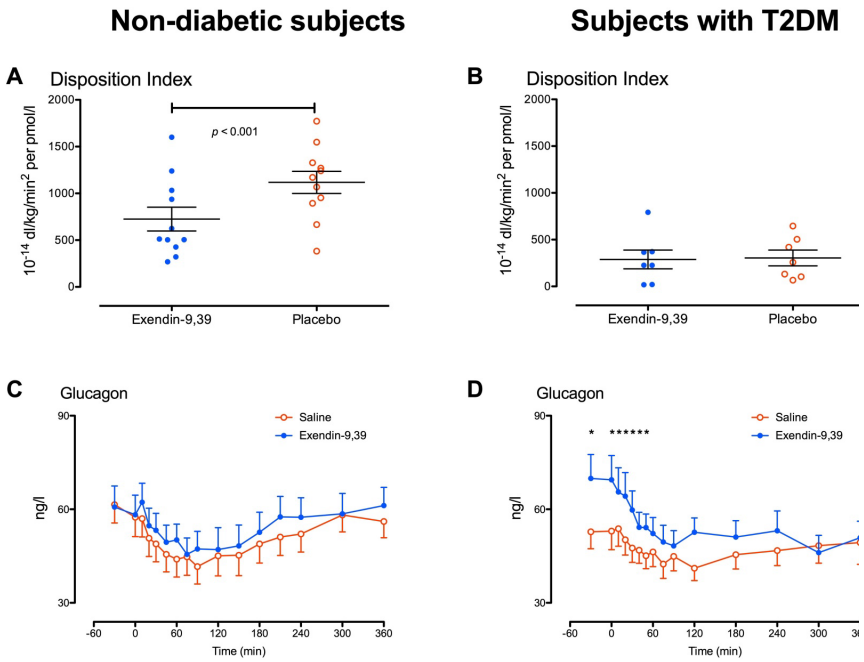

Figure 1: Effect of GLP-1 receptor blockade using exendin-9,39. At time 0, subjects received an intravenous glucose challenge. Exendin-9,39 raised post-challenge glucose concentrations (also fasting in T2DM). In people without diabetes, β-cell function (**A**) is impaired but this is not apparent (**B**) in people with T2DM. On the other hand, in non-diabetic subjects there is no effect on glucagon (**C**) whereas in T2DM, glucagon is increased during fasting and the first 50 minutes after the glucose challenge (**D**). * $p < 0.05$. Non diabetic data from Sathananthan, M., et al. (2013) *Diabetes* 62(8): 2752-2756

Although the source of fasting GLP-1 secretion is uncertain, complementary experiments in isolated human islets (and the literature[14,16,17,24]) strongly suggest an islet source for GLP-1. O'Malley et al. have suggested that islet GLP-1 expression increases with the development of diabetes[13]. This is borne out by our data in human islets documenting increased GLP-1 in T2DM. It is also notable that exposure of islets to FFA and hyperglycemia also increases GLP-1, in keeping with prior reports[28-30]. Our published[11] and unpublished preliminary data, as well as genetic data[23] shows that GLP-1 signaling through its cognate receptor in the fasting state (or during an intravenous glucose challenge) plays a role in islet function. **Understanding the physiologic role of GLP-1 secretion independently of oral intake in humans will allow us to better modulate this pathway and prevent / treat islet dysfunction in prediabetes and T2DM.**

Because the study of the GLP-1 system can be confounded by a lack of antibody specificity[31], investigators have made use of genetic models or of GLP1R blockade with exendin-9,39. Chambers et al. used selective reintroduction of glucagon gene (*Gcg*) expression in the intestine versus the pancreas of *Gcg*$^{-/-}$ mice[32]. They reported that exendin-9, 39 infusion after selective reactivation of pancreatic *Gcg* expression impaired the response to intraperitoneal (i.e. not delivered via the intestine) glucose[32]. In contrast, exendin-9,39 had no effect on the insulin response to glucose after selective reactivation of intestinal *Gcg* expression[32]. To ensure that these experimental results were not influenced by the α-cell hyperplasia present in *Gcg*$^{-/-}$ mice – conditions that favor GLP-1 production in dedifferentiated α-cells[33] – two new lines of mice with the elimination of *Gcg* in the distal gut or throughout the small and large intestine were developed. These were used to re-examine the importance of glucagon-derived peptides in the gut and in the pancreas. Using these models, without α-cell hyperplasia, the same investigators suggest that while gut GLP-1 is a contributor to glucose homeostasis in healthy mice without α-cell hyperplasia, detectable GLP-1 is present in mouse and human pancreas[17]. This led Hutch et al. to conclude that the response to DPP-4i is dependent on pancreatic production of GLP-1 and also (in the presence of an oral challenge) of intestinal Glucose-dependent Insulinotropic Polypeptide (GIP)[16].

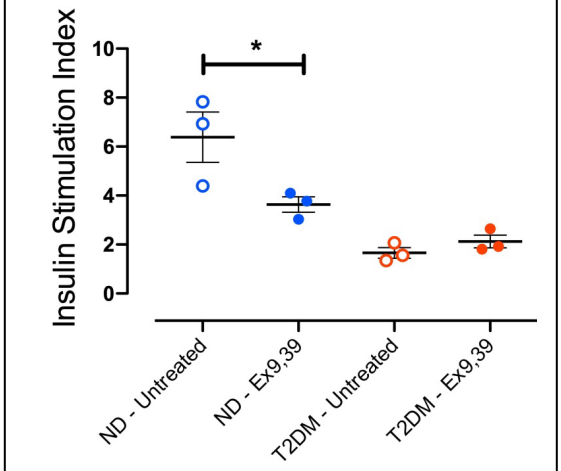

Figure 2: Acute GLP1R blockade with Ex-9,39 in isolated human cadaveric islets (obtained from Prodo labs) from non-diabetic subjects (ND) decreased insulin secretion in response to hyperglycemia. This was not apparent in T2DM. Glucose-stimulated insulin secretion was assessed by static incubation of islets at low (4mM) and high (16mM) glucose ± 100nM Ex-9,39. Insulin Stimulation Index represents fold change in insulin secretion at 16/4 mM glucose. *p<0.05 (n=3 technical repeats).

In animal models where GLP-1 signaling is impeded, GIP secretion in response to an oral (not I.V.) challenge increases to compensate[16,32,34]. Understanding the physiologic role of GIP (unlike GLP-1) has been hampered by the lack of a competitive antagonist useful in humans. Gasbjerg et al. have recently described such a compound but as yet it has not been used by other investigators[35]. However, the insulinotropic effect of GIP seems to be lost in T2DM[36]. In addition, although it is possible that intestinal GIP compensates for GLP1R blockade, a negative effect of exendin-9,39 is still observed in non-diabetic human islets and humans (± T2DM) exposed to glucose – given intravenously in the case of the *in vivo* experiments[11,12].

Intra-islet expression of GLP-1 seems to be enhanced in T2DM[13]. Certainly, in rodent models and human islets exposure to substrate excess or inflammation increases the presence of GLP-1 within the islet[14,24]. Perhaps as islet cells dedifferentiate or transdifferentiate[25,26] in T2DM, GLP-1 production increases. Whether this is an adaptive or maladaptive response is uncertain. We will compare the effects of GLP1R blockade in people with and without T2DM. An acute rise in FFA decreases β-cell function (as quantified by Disposition Index) in humans[27]. In addition, fasting and nadir post-prandial glucagon concentrations are increased by FFA elevation[18]. It is unknown if 'pancreatic' GLP-1 helps dampen these deleterious effects on islet function and if this applies equally to α-cell and β-cell function. By comparing the effect of FFA elevation on islet function in the presence and absence of GLP-1R blockade in people without T2DM, the experiments will help determine the role of GLP-1 in protecting islet function during metabolic stress (as recreated by FFA elevation).

III. Preliminary Data

The rationale and rigor of our hypotheses are supported by our Data **(In addition to Fig.1-3): -**

1. Manipulation of FFA concentrations; measurement of β-cell function and of glucose metabolism: We have experience in manipulating FFA[27,37] to alter β-cell function. We have extensive experience with tracer methodology[38-41] and with deconvolution of insulin secretion from peripheral C-peptide concentrations[42-44]. We also have extensive experience[7,45-48] with the application of the minimal model[49] to experimental data.

2. Use of exendin-9,39 and GLP-1: We have used these peptides extensively as attested by our preliminary data and publication record[7,11,39-41,50-52].

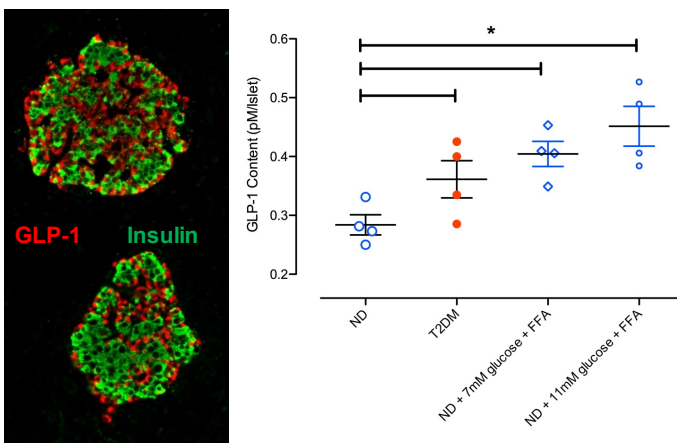

Figure 3: Human islets exhibit GLP-1 immunoreactivity. (**Left panel**). Images show examples of sections from cadaveric pancreata from 2 patients with T2DM stained with a GLP1 specific antibody and counterstained with insulin. (**Right panel**) In addition, total islet GLP1 content (measured by GLP-1-specific ELISA) is increased in human islets with T2DM compared to non-diabetic (ND), (n=4 repeats). Islet GLP-1 content is also increased after 72 hours incubation in hyperglycemic (7 and 11 mM glucose) conditions + FFA (0.5 mM palmitate). *p<0.05 for all conditions vs. ND islets.

IV. Research Design and Methods

Subjects: After approval from the Mayo Clinic Institutional Review Board, we will recruit 20 weight-stable, subjects with T2DM managed with diet or with metformin alone using local advertising, EHR search tools and other methods employed previously[27]. Subjects will have no history of microvascular or macrovascular complications of T2DM. Individuals who have expressed interest in participating in research will also be contacted and invited to participate in the study. In addition, we will recruit 20

age- weight- and sex-matched subjects without diabetes using similar methods. Subjects <18 years of age or >65 years of age will not be studied.

Inclusion criteria – non-diabetic subjects.
We will recruit up to 20 weight-stable, non-diabetic subjects using the methods described above.

The following are **Exclusion Criteria: -**

(a) Age < 25 or > 65 years (to avoid studying subjects who could have latent type 1 diabetes, or the effects of age extremes in subjects with normal or impaired fasting glucose).
(b) $HbA_{1c} \geq 6.5\%$
(c) Use of glucose-lowering agents.
(d) For female subjects: positive pregnancy test at the time of enrollment or study
(e) History of prior upper abdominal surgery such as adjustable gastric banding, pyloroplasty and vagotomy.
(f) Active systemic illness or malignancy.
(g) Symptomatic macrovascular or microvascular disease.

Subsequently, subjects will then undergo an Oral Glucose Tolerance Test to ensure that they have a fasting glucose ≤ 125mg/dl and a two-hour glucose ≤ 200mg/dl.

Inclusion criteria – diabetic subjects.
We will recruit up to 20 weight-stable, diabetic subjects treated with diet and lifestyle alone or with metformin monotherapy using the methods described above.

The following are **Exclusion Criteria: -**

(a) Age < 25 or > 65 years (to avoid studying subjects who could have latent type 1 diabetes, or the effects of age extremes in subjects with normal or impaired fasting glucose).
(b) Use of any glucose-lowering agent other than metformin.
(c) 2 or more fasting glucose values > 250mg/dl on medication or after medication withdrawal.
(d) Unwillingness or inability to withdraw medication for three weeks prior to, and for the duration of the study.
(e) For female subjects: positive pregnancy test at the time of enrollment or study
(f) History of prior upper abdominal surgery such as adjustable gastric banding, pyloroplasty and vagotomy.
(g) Active systemic illness or malignancy.
(h) Symptomatic macrovascular or microvascular disease.

The two groups will be matched for age, sex and weight.

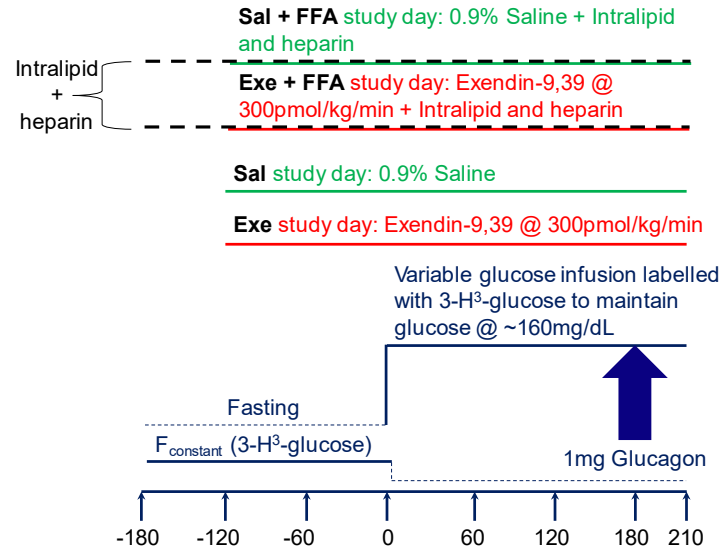

Figure 4: Experimental Design

Screening Visit After an overnight fast of 8 hours, subjects will present to the Clinical Research Trials Unit (CRTU) at approximately 0700: Subjects will provide written, informed consent. To ensure they are otherwise healthy, subjects will undergo a history and limited physical examination; blood collection for complete blood count, fasting glucose, $HbA_{1c,}$ sodium, potassium, creatinine and urine collection to exclude pregnancy. An ECG will also be performed. The Minnesota Leisure-Time Physical Activity questionnaire will be used to assess habitual activity levels[53]. **Body Composition:** After informed written consent is obtained, body composition will be measured at the screening visit using dual-energy X-ray absorptiometry (iDXA scanner; GE, Wauwatosa, WI). All subjects will be instructed to follow a weight-maintenance diet (55% carbohydrate, 30% fat and 15% protein) for at least 3 days prior to each study. **Screening OGTT:** At approximately 0900 subjects without T2DM will ingest 75g glucose. Subjects with T2DM will not undergo OGTT. Arterialized venous blood will be sampled over 2 hrs[54]. Glucose values at 0 and 120 minutes will be used to categorize glucose tolerance status. Subjects who have had a DEXA or OGTT in the last 3 months as part the Vella Lab research studies or another Mayo research group, and weight is stable (+) or (-) 5 pounds, will not be retested, and previously obtained values will be used

Medication withdrawal: Participants (with T2DM) who are taking metformin will be asked to discontinue medication for three weeks before and then continue off medication for the duration of the study. While off medication, they will be asked to self-monitor their fasting glucose at least twice daily. Values consistently > 250mg/dL will result in discontinuation of their participation in the study.

Experimental Design (Fig.4): A total of 40 subjects (20 with T2DM and 20 age- weight- and sex-matched subjects without diabetes) will undergo 2 experiments similar to those described in **Specific Aim 1** in random order. The two study days will be similar to the **Sal** and **Exe** days outlined in **Specific Aim 1**. The nondiabetic subjects will undergo two additional study days where at approximately 0600 (-180 min) an additional infusion of Intralipid (20%, 0.011ml/kg/min; Baxter, Healthcare, Deerfield, IL) and heparin (200 units prime, 0.2 unit/kg/min continuous) will commence as previously described[55]. The infusion will continue till the end of study (1230 – 210 min). In previous experiments, these infusions raised FFA concentrations three-fold (~1200pmol/l) impairing insulin secretion and action as well as raising fasting and post-prandial glucagon concentrations[27]. The two study days will be referred to as the **Sal+FFA** and the **Exe+FFA** study days.

Subjects with T2DM will undergo point of care glucose testing at 2000, 0000, and 0400. If the glucose value is found to be greater than 180 mg/dL, a mild correction scale as outlined in the existing “Subcutaneous Insulin Order Set” will be used to administer aspart insulin subcutaneously. The mild correction scale reads as follows: glucose 180-219 mg/dL, give 2 units, 220-259 mg/dL give 3 units, and so on up to 7 units of rapid acting insulin above 380 mg/dL. This order set also includes orders for nurses to check glucose at anytime if there is concern for hypoglycemia. If hypoglycemia is detected at any point, nurses will administer 15 grams of oral carbohydrate and recheck point of care glucose 15 minutes later to monitor for improvement in glucose and symptoms. The goal is to prevent morning glucose values above 180 mg/dL which have occurred after the standardized meal in some subjects with T2DM who control their diabetes with strict carbohydrate restriction. Administering subcutaneous insulin at 0400 would give sufficient time for exogenous insulin to be metabolized before blood draws are performed to measure endogenous insulin levels occur at 0830.

Analytical Techniques: All blood will be immediately placed on ice, centrifuged at 4ºC, separated and stored at -80ºC until assay. *Glucose* will be measured using a Yellow Springs glucose analyzer. *Glucagon* will be collected in protease inhibitor-containing tubes (BD800, BD Franklin Lakes, NJ) and measured using an ELISA (Mercodia, Winston-Salem, NC). *C-peptide* will be measured using EMD Millipore (Billerica, MA) reagents. *Insulin* will be measured using a chemiluminescence assay with reagents obtained from Beckman (Access Assay, Beckman, Chaska, MN). *Total and Intact GLP-1* will be collected in protease inhibitor-containing tubes (BD800, BD Franklin Lakes, NJ) and measured using an ELISA (ALPCO Diagnostics, Salem, NH). *[3-^{3}H] glucose specific activity* will be measured by liquid scintillation counting after deproteinization and passage over anion- and cation-exchange columns[48]. *FFA concentrations* will be measured using LC/MS as previously[56].

Power Calculation	Mean ± SD	Detectable difference
(*n* = 20 / 20)		
Φ (10^{-9}min^{-1}) - hyperglycemia	149 ± 45	41 (28%)
Secondary Endpoints	**Mean ± SD**	**Detectable difference**
ϕ_b (10^{-9}) - fasting	8 ± 2	1.8 (22%)
Fasting glucagon (ng/l)	71 ± 17	15 (21%)
Fasting glucose (mmol/l)	5.0 ± 0.4	0.4 (8%)
Fasting EGP (μmol/kg/min)	14.4 ± 2.2	1.5 (10%)
Φ (10^{-9}min^{-1}) – GLP-1	316 ± 124	113 (36%)

Table 1: Means ± SD used to estimate power

Calculations: In addition to fasting, peak and nadir *hormone concentrations*, we will calculate area above basal (AAB) or area under the curve (AUC) during the experiment using the trapezoidal rule as previously described[48]. *S_i, Insulin Secretion Rate (ISR) and β-Cell responsivity (Φ)* indices will be calculated[49] from the glucose, insulin and C-peptide concentrations observed during the experiments using the minimal model[57], incorporating age-associated changes in C-peptide kinetics[58]. Φ is derived from the sum of ϕ_1 (1st phase insulin secretion) and ϕ_2 (2nd phase insulin secretion) as before[50]. Disposition index (DI) will express β-cell function as a function of the prevailing insulin action[49]. *Glucose disappearance (Rd)* will be calculated using steady state Steele equations[59,60] after *Specific activity* is smoothed as previously described[61]. *Endogenous glucose production (EGP)* will be calculated as the difference between the tracer determined rate of glucose appearance and the glucose infusion rate. All infusion rates will be expressed as Kg per lean body mass. The *GLP-1 model*[42,62], will be used to quantify the potentiation of insulin secretion by GLP-1[51]. The model assumes that insulin secretion is linearly modulated by GLP-1 through an index (π), which quantifies the increase of insulin secretion due to (changes in) circulating GLP-1concentrations[50].

Statistical Analysis: The primary endpoints for the hypotheses being tested represent differences in a given parameter e.g. glucagon for each subject between the **Sal** and **Exe** days. These will be used in an analysis of covariance (ANCOVA), which will include the baseline value (**Sal**) as a covariate together with age, BMI and sex, to test for differences attributable to T2DM. Within-group differences attributable to study conditions will be assessed using a Student's paired *t*-test (parametric) or Wilcoxon matched-pairs signed rank test (non-parametric). Other response variables (e.g. hormone area under the curve) will also be examined.

Power Calculation: Please refer to **Table 1** for the relevant effect size that is detectable for fasting glucose, glucagon and EGP – since the primary analysis will use ANCOVA to detect between group differences in response to exendin-9,39 we anticipate the ability to detect even smaller changes in these parameters. As regards the ability to detect an effect on Φ – prior studies using a meal challenge show values of 50±17[45] vs. 29±19[10] 10^{-9}min^{-1} – non-diabetic vs. T2DM respectively – a ~40% difference. Assuming similar between-group differences and similar variation in Φ, we will be amply powered to detect biologically significant variation in β-cell responses.

Interpretation: To test our 1° hypothesis, we will compare differences (T2DM vs. non-diabetic controls) in the change between **Sal** and **Exe** days for fasting glucagon. Our preliminary data (**Fig.1**) has suggested a greater effect of GLP-1 receptor blockade on α-cell function in people with T2DM compared to non-diabetic subjects. If the increase in fasting glucagon concentrations is higher in people with T2DM we will accept our 1° hypothesis. If this is the case, we will then determine if this is accompanied by a corresponding change in fasting glucose. Glucose concentrations could remain unchanged because of a compensatory rise in fasting insulin secretion, preventing an increase in EGP. An alternative scenario would be that the glucagon increase raises EGP, unrestrained by insulin secretion but this is counteracted by a reciprocal and equal increase in glucose disappearance. The use of tracer-based measurement will enable us to measure glucose uptake and glucose release separately. It is also possible, but unlikely[2], that GLP1R blockade changes hepatic insulin sensitivity. This will be quantified by comparing EGP with prevailing insulin concentrations on each study day.

To test our 2° hypothesis (i) we will compare (T2DM vs. non-diabetic controls) the change in nadir glucagon concentrations, between **Sal** and **Exe** days, during the hyperglycemic clamp (120 – 180 min). Previously, we

had shown heterogeneity in glucagon suppression to hyperglycemia in subjects across the spectrum of glucose tolerance – with some of the heterogeneity explained by impaired insulin action[52]. In our preliminary experiments in people with T2DM, inhibition of endogenous GLP-1 secretion by exendin-9,39 raised both fasting and nadir glucagon in response to an intravenous glucose profile that mimics the systemic appearance of a 50g OGTT[40] (**Fig.1**). If this is the case, then we will accept our 2° hypothesis (i). It is possible that the nadir observed is a reflection of fasting glucagon concentrations and therefore we will also compare the % suppression from fasting values to determine whether effects on α-cell suppression by hyperglycemia are due to differences in dysregulation of baseline glucagon secretion. If we reject our 2° hypothesis (i), we will design studies to determine if α-cell suppression in response to protein and fat (greater stimuli of post-prandial glucagon secretion) is also impaired by GLP1R blockade with exendin-9,39. This is relevant to people with T2DM who have paradoxical rises in glucagon after mixed meals which cause postprandial hyperglycemia[63,64].

The relative disparity in response (between people with and without T2DM) to exendin-9,39 in the fasting state observed previously may reflect the underlying degree of β-cell dysfunction. People with T2DM have impaired β-cell function perhaps to the degree that they are much less responsive to any change in endogenous GLP-1 secretion and action – reflecting a general decreased response to secretagogues[65,66]. Accordingly, we will test our 2° hypothesis (ii) by comparing the change in Φ (T2DM vs. non-diabetic controls) between **Sal** and **Exe** days during the hyperglycemic clamp. If we accept our hypothesis, in the non-diabetic subjects, we will correlate the change in Φ with β-cell function as measured by the Disposition Index[49] (calculated from the screening OGTT[67]) to determine if the effect of exendin-9,39 on insulin secretion is a function of baseline DI. We would expect the effect of GLP1R blockade on Φ to be less marked as DI decreases. Examination of the effect on the different components of Φ i.e. ϕ_1 and ϕ_2 will enable us to determine whether these effects occur via increased storage of preformed insulin granules (ϕ_1 – 1st phase), increased synthesis during a challenge (ϕ_2– 2nd phase), or a combination.

People with T2DM have elevated FFA concentrations and acute elevation of FFA impairs β-cell function in non-diabetic individuals[18,27]. There are minimal effects of raising FFA in people with already elevated FFA concentrations[68] hence our experimental design where we will only raise FFA in people without T2DM to test 2° hypothesis (iii). Intra-islet GLP-1 seems to increase after β-cell exposure to FFA (**Fig.3**). We will compare the α-cell and β-cell responses to exendin-9,39 infusion in the presence and absence of FFA elevation. If we accept our 2° hypothesis (iii) this would imply that endogenous secretion of GLP-1 (in the fasting state) serves as an adaptive response to dampen the negative effects of acute FFA elevation on islet function[18].

Bibliography

1. Kieffer, T.J. & Habener, J.F. The glucagon-like peptides. *Endocr Rev* **20**, 876-913 (1999).
2. Vella, A. & Rizza, R.A. Extrapancreatic effects of GIP and GLP-1. *Horm Metab Res* **36**, 830-836 (2004).
3. Drucker, D.J. & Nauck, M.A. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* **368**, 1696-1705 (2006).
4. Drucker, D.J. Evolving Concepts and Translational Relevance of Enteroendocrine Cell Biology. *The Journal of clinical endocrinology and metabolism*, jc20153449 (2015).
5. Smushkin, G.*, et al.* Defects in GLP-1 Response to an Oral Challenge Do Not Play a Significant Role in the Pathogenesis of Prediabetes. *J Clin Endocrinol Metab* **97**, 589-598 (2012).
6. Orskov, C., Holst, J.J., Poulsen, S.S. & Kirkegaard, P. Pancreatic and intestinal processing of proglucagon in man. *Diabetologia* **30**, 874-881 (1987).
7. Shah, M.*, et al.* Contribution of endogenous glucagon-like peptide 1 to glucose metabolism after Roux-en-Y gastric bypass. *Diabetes* **63**, 483-493 (2014).
8. Laferrere, B.*, et al.* Effect of weight loss by gastric bypass surgery versus hypocaloric diet on glucose and incretin levels in patients with type 2 diabetes. *J Clin Endocrinol Metab* **93**, 2479-2485 (2008).
9. Jimenez, A.*, et al.* GLP-1 and glucose tolerance after sleeve gastrectomy in morbidly obese subjects with type 2 diabetes. *Diabetes* **63**, 3372-3377 (2014).
10. Dalla Man, C.*, et al.* Dipeptidyl peptidase-4 inhibition by vildagliptin and the effect on insulin secretion and action in response to meal ingestion in type 2 diabetes. *Diabetes Care* **32**, 14-18 (2009).
11. Sathananthan, M.*, et al.* Direct Effects of Exendin-(9,39) and GLP-1-(9,36)amide on Insulin Action, beta-Cell Function, and Glucose Metabolism in Nondiabetic Subjects. *Diabetes* **62**, 2752-2756 (2013).
12. Salehi, M., Aulinger, B., Prigeon, R.L. & D'Alessio, D.A. Effect of endogenous GLP-1 on insulin secretion in type 2 diabetes. *Diabetes* **59**, 1330-1337 (2010).
13. O'Malley, T.J., Fava, G.E., Zhang, Y., Fonseca, V.A. & Wu, H. Progressive change of intra-islet GLP-1 production during diabetes development. *Diabetes/metabolism research and reviews* **30**, 661-668 (2014).
14. Traub, S.*, et al.* Pancreatic alpha Cell-Derived Glucagon-Related Peptides Are Required for beta Cell Adaptation and Glucose Homeostasis. *Cell Rep* **18**, 3192-3203 (2017).
15. Marchetti, P.*, et al.* A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets. *Diabetologia* **55**, 3262-3272 (2012).
16. Hutch, C.R.*, et al.* The role of GIP and pancreatic GLP-1 in the glucoregulatory effect of DPP-4 inhibition in mice. *Diabetologia* **62**, 1928-1937 (2019).
17. Song, Y.*, et al.* Gut-Proglucagon-Derived Peptides Are Essential for Regulating Glucose Homeostasis in Mice. *Cell Metab* (2019).
18. Sharma, A.*, et al.* Impaired Insulin Action is Associated with Increased Glucagon Concentrations in Non-Diabetic Humans. *J Clin Endocrinol Metab* **103**, 314-319 (2018).
19. Bock, G.*, et al.* Pathogenesis of pre-diabetes: mechanisms of fasting and postprandial hyperglycemia in people with impaired fasting glucose and/or impaired glucose tolerance. *Diabetes* **55**, 3536-3549 (2006).
20. Dinneen, S.F.*, et al.* Effects of changing diagnostic criteria on the risk of developing diabetes. *Diabetes Care* **21**, 1408-1413 (1998).
21. Ellingsgaard, H.*, et al.* Interleukin-6 regulates pancreatic alpha-cell mass expansion. *Proc Natl Acad Sci U S A* **105**, 13163-13168 (2008).
22. Suzuki, K.*, et al.* Identification of 28 new susceptibility loci for type 2 diabetes in the Japanese population. *Nat Genet* **51**, 379-386 (2019).
23. Kwak, S.H.*, et al.* Nonsynonymous Variants in PAX4 and GLP1R Are Associated With Type 2 Diabetes in an East Asian Population. *Diabetes* **67**, 1892-1902 (2018).

24. Ellingsgaard, H.*, et al.* Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat Med* **17**, 1481-1489 (2011).

25. Cinti, F.*, et al.* Evidence of beta-Cell Dedifferentiation in Human Type 2 Diabetes. *J Clin Endocrinol Metab* **101**, 1044-1054 (2016).

26. Talchai, C., Xuan, S., Lin, H.V., Sussel, L. & Accili, D. Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. *Cell* **150**, 1223-1234 (2012).

27. Shah, M.*, et al.* TCF7L2 Genotype and alpha-Cell Function in Humans Without Diabetes. *Diabetes* **65**, 371-380 (2016).

28. Nie, Y.*, et al.* Regulation of pancreatic PC1 and PC2 associated with increased glucagon-like peptide 1 in diabetic rats. *J Clin Invest* **105**, 955-965 (2000).

29. Kilimnik, G., Kim, A., Steiner, D.F., Friedman, T.C. & Hara, M. Intraislet production of GLP-1 by activation of prohormone convertase 1/3 in pancreatic alpha-cells in mouse models of ss-cell regeneration. *Islets* **2**, 149-155 (2010).

30. Vasu, S., Moffett, R.C., McClenaghan, N.H. & Flatt, P.R. Responses of GLP1-secreting L-cells to cytotoxicity resemble pancreatic beta-cells but not alpha-cells. *J Mol Endocrinol* **54**, 91-104 (2015).

31. Drucker, D.J. Incretin action in the pancreas: potential promise, possible perils, and pathological pitfalls. *Diabetes* **62**, 3316-3323 (2013).

32. Chambers, A.P.*, et al.* The Role of Pancreatic Preproglucagon in Glucose Homeostasis in Mice. *Cell Metab* **25**, 927-934 e923 (2017).

33. Hayashi, Y.*, et al.* Mice deficient for glucagon gene-derived peptides display normoglycemia and hyperplasia of islet {alpha}-cells but not of intestinal L-cells. *Mol Endocrinol* **23**, 1990-1999 (2009).

34. Pederson, R.A.*, et al.* Enhanced glucose-dependent insulinotropic polypeptide secretion and insulinotropic action in glucagon-like peptide 1 receptor -/- mice. *Diabetes* **47**, 1046-1052 (1998).

35. Gasbjerg, L.S.*, et al.* GIP(3-30)NH2 is an efficacious GIP receptor antagonist in humans: a randomised, double-blinded, placebo-controlled, crossover study. *Diabetologia* **61**, 413-423 (2018).

36. Nauck, M.A.*, et al.* Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* **91**, 301-307 (1993).

37. Varghese, R.T.*, et al.* Mechanisms Underlying the Pathogenesis of Isolated Impaired Glucose Tolerance in Humans. *J Clin Endocrinol Metab* **101**, 4816-4824 (2016).

38. Vella, A.*, et al.* Glucose-induced suppression of endogenous glucose production: dynamic response to differing glucose profiles. *Am J Physiol Endocrinol Metab* **285**, E25-E30 (2003).

39. Vella, A.*, et al.* Effect of glucagon-like peptide-1(7-36)-amide on initial splanchnic glucose uptake and insulin action in humans with type 1 diabetes. *Diabetes* **50**, 565-572 (2001).

40. Vella, A.*, et al.* Effect of glucagon-like peptide 1(7-36) amide on glucose effectiveness and insulin action in people with type 2 diabetes. *Diabetes* **49**, 611-617 (2000).

41. Vella, A.*, et al.* Lack of effect of exendin-4 and glucagon-like peptide-1-(7,36)-amide on insulin action in non-diabetic humans. *Diabetologia* **45**, 1410-1415 (2002).

42. Dalla Man, C.*, et al.* A model of GLP-1 action on insulin secretion in nondiabetic subjects. *Am J Physiol Endocrinol Metab* **298**, E1115-E1121 (2010).

43. Laurenti, M.C.*, et al.* Assessment of pulsatile insulin secretion derived from peripheral plasma C-peptide concentrations by nonparametric stochastic deconvolution. *Am J Physiol Endocrinol Metab* **316**, E687-E694 (2019).

44. Varghese, R.T.*, et al.* Performance of individually measured vs population-based C-peptide kinetics to assess beta-cell function in the presence and absence of acute insulin resistance. *Diabetes Obes Metab* **20**, 549-555 (2018).

45. Bock, G.*, et al.* The effect of DPP-4 inhibition with sitagliptin on incretin secretion and on fasting and postprandial glucose turnover in subjects with impaired fasting glucose. *Clin Endocrinol (Oxf)* **73**, 189-196 (2010).

46. Sathananthan, M.*, et al.* The effect of vagal nerve blockade using electrical impulses on glucose metabolism in nondiabetic subjects. *Diabetes Metab Syndr Obes* **7**, 305-312 (2014).

47. Sathananthan, M.*, et al.* Six and 12 Weeks of Caloric Restriction Increases beta Cell Function and Lowers Fasting and Postprandial Glucose Concentrations in People with Type 2 Diabetes. *J Nutr* **145**, 2046-2051 (2015).

48. Smushkin, G.*, et al.* The effect of a bile acid sequestrant on glucose metabolism in subjects with type 2 diabetes. *Diabetes* **62**, 1094-1101 (2013).

49. Cobelli, C.*, et al.* The oral minimal model method. *Diabetes* **63**, 1203-1213 (2014).

50. Sathananthan, A.*, et al.* Common genetic variation in GLP1R and insulin secretion in response to exogenous GLP-1 in nondiabetic subjects: a pilot study. *Diabetes Care* **33**, 2074-2076 (2010).

51. Shah, M.*, et al.* Contribution of endogenous glucagon-like peptide-1 to changes in glucose metabolism and islet function in people with type 2 diabetes four weeks after Roux-en-Y gastric bypass (RYGB). *Metabolism* **93**, 10-17 (2019).

52. Smushkin, G.*, et al.* Diabetes-associated common genetic variation and its association with GLP-1 concentrations and response to exogenous GLP-1. *Diabetes* **61**, 1082-1089 (2012).

53. Taylor, H.L.*, et al.* A questionnaire for the assessment of leisure time physical activities. *J Chronic Dis* **31**, 741-755 (1978).

54. Sathananthan, A.*, et al.* A concerted decline in insulin secretion and action occurs across the spectrum of fasting and postchallenge glucose concentrations. *Clin Endocrinol (Oxf)* **76**, 212-219 (2012).

55. Shah, P.*, et al.* Effects of free fatty acids and glycerol on splanchnic glucose metabolism and insulin extraction in nondiabetic humans. *Diabetes* **51**, 301-310 (2002).

56. Lu, J., Varghese, R.T., Zhou, L., Vella, A. & Jensen, M.D. Glucose tolerance and free fatty acid metabolism in adults with variations in TCF7L2 rs7903146. *Metabolism* **68**, 55-63 (2017).

57. Breda, E., Cavaghan, M.K., Toffolo, G., Polonsky, K.S. & Cobelli, C. Oral glucose tolerance test minimal model indexes of beta-cell function and insulin sensitivity. *Diabetes* **50**, 150-158 (2001).

58. Van Cauter, E., Mestrez, F., Sturis, J. & Polonsky, K.S. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* **41**, 368-377 (1992).

59. Steele, R., Bjerknes, C., Rathgeb, I. & Altszuler, N. Glucose uptake and production during the oral glucose tolerance test. *Diabetes* **17**, 415-421 (1968).

60. Steele, R., Wall, J.S., De Bodo, R.C. & Altszuler, N. Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* **187**, 15-24 (1956).

61. Bradley, D.C., Steil, G.M. & Bergman, R.N. OOPSEG: a data smoothing program for quantitation and isolation of random measurement error. *Comput Methods Programs Biomed* **46**, 67-77 (1995).

62. Dalla Man, C., Micheletto, F., Sathananthan, M., Vella, A. & Cobelli, C. Model-Based Quantification of Glucagon-Like Peptide-1-Induced Potentiation of Insulin Secretion in Response to a Mixed Meal Challenge. *Diabetes Technol Ther* **18**, 39-46 (2016).

63. Shah, P., Basu, A., Basu, R. & Rizza, R. Impact of lack of suppression of glucagon on glucose tolerance in humans. *Am J Physiol* **277**, E283-290 (1999).

64. Shah, P.*, et al.* Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetes mellitus. *J Clin Endocrinol Metab* **85**, 4053-4059 (2000).

65. Chen, M., Bergman, R.N., Pacini, G. & Porte, D., Jr. Pathogenesis of age-related glucose intolerance in man: insulin resistance and decreased beta-cell function. *J Clin Endocrinol Metab* **60**, 13-20 (1985).

66. Shankar, S.S.*, et al.* Standardized Mixed-Meal Tolerance and Arginine Stimulation Tests Provide Reproducible and Complementary Measures of beta-Cell Function: Results From the Foundation for the National Institutes of Health Biomarkers Consortium Investigative Series. *Diabetes Care* **39**, 1602-1613 (2016).

67. Dalla Man, C.*, et al.* Two-hour seven-sample oral glucose tolerance test and meal protocol: minimal model assessment of beta-cell responsivity and insulin sensitivity in nondiabetic individuals. *Diabetes* **54**, 3265-3273 (2005).

68. Giacca, A., Xiao, C., Oprescu, A.I., Carpentier, A.C. & Lewis, G.F. Lipid-induced pancreatic beta-cell dysfunction: focus on in vivo studies. *Am J Physiol Endocrinol Metab* **300**, E255-262 (2011).