REGULATION OF ALPHA CELL FUNCTION BY GP130 RECEPTOR SIGNALLING
IN A RODENT MODEL OF TYPE 2 DIABETES

by

Samuel Zhong Wei Chow

B.Sc., The University of British Columbia, 2012

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES
(Experimental Medicine)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

December 2014

© Samuel Zhong Wei Chow, 2014
Abstract

Dysregulated α cell glucagon secretion contributes to post-meal hyperglycemia in prediabetes and to hyperglycemia in type 2 diabetes (T2D). Islets in T2D are characterized by chronic inflammation, and recent human data showed increased IL-6 family cytokine expression in T2D islets in a global gene expression study (IL6 mRNA increased 2.75-fold, IL11 mRNA increased 1.61-fold). We recently discovered that IL-6 stimulates glucagon secretion from human and rodent islets. Cytokines of the IL-6 family all require the gp130 receptor to signal. Therefore, we were interested in elucidating the effects of α cell gp130 receptor signalling on glycemic control in T2D. IL-6 family cytokines were elevated in islets in rodent models of T2D. IL-6 induced STAT3 activation in primary α cells and stimulated glucagon secretion in a gp130 receptor-dependent manner. Pancreatic α cell specific gp130 knockout (αgp130KO) mice showed no differences in glycemic control, α cell function or α cell mass. However, when subjected to streptozotocin (STZ) plus high fat diet (HFD) to induce islet inflammation and pathophysiology modelling T2D, αgp130KO mice had reduced fasting glycemia, improved glucose tolerance, and improved α cell function. Hyperinsulinemic-euglycemic clamps revealed no differences in insulin sensitivity. Our data strongly suggest that reduced glycemia and improved glucose tolerance in our αgp130KO mice is due to improved α cell function, however further studies are required to elucidate the mechanism of action of gp130 receptor signalling in α cells. We conclude that in a setting of increased islet inflammation such as observed in T2D, activation of α cell gp130 receptor signalling has long-lasting deleterious effects on α cell function, promoting hyperglycemia. Antagonism of α cell gp130 receptor signalling may be useful for the treatment of T2D.
Preface

S. Chow was involved in the designing, conducting, and analyzing the research data with guidance from Dr. J.A. Ehses and with technical assistance from M. Speck and M. Komba. Specifically, M. Speck was responsible for the data generated in Figures 3.2B-F, 3.3D-E, 3.4Q-S, and for assisting with islet isolations, and glucose tolerance tests needed to generate data for Figures 3.2-3.11. M. Komba also assisted with islet isolations. The hyperinsulinemic-euglycemic clamping was performed with the technical assistance of M. Speck (Figure 3.6)

Some data presented in this thesis is published by the author, S. Chow, in Chow et al. (2014) *Diabetes* 63(9): 2984-2995.

All animal work conducted for this study was approved by the University of British Columbia Animal Care Committee. Biology and Husbandry of the Laboratory Rodent training was completed through the UBC Animal Care Centre (certificate #RBH-201-12). Ethics training was completed and met the requirements of the Canadian Council of Animal Care (certificate #5714-13).
Table of Contents

Abstract ........................................................................................................................................... ii
Preface ................................................................................................................................................ iii
Table of Contents .............................................................................................................................. iv
List of Tables ...................................................................................................................................... viii
List of Figures ................................................................................................................................... ix
List of Abbreviations ........................................................................................................................ x
Acknowledgements .......................................................................................................................... xii

Chapter 1: Introduction ...................................................................................................................... 1

1.1 The pancreatic α cell and glucagon .......................................................................................... 1
  1.1.1 The pancreatic α cell ......................................................................................................... 1
  1.1.2 Glucagon synthesis ......................................................................................................... 2
  1.1.3 Glucagon secretion ......................................................................................................... 4
  1.1.4 Glucagon action and the glucagon receptor ................................................................. 5
1.2 The gp130 receptor ............................................................................................................... 6
  1.2.1 The gp130 receptor and gp130 family cytokines ......................................................... 6
  1.2.2 Gp130 receptor signalling ............................................................................................ 8
1.3 Diabetes mellitus .................................................................................................................... 10
  1.3.1 Disease burden ............................................................................................................ 10
  1.3.2 Type 1 diabetes .......................................................................................................... 11
  1.3.3 Type 2 diabetes .......................................................................................................... 11
  1.3.4 Pancreatic α cell dysfunction in T2D ........................................................................ 12


1.4 Islet inflammation in T2D ................................................................. 13
  1.4.1 Gp130 cytokines in T2D ............................................................... 13
  1.4.2 Islet inflammation and the pancreatic α cell................................. 14

Chapter 2: Methodology ........................................................................ 17
  2.1 In vivo studies ................................................................................ 17
    2.1.1 Animals ...................................................................................... 17
    2.1.2 Genotyping ................................................................................ 18
    2.1.3 Streptozotocin, high-fat diet, and streptozotocin/high-fat diet mouse models .... 20
    2.1.4 Physiological measurements ..................................................... 20
    2.1.5 Hyperinsulinemic-euglycemic clamps ........................................ 21
  2.2 In Vitro Studies ................................................................................ 23
    2.2.1 Pancreatic islet isolation ............................................................ 23
    2.2.2 Islet dispersion .......................................................................... 24
    2.2.3 αTC1-9 cells .............................................................................. 24
    2.2.4 Glucagon, GLP-1, and insulin secretion assays .......................... 25
    2.2.5 Islet secretion of IL-6, LIF, and sIL-6R ...................................... 26
    2.2.6 Gp130-mediated gene expression in islet and αTC1.9 cells.......... 27
    2.2.7 Gene expression analysis ............................................................ 27
    2.2.8 Western blotting ........................................................................ 30
    2.2.9 Immunostaining ........................................................................ 31
      2.2.9.1 Immunohistochemistry of paraffin-embedded pancreatic sections .... 31
      2.2.9.2 Immunohistochemistry of frozen pancreatic sections .................. 31
      2.2.9.3 Pancreatic β and α cell mass analysis ..................................... 32
2.2.9.4 Immunocytochemistry of dispersed islets ..............................................32
2.2.9.5 Flow cytometry .........................................................................................34
2.2.10 Statistical Analysis ....................................................................................34

Chapter 3: Results ..................................................................................................35

3.1 Gp130 family cytokines are elevated in pancreatic islets from rodent models of T2D ...35
3.2 Gp130 receptor activation stimulates STAT3 phosphorylation in and glucagon secretion from α cells .........................................................................................................................37
3.3 Generation and validation of α cell specific gp130 receptor KO mice ..................39
  3.3.1 Analysis of Cre recombinase expression in Gcg-Cre mice ..............................39
  3.3.2 Partial knockout of the α cell gp130 receptor impairs pSTAT3 activation and glucagon secretion ..........................................................................................................................39
  3.3.3 Partial knockout of the α cell gp130 receptor has no effect on normal α cell function and fasting GLP-1 levels ................................................................................................................40
3.4 Pancreatic α cell dysfunction and islet inflammation in the STZ/HFD mouse model of T2D 43
  3.4.1 The STZ/HFD mouse: a non-genetic mouse model of T2D .............................43
  3.4.2 Characterization of islet inflammation in the STZ/HFD mouse model .............44
3.5 Characterization of αgp130KO mice in a non-genetic model of T2D ....................48
  3.5.1 Partial knockout of the α cell gp130 receptor protects from hyperglycemia following STZ and STZ/HFD treatment ........................................................................................................48
  3.5.2 STZ/HFD treated αgp130KO mice display no changes in insulin sensitivity ....49
3.6 Mechanism of action of gp130 receptor signalling in α cells ..............................53
  3.6.1 Gp130 receptor signalling may influence α cell identity ...............................53
3.6.2  Gp130 receptor signalling induces SOCS3 mRNA expression .......................... 55

Chapter 4: Discussion ........................................................................................................ 58

References .......................................................................................................................... 66
List of Tables

Table 2.1 Rodent strains used in this study ..............................................................................18
Table 2.2 Genotyping primer sequences ...................................................................................19
Table 2.3 Mouse RT-qPCR forward and reverse primers ..........................................................28
Table 2.4 Rat RT-qPCR forward and reverse primers ...............................................................29
Table 2.5 Human RT-qPCR forward and reverse primers .......................................................29
List of Figures

Figure 1.1 Proglucagon processing ........................................................................................................3
Figure 1.2 Gp130 family cytokines and their cognate receptors ......................................................7
Figure 1.3 Gp130 receptor signal transduction ....................................................................................9
Figure 2.1 Genotyping of Gcg-Cre (A), gp130\textsuperscript{fl/fl} (B), and mT/mG (C) mice ...................20
Figure 3.1 IL-6 family cytokine expression is increased in rodents with T2D .................................36
Figure 3.2 IL-6 and HIL-6 activate α cell STAT3 signaling and stimulate glucagon secretion from islets ......................................................................................................................................................38
Figure 3.3 αgp130KO mice exhibit partial loss of α cell gp130 receptor function and normal glucose homeostasis and α cell function under chow fed conditions ............................................42
Figure 3.4 Pancreatic α cell dysfunction and islet inflammation in STZ, HFD, and STZ/HFD treated mice ......................................................................................................................................................................................47
Figure 3.5 αgp130KO mice display improved glucose homeostasis and α cell function following STZ/HFD ................................................................................................................................................................................51
Figure 3.6 αgp130KO mice display no difference in glucose homeostasis or α cell function on HFD ................................................................................................................................................................................52
Figure 3.7 No difference in insulin sensitivity in αgp130KO mice following STZ/HFD ..........53
Figure 3.8 Gp130 receptor signalling may influence α cell identity .................................................55
Figure 3.9 IL-6 induced \textit{SoCs}3 mRNA expression ........................................................................56
Figure 3.10 Gp130 receptor activation induces STAT3 phosphorylation and \textit{SoCs}3 mRNA expression in αTC1.9 cells .......................................................................................................................................................57
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP-activated protein kinase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>K&lt;sub&gt;ATP&lt;/sub&gt;</td>
<td>ATP-sensitive K&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CT</td>
<td>Cardiotrophin</td>
</tr>
<tr>
<td>CLC</td>
<td>Cardiotrophin-like cytokine</td>
</tr>
<tr>
<td>CNTF</td>
<td>Ciliary neurotrophic factor</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EGP</td>
<td>Endogenous glucose production</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ECM</td>
<td>Extra-cellular matrix</td>
</tr>
<tr>
<td>ERK</td>
<td>Extracellular regulated kinase</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>FMO</td>
<td>Fluorescence minus one</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>GLP-1, GLP-2</td>
<td>Glucagon-like peptide 1 and 2</td>
</tr>
<tr>
<td>GSIS</td>
<td>Glucose stimulated insulin secretion</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Glucose transporter</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycated hemoglobin</td>
</tr>
<tr>
<td>Gp130</td>
<td>Glycoprotein 130</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hank’s balanced salt solution</td>
</tr>
<tr>
<td>HFD</td>
<td>High fat diet</td>
</tr>
<tr>
<td>HIL6</td>
<td>Hyper-Interleukin-6</td>
</tr>
<tr>
<td>ITT</td>
<td>Insulin tolerance test</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IL-6R</td>
<td>Interleukin 6 receptor</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intra-peritoneal</td>
</tr>
<tr>
<td>IPGTT</td>
<td>Intra-peritoneal glucagon tolerance test</td>
</tr>
<tr>
<td>IAPP</td>
<td>Islet amyloid polypeptide</td>
</tr>
<tr>
<td>JAK</td>
<td>Janus kinase</td>
</tr>
<tr>
<td>KO</td>
<td>Knockout</td>
</tr>
<tr>
<td>KRB</td>
<td>Krebs ringer buffer</td>
</tr>
<tr>
<td>LIF</td>
<td>Leukemia inhibitory factor</td>
</tr>
<tr>
<td>mG</td>
<td>Membrane eGFP</td>
</tr>
<tr>
<td>mT</td>
<td>Membrane tomato</td>
</tr>
<tr>
<td>OSM</td>
<td>Oncostatin M</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PFA</td>
<td>Paraformaldehyde</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and tensin homolog</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphoinositol 3-kinase</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PC2, PC1/3</td>
<td>Proconvertase 2 and 1/3</td>
</tr>
<tr>
<td>Gcg</td>
<td>Proglucagon</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein kinase A</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative-polymerase chain reaction</td>
</tr>
<tr>
<td>RT</td>
<td>Reverse transcriptase</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RPM</td>
<td>Rotation per minute</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal transducer and activator of transcription</td>
</tr>
<tr>
<td>sIL-6R</td>
<td>Soluble interleukin 6 receptor</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>STZ</td>
<td>Streptozotocin</td>
</tr>
<tr>
<td>SOCS</td>
<td>Suppressor of cytokine signalling</td>
</tr>
<tr>
<td>TUNEL</td>
<td>Terminal uridine nick-end labeling</td>
</tr>
<tr>
<td>TBS</td>
<td>Tris buffer saline</td>
</tr>
<tr>
<td>T1D</td>
<td>Type 1 diabetes</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 diabetes</td>
</tr>
<tr>
<td>USD</td>
<td>United States dollars</td>
</tr>
<tr>
<td>αgp130KO</td>
<td>α cell specific gp130 knockout</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
</tbody>
</table>
Acknowledgements

As I ponder over the last few months of this journey, I am greatly indebted to the many people that have played a role in the development of my skills as a scientist and my character as a person. First and foremost, I thank God for strength and grace throughout my life. Although I may have been weak and feeble, He has been upholding me by His Hand. My years of graduate studies have been enjoyable and a delight, but during the adversities, which were rare, I knew I had someone to lean on.

My supervisor and mentor, Dr. Jan Ehses, you have certainly fulfilled that last role. I wouldn’t be surprised if you have lost all your hair after taking me on as a student. You have shown patience when I have been rash in my interpretations and conclusions, and I have enjoyed the conversations of sports and life. Indeed, wherever I go on from here, I will be spoiled by your example as a mentor, for I cannot see how I could have any better.

Maddy, you are the master when it comes to animal work and there is no one that can match your expertise. Words cannot express my gratitude to you for your time and help with this work. To my lab members, Dominika and Meixia, I have enjoyed your friendship and the lighthearted conversations we had over the many cakes and house gatherings. To the colleagues, thank you for brightening up the mood when the weather and experiments prove otherwise. I have felt part of a greater community, and it has truly enriched my experience during my studies.

Lastly, I am grateful to my family, who has always supported me to this day. You have always encouraged me to strive and push forward when everything seems to be pushing back. To everyone else, thank you for your thoughts and prayers.
Chapter 1: Introduction

1.1 The pancreatic α cell and glucagon

1.1.1 The pancreatic α cell

The pancreatic islets of Langerhans are clusters of endocrine cells located in the pancreas, discovered and named after Paul Langerhans in 1869\(^1\). Within these islets, endocrine α cells, β cells, δ cells, PP cells, and ε cells reside and secrete glucagon, insulin, somatostatin, pancreatic polypeptide, and ghrelin respectively. The discovery of the α cell dates back to the discovery of the β cell. In 1907, Michael Lane took pancreatic sections from guinea pigs and fixed them in 70% alcohol or aqueous-chrome-sublimate. Staining with neutral gentian showed two distinct populations of islet cells as determined by the presence or absence of violet-stained granules. Despite not knowing the content stored within these granules, Lane postulated that the pancreatic islet was comprised of two different cell types, henceforth calling these populations the α and β cell\(^2\).

The pancreatic α cell is often neglected compared to the β cell, perhaps due to the fact that the pancreatic α cell only makes up 10-20% of the mouse islet, and is predominantly located at the periphery of the islet. However, this proportion is increased in human islets, where up to 40% of the islet is comprised of α cells, and the distribution of α cells is dispersed throughout the whole islet\(^3\). This may suggest a more prominent role for the α cell in human physiology compared to the rodent.

Although the discovery and isolation of insulin would precede that of glucagon, the presence of glucagon was still made known. In 1921, Banting and Best injected crude pancreatic extracts into pancreatectomized canines, only to observe a transient increase in glycemia followed by a drop in blood glucose levels\(^4\). This transient hyperglycemia-promoting
contaminant would be none other than glucagon. It was not until 1923 that Kimball and Murlin extracted insulin from pancreatic extracts by liquid-liquid extraction, and in doing so precipitated glucagon in one of their filtrates. This filtrate’s ability to act as a glucose agonist was named glucagon. Glucagon would be subsequently purified in 1949. Despite great advances in the identification and purification of this substance from α cells, there remained several hurdles in assessing α cell function. The amino acid sequence of glucagon was determined in 1957, and shortly thereafter, antibodies and the development of a radioimmunoassay made it possible to determine glucagon concentrations and assess α cell function in 1961.

In spite of the long history of glucagon and the pancreatic α cell, much remains unknown regarding α cell biology and its role in the pathophysiology of diabetes. This is likely due to the scarcity of α cells within the islet, the confounding factors in studying a heterogeneous population of endocrine cells in the islets, and the lack of reliable immortalized α cell lines. However, recent advances such as therapeutics targeting glucagon in type 2 diabetes (T2D), and the finding that α cells can be reprogrammed into β cells in rodents, have once again drawn attention to the neglected α cell and have highlighted its potential as a therapeutic target for diabetes treatment.

1.1.2 Glucagon synthesis

Following the discovery of glucagon and its amino acid sequence, the proglucagon (gcg) gene was sequenced in 1983 by Bell and colleagues. Transcription of the proglucagon gene encodes glucagon, glucagon-like peptide (GLP)-1 and GLP-2. Upon translation, the proglucagon peptide is cleaved by prohormone convertase (PC)-2 to produce the 29-amino acid hormone glucagon in pancreatic α cells (Figure 1.1). The proteolytic cleavage of proglucagon to mature glucagon is absent in PC2 knockout mice where pulse-chase experiments demonstrated
the accumulation of the proglucagon precursor. The proglucagon gene is also expressed in intestinal L-cells, with PC1/3 cleaving the proglucagon peptide to produce GLP-1 and GLP-2. The glucagon peptide itself is short-lived. Having a half-life of six minutes, glucagon is quickly degraded by neutral endopeptidase and dipeptidyl peptidase-4.

Figure 1.1 Proglucagon processing. Alternative post-translational cleavage of proglucagon peptide into glucagon, GLP-1 and GLP-2. Pancreatic α cells express PC2, allowing for glucagon production in the islet. PC1/3 expression in intestinal L cells allows for GLP-1 and GLP-2 production in the ileum and distal colon.
1.1.3 Glucagon secretion

Postprandial hyperglycemia stimulates β cell insulin secretion, while suppressing glucagon secretion in counter-regulatory fashion. The inverse situation also holds true, whereby hypoglycemia induces glucagon secretion in vivo. Despite knowing much about the mechanism of glucose-induced insulin secretion, it is still unclear how glucose regulates glucagon secretion, or whether glucose directly regulates glucagon secretion at all. For example, glucose has been shown to paradoxically stimulate glucagon secretion in isolated α cells19,20. In contrast, glucose deprivation has been shown to induce AMP-activated protein kinase (AMPK) activation and stimulate glucagon secretion21. The latter study suggests that α cells do possess an intrinsic glucose-sensing mechanism mediated by ATP-sensitive K⁺ (K\textsubscript{ATP}) channels, however, the exact mechanisms remain enigmatic and elusive.

Pancreatic α and β cells appear to possess the same exocytosis secretory machinery22,23. In β cells, metabolized glucose induces closure of K\textsubscript{ATP} channels, membrane depolarization, opening of Ca\textsuperscript{2+} channels, and influx of Ca\textsuperscript{2+} into the cells. This process renders β cells electrically active and triggers insulin exocytosis24,25, while α cells remain electrically inactive under high glucose conditions22,26. During hypoglycemic episodes, the same mechanism is thought to occur in α cells, where Ca\textsuperscript{2+} influx and an electrically active α cell is coupled with glucagon release27. Yet it would seem contradictory that with the same excitatory machinery, glucose would stimulate insulin secretion in one cell, while inhibiting glucagon secretion in another. One possible explanation is that α cells contain a relatively elevated ATP:ADP ratio under low glucose conditions, which results in closure of most K\textsubscript{ATP} channels23. After a meal, metabolized glucose further increases the ATP:ADP ratio, which then induces closure of all K\textsubscript{ATP} channels, leading to the inactivation of Na\textsuperscript{+} and Ca\textsuperscript{2+} channels, and inhibition of Ca\textsuperscript{2+} influx28,29.
Indeed, experiments titrating $K_{ATP}$ channel activity in $\alpha$ cells demonstrated that excessive, i.e. after a meal, or insufficient $K_{ATP}$ conductance inhibits glucagon secretion$^{22,30}$. Therefore, regulation of $K_{ATP}$ channel activity and sensitivity to changes in ATP:ADP concentrations may underlie glucose regulation of glucagon secretion.

In addition to direct effects of glucose on $\alpha$ cells, the autonomic nervous system$^{31,32}$ and glutamate$^{33}$ have been convincingly shown to increase glucagon secretion. Indeed, glucose sensing by the autonomic nervous may represent one of the major mechanisms regulating glucagon secretion$^{32}$. Several factors have also been demonstrated to inhibit glucagon secretion, including insulin, somatostatin, $\gamma$-aminobutyric acid (GABA), zinc ions, leptin, and GLP-1$^{34-46}$. Research on the cognate receptors of these factors in $\alpha$ cells have strengthened the hypothesis that $\alpha$ cells are regulated in a paracrine manner. Mice with knockout of the $\alpha$ cell insulin receptor displayed mild glucose intolerance, and hyperglucagonemia in the fed state$^{47}$. Using a guinea pig $\alpha$ cell line, InR1-G9, siRNA mediated knockdown of the insulin receptor demonstrated enhanced glucagon secretion$^{47}$. In vitro, leptin reduced $\alpha$ cell Ca$^{2+}$ signalling in the $\alpha$TC1-9 murine cell line, mouse islets, and human islets, resulting in a decrease in glucagon secretion$^{46}$. Follow-up studies in $\alpha$ cell specific knockout mice for the leptin receptor demonstrated no differences in glucose metabolism$^{48}$. However, the inconsistencies in these studies may be the result of only partial deletion of the leptin receptor, so leptin may still regulate glucagon secretion in vivo. Thus, as advanced research tools become available (e.g. $\alpha$ cell specific knockout mice) we have begun to unravel the cell autonomous mechanisms regulating glucagon secretion in vivo.

1.1.4 Glucagon action and the glucagon receptor

Glucagon primarily acts on the liver to increase hepatic glucose output by increasing glycogenolysis. Accounting for 70% of total hepatic glucose output, glucagon is secreted into the
hepatic portal vein and binds the glucagon receptor\textsuperscript{49,50}. The glucagon receptor is a G-protein-coupled receptor (GPCR), located on the cell membrane. Upon ligand-receptor binding, adenylate cyclase is activated, intracellular cyclic adenosine monophosphate (cAMP) concentrations are increased, followed by activation of protein kinase A (PKA)\textsuperscript{51,52}. This results in an increase in glycogenolysis to increase glycemia. Mice with global knockout of the glucagon receptor displayed α cell hyperplasia and reduced glycemia\textsuperscript{53}, while mice with tissue specific deletion of the glucagon receptor in the liver showed a similar phenotype\textsuperscript{54}. Furthermore, wildtype islets transplanted in liver glucagon receptor knockout (KO) mice displayed increased α cell mass\textsuperscript{54}. These data demonstrate that the liver is the main tissue whereby glucagon exerts its effects and in turn, the liver may secrete an unknown growth factor that regulates α cell mass. However, a recent study by Mighiu et al. (2013) demonstrated that glucagon infusion in the mediobasal hypothalamus inhibited hepatic glucose production and acts to regulate glycemia in a counter-regulatory fashion\textsuperscript{55}. Thus, in addition to liver-specific effects of glucagon, there may be a role for glucagon in the central nervous system with effects on glucose homeostasis.

1.2 \textbf{The gp130 receptor}

1.2.1 \textbf{The gp130 receptor and gp130 family cytokines}

The gp130 family of cytokines exhibit structural similarity and belong to the class I cytokine receptor family\textsuperscript{56}. To date, this receptor family includes interleukin (IL)-6, IL-11, IL-27, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin (CT)-1, CT-2, and cardiotrophin-like cytokine (CLC). Each cytokine binds its respective α-receptor (e.g. IL-6 receptor) with the exception of the LIF receptor, which can bind to CNTF, CT-1, CT-2, and CLC (Figure 1.2). All gp130 family members and their respective α-
receptor complex must bind to the gp130 receptor, referred to as the β-receptor, to induce intracellular signal transduction. As gp130 is ubiquitously expressed, it is the α-receptor that confers tissue specificity\textsuperscript{57}. Whereas knockout of the gp130 receptor results in embryonic lethality at day 12.5, deletion of individual gp130 family cytokines show mild defects suggesting overlapping functions and redundancy within this receptor family\textsuperscript{58,59}. For example, LIF\textsuperscript{−/−} mice are sterile due to defects in blastocyst implantation and hematopoiesis. IL-6\textsuperscript{−/−} mice have defects in hematopoiesis, acute phase protein synthesis, antigen-specific antibody production, chemokine induction and leukocyte recruitment and hepatocyte regeneration, and CNTF\textsuperscript{−/−}, OSM\textsuperscript{−/−}, and CT-1\textsuperscript{−/−} mice show various neuronal defects\textsuperscript{59}. In general, several roles in tissue development, tissue homeostasis, hematopoiesis, reproduction, and inflammation have been shown for gp130 family members\textsuperscript{59}.

\textbf{Figure 1.2 Gp130 family cytokines and their cognate receptors.} Gp130 receptor ligands and their specific ligand receptors. All gp130 family cytokines signal through the ubiquitous gp130 receptor. Cell specificity is conferred by the cognate cytokine receptor. Cytoplasmic regions of gp130 receptor are responsible for signal transduction upon receptor activation.
1.2.2 Gp130 receptor signalling

The gp130 receptor was first cloned in 1990 by Hibi and colleagues\textsuperscript{57}. Unlike other cytokine receptors, gp130 does not contain an intrinsic kinase domain. Instead, gp130 contains Janus kinase (JAK) association domains that recruit JAK for auto-phosphorylation and initiation of signalling cascades\textsuperscript{57}. Gp130 signalling is initiated when a gp130 family cytokine binds to its transmembrane non-signalling $\alpha$-receptor, e.g. the IL-6 receptor (IL-6R). Thereafter, the bound IL-6R causes homodimerization of two gp130 receptors and this results in activation of JAK/STAT, ERK, and PI3K signal transduction cascades (Figure 1.3). Gp130 has been shown to activate the Janus kinases JAK1, JAK2, and TYK2. Thereafter, signal transducer and activator of transcription (STAT) proteins are recruited to phosphorylated tyrosines present on the cytoplasmic domain of gp130. STAT3 is most potently activated, while STAT1 is induced by IL-27 only. These signalling cascades are under tight control of negative feedback mechanisms. Suppressor of cytokines signalling (SOCS) proteins, SOCS1 and SOCS3, directly bind to phosphorylated JAK and/or to the SHP2- binding site of gp130 to inhibit the JAK/STAT and ERK pathway respectively\textsuperscript{59}.

To avoid early lethality, gp130\textsuperscript{flox} mice were generated in which exon 16 encoding the transmembrane domain of gp130 was floxed\textsuperscript{60}. This has allowed the generation of numerous tissue-specific gp130 receptor knockout mice including, liver, lung, neuron, cardiac, and T cell-specific knockout mice. In general, gp130 receptor expression is not required for the development of these tissues, but is required in the adaptive responses of these tissues to injury (liver, lung)\textsuperscript{59} and biomechanical stress (cardiac myocytes)\textsuperscript{61}. Other common findings among tissue specific mutants are that gp130 signalling protects cells from inflammation-induced apoptosis, and depletion of gp130 in one cell type can have consequences on a non-mutated cell.
type. The liver-specific gp130 mutant illustrates the latter, where the absence of an acute phase response protected from atherosclerosis in the aorta\textsuperscript{62}.

Figure 1.3 Gp130 receptor signal transduction. Activation of gp130 receptor signalling causes homodimerization of two gp130 receptors. JAK is recruited to cytoplasmic regions of gp130, where activation by gp130 causes JAK to phosphorylate gp130-bound JAK and tyrosine residues on intracellular regions of gp130. STAT3 is recruited to phosphorylated tyrosine residues and phosphorylated by JAK. Dimerized activated pSTAT3 translocates into the nucleus, acting as a transcription factor and enhancing transcription. Activation of gp130 can also activate the PI3K and MEK signalling pathway. A negative feedback regulator of gp130 signalling, SOCS1/3, inhibits JAK-mediated STAT3 phosphorylation and ERK pathway activation by binding JAK and SHP2.
1.3 Diabetes mellitus

1.3.1 Disease burden

Diabetes mellitus is a huge global economic burden, with an estimated 285 million adults diagnosed with this disease in 2010. Diabetes cost the global health care system 376 billion healthcare dollars (USD) in 2010, and this figure is projected to increase to 490 billion healthcare dollars (USD) in 2030\(^6\). Adding to the economic burden of this disease are the debilitating secondary complications that follow. Patients with diabetes frequently encounter secondary complications such as kidney failure, cardiovascular diseases, stroke, retinopathy, and neuropathy, resulting in 10-20 years of premature mortality\(^{64,65}\). It is the fatalities due to these secondary diseases that mask the severity of diabetes, underscoring the potential dangers caused by this disease. In Canada alone, approximately 2.4 million people had diagnosed diabetes in 2009, with an expected 56% increase in disease prevalence by 2019 (3.7 million)\(^6\). An additional daunting fact is that a further 5 million Canadians have impaired glucose tolerance and fasting glucose, putting these individuals at 30% increased risk for developing diabetes\(^6\).

Diabetes is a metabolic disorder characterized by marked hyperglycemia, attributable to the body’s inability to produce or respond to insulin and due to inappropriately elevated glucagon secretion. In response to elevated blood glucose levels, insulin is produced and secreted by pancreatic \(\beta\) cells. Acting predominantly on the liver, muscle, and adipose tissue, insulin stimulates the uptake of glucose for storage in the form of glycogen, and the accumulation of triglycerides within adipose tissue. In this manner, insulin is one of the body’s main anabolic hormones, required for extracting energy from nutrition.

Opposing the anabolic actions of insulin is the catabolic hormone glucagon. Secreted by pancreatic \(\alpha\) cells, glucagon mainly acts on hepatocytes to increase gluconeogenesis and
glycogenolysis. The counter-regulatory action of glucagon plays an important role in preventing fatal hypoglycemia. Together, impairments in insulin secretion or action, and inappropriate levels of glucagon may result in elevated blood glucose levels and the manifestation of diabetes.

1.3.2 Type 1 diabetes

Roughly 10% of diabetes patients have type 1 diabetes (T1D), a disease where the individual’s own immune cells target and destroy the insulin-producing β cells. Patients devoid of β cells can no longer maintain glucose homeostasis, and must be supplemented with insulin to survive. A study on patients with T1D under a euglycemic (5.3 mmol/l) clamp with variable insulin infusion demonstrated that glucagon suppression was induced by insulin. Thus, the lack of insulin-producing β cells in T1D may result in an unrestrained α cell, also contributing to hyperglycemia. Additionally, glucagon receptor-deficient mice were protected from hyperglycemia after streptozotocin (STZ)-induced β cell death, an effect that was reversed by re-expression of the glucagon receptor in hepatocytes. This demonstrates that glucagon action is required for hyperglycemia in a non-genetic model of T1D. Lastly, individuals with T1D are more prone to the fatal consequences of hypoglycemia likely due to a dysfunctional counter-regulatory glucagon response. This “hypoglycemia blindness” is thought to be the result of heightened insulin sensitivity and an impaired autonomic response. Collectively, these studies suggest that an unrestrained α cell along with insulin deficiency, may contribute to hyperglycemia during T1D, however this has not been confirmed in humans.

1.3.3 Type 2 diabetes

The most common form of diabetes, T2D, accounts for approximately 90% of all cases. Prior to the onset of T2D (i.e. in prediabetic individuals), peripheral tissues require increased insulin concentrations –termed insulin resistance– to induce insulin receptor signalling and
maintain euglycemia. As the disease progresses, adaptive insulin secretion declines due to β cell exhaustion, dysfunction, failure and subsequent death\textsuperscript{70-72}. Endoplasmic reticulum (ER) stress, glucolipotoxicity, islet amyloid polypeptide (IAPP), and islet inflammation have all been shown to contribute to β cell failure\textsuperscript{71,73-78}. This subsequent loss of insulin secretion in the face of insulin resistance further exacerbates the disease, and is thought to represent the clinical onset of hyperglycemia.

1.3.4 Pancreatic α cell dysfunction in T2D

Diabetes research has largely focused on the β cell and taken an “insulinocentric” approach, namely that insulin deficiency gives rise to all diabetic symptoms\textsuperscript{11}. However, in individuals diagnosed with prediabetes, the inverse relationship of pulsatile insulin and glucagon is impaired suggesting α cell function or intra-islet crosstalk is impaired even prior to the onset of T2D\textsuperscript{79}. Indeed, numerous studies in rodent models of T2D have targeted glucagon and the glucagon receptor using neutralizing antibodies\textsuperscript{80}, receptor antagonists\textsuperscript{81,82}, and antisense oligonucleotides\textsuperscript{83,84} and shown improvements in glycemia. These data strongly support the hyperglycemia-promoting effects of glucagon and implicate glucagon in the pathogenesis of T2D in rodents.

A similar case can be made for human subjects with T2D. When patients with T2D were infused with insulin and somatostatin to reproduce a diabetic profile during a glucose challenge with either non-suppressed or suppressed glucagon levels, those individuals with non-suppressed glucagon had impaired glucose tolerance following an oral glucose challenge. One possible explanation for this dysglycemia is the glucagon:insulin ratio that the liver is exposed to. In the face of β cell death and relative hyperglucagonemia, a greater glucagon:insulin ratio has greater effects on hepatic glucose production and thus promotes hyperglycemia\textsuperscript{85,86}. Furthermore, fasted
glucagon has been reported to be elevated in individuals with T2D, suggesting that an unrestrained \( \alpha \) cell contributes to fasting hyperglycemia\(^{60} \) in addition to postprandial hyperglycemia. The hyperglycemia-promoting effects of glucagon in T2D have also been confirmed in the clinic, where recent trials with glucagon receptor antagonists have robustly reduced hyperglycemia in subjects with T2D\(^ {87} \).

The main mechanism underlying hyperglucagonemia in T2D is thought to be \( \beta \) cell dysfunction and the lack of effects of \( \beta \) cell-derived secretory products on \( \alpha \) cells (e.g. insulin, GABA, zinc ions)\(^ {39} \). It has been demonstrated that all of these factors inhibit glucagon secretion, and their secretion is likely decreased in frank T2D. However, it is less clear whether these factors are reduced early in T2D, prior to the development of marked \( \beta \) cell dysfunction. Recent data have demonstrated that inflammation is a hallmark of the islet pathology during T2D\(^ {75,88,89} \), and that IL-6 is a key cytokine with effects on \( \alpha \) cell glucagon secretion, survival, and differentiation\(^ {90,91} \). Thus, inflammation may be a key driver of \( \alpha \) cell dysfunction during the development of diabetes and further knowledge of the role of the gp130 receptor in \( \alpha \) cells may generate a greater understanding of how the \( \alpha \) cell contributes to hyperglycemia in this disease.

1.4 Islet inflammation in T2D

1.4.1 Gp130 cytokines in T2D

A chronic low-grade inflammatory state results in increased systemic levels of pro-inflammatory cytokines in T2D, with adipose tissue being the main contributor of increased circulating IL-6 levels\(^ {92} \). Indeed, elevated systemic levels of IL-1\( \beta \) together with IL-6 are predictive of increased risk to develop T2D\(^ {93} \). In diabetic subjects and rodent models of T2D, OSM is also secreted by white adipose tissue macrophages, stimulating neighbouring adipocytes to secrete IL-6\(^ {94} \). This increase in IL-6 promotes insulin resistance in the liver\(^ {95} \), with IL-6-
induced SOCS3 mediating these negative effects in rodents\textsuperscript{96,97}. However, exercise-induced IL-6 mediates a contrasting effect in skeletal muscle. IL-6 secreted by skeletal muscle during exercise modulates insulin action by increasing insulin-stimulated glucose uptake\textsuperscript{98}. CNTF can similarly increase glucose uptake in muscle, while this effect is absent in \textit{ob/ob} and HFD-fed obese mice\textsuperscript{99}. Another study has demonstrated that exercise-induced IL-6 also stimulates GLP-1 secretion from intestinal L cells, and thereby potentiates insulin secretion upon subsequent glucose challenge\textsuperscript{91}. Thus, post-exercise IL-6 appears to have favorable effects on glycemic control, conflicting with the adverse effects of IL-6-induced insulin resistance in the liver during obesity. Global knockout of IL-6 did not provide any insight into the hierarchy of these effects; IL-6 deficiency exacerbated HFD-induced hepatic insulin resistance\textsuperscript{100}. Because IL-6 deletion still resulted in hepatic insulin resistance, other factors may compensate to promote insulin resistance such as TNF-\textit{\alpha}\textsuperscript{101}. In T2D, several gp130 family cytokine genes are expressed at elevated levels in islets\textsuperscript{102}. However, it is not known how these cytokines contribute to disease progression. These studies suggest that more targeted approaches are required to determine the tissue-specific effects of gp130 cytokines.

1.4.2 Islet inflammation and the pancreatic \textit{\alpha} cell

It is becoming increasingly apparent that inflammation characterizes islet pathology during T2D and contributes to hyperglycemia in this disease\textsuperscript{73,103,104}. Islets from individuals with T2D and animal models of this disease show increased macrophage infiltration and expression of proinflammatory cytokines\textsuperscript{75,103,105-107}. Depletion of macrophages using clodronate-loaded liposomes decreased islet inflammation and improved \textit{\beta} cell function in three recent studies examining the palmitate-infused mouse, the \textit{db/db} mouse, the KKAy mouse, the Zucker fatty diabetic rat, and the \textit{\beta} cell human islet amyloid polypeptide overexpressing mouse\textsuperscript{73,108,109}. There
were no effects of macrophage depletion on insulin sensitivity. Indeed, neutralization of IL-1 or IL-1β in individuals with T2D has demonstrated improved β cell function and reduced glycemia in a number of clinical studies. Thus, preclinical and human data indicate that islet inflammation is a hallmark of T2D pathology and that β cell function is negatively affected by IL-1-driven islet inflammation.

In addition to up-regulated il1b mRNA expression, islets from rodent models of T2D also have highly up-regulated il6 mRNA expression and IL-6 secretion\textsuperscript{106,110}. The same holds true for the human disease, where recently published data from a global gene expression study in islets from T2D subjects showed increased expression of il6 and il11 mRNA by 2.75-fold and 1.61-fold respectively\textsuperscript{102}.

It was recently discovered that mouse islets express high levels of IL-6 receptor (IL-6R) mRNA compared to other mouse tissues, with very high expression in rat purified α and β cells relative to other rat tissues\textsuperscript{90}. Gp130 receptor mRNA was also expressed in mouse islets, and in rat α cells levels were higher than in numerous other rat tissues\textsuperscript{90}. IL-6 increased α cell proliferation and prevented nutrient induced α cell apoptosis, while increasing proglucagon gene expression and glucagon secretion in human and rodent islets\textsuperscript{90}. Consistent with these results, HFD-induced α cell mass expansion was absent and circulating glucagon was decreased in IL-6 knockout mice on HFD. However, prolonged exposure to IL-6 (up to 4 days) stimulated GLP-1 secretion from purified α cells, perhaps due to increased PC1/3 expression\textsuperscript{91}. Despite not elucidating the cellular mechanism of these various effects, these studies demonstrate that IL-6-induced gp130 activation has effects on α cell function, mass, and differentiation. The latter may be an adaptive response to chronic exposure to IL-6 in obesity and T2D, acting to preserve β cell function.
Other studies have also linked IL-6 to the pancreatic α cell. Tweedell et al. (2011) demonstrated that IL-6 deficient mice have a blunted glucagon response to endotoxin\textsuperscript{111}. They went on to demonstrate that IL-6 amplifies glucagon secretion in response to adrenergic stimuli\textsuperscript{112}. Another study in rat neonates demonstrated that IL-6 neutralization decreased α cell mass expansion and glucagon production, corroborating previous results\textsuperscript{113}.

Overall, activation of the gp130 receptor appears to have tissue-specific effects with respect to the regulation of glucose homeostasis. These include effects on insulin action, entero-endocrine cells, the systemic immune response, and pancreatic α cell mass, function, and differentiation. However, studies have not clarified how gp130 family cytokines regulate glycemia via actions on α cell glucagon or GLP-1 secretion in a setting of islet inflammation and decreased β cell mass modeling T2D. To study this, we generated α cell gp130 receptor knockout (agp130KO) mice and investigated the role of the α cell in a non-genetic rodent model of T2D. The following hypothesis was tested:

**Hypothesis:** Pancreatic α cell gp130 receptor signalling contributes to α cell dysfunction and regulates glucose homeostasis in T2D.

**Objective:** To determine the role of α cell gp130 receptor signalling in α cell dysfunction and glycemic control in an animal model of T2D.
Chapter 2: Methodology

2.1 In vivo studies

2.1.1 Animals

Male rodents were used for all experiments, and all rodent strains used are listed in Table 2.1. To delete the gp130 receptor from pancreatic α-cells, floxed gp130 receptor mice (gp130^{fl/fl}; provided by Dr. Werner Müller, University of Manchester, UK) and mice expressing Cre recombinase under the control of the rat glucagon promoter (Gcg-Cre; provided by Dr. Pedro Herrera, University of Geneva, Switzerland) were housed and bred at the Child & Family Research Institute (CFRI) animal care facility. Gp130^{fl/fl} mice were bred with Gcg-Cre mice to obtain Gcg-Cre mice heterozygous for the gp130 receptor floxed gene (Gcg-Cre^{+/-} gp130^{fl/+}). Gcg-Cre^{+/-} gp130^{fl/+} mice were then backcrossed with gp130^{fl/fl} mice to obtain mice homozygous for the gp130 receptor floxed gene, thus creating mice with α-cell specific deletion of the gp130 receptor (Gcg-Cre^{+/-} gp130^{fl/fl}: hereafter referred to as αgp130KO mice). These αgp130KO mice were bred with gp130^{fl/fl} mice to obtain the strains used for the present studies (gp130^{fl/fl} and αgp130KO mice). All rodents were housed at the CFRI animal facility, maintained on a 12 hour light/dark cycle, and fed normal chow diet (Chow; cat. #20-5053, 13 kcal% fat, PicoLab Rodent Diet, Lab Diet, St. Louis, USA).

Mice deficient for the leptin receptor, BKS.Cg-m+/-Lepr^{db}/BomTac (db/db), were housed at Novo Nordisk, Måløv, Denmark. All procedures were approved by the University of British Columbia Committee on Animal Care or the Principles of Laboratory Care (Denmark).

To assess the expression of the Cre recombinase, Gcg-Cre^{+/-} mice were bred with homozygous mT/mG mice to generate heterozygous Gcg-Cre^{+/-} mT/mG^{+/-} mice. Heterozygous
expression of the mT/mG cassette expresses sufficient protein fluorescence to be visualized by microscopy.

Table 2.1 Rodent strains used in this study.

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Abbreviation</th>
<th>Source</th>
<th>Catalog number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goto Kakizaki rat</td>
<td>GK</td>
<td>Taconic, Hudson, NY USA</td>
<td>-</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>-</td>
<td>Taconic, Hudson, NY, USA</td>
<td>-</td>
</tr>
<tr>
<td>BKS.Cg-m+/+Leprdb/BomTac</td>
<td>db/db</td>
<td>Taconic, Ejby, Denmark</td>
<td>-</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>C57BL/6J</td>
<td>Centre for Disease Modeling, Vancouver, Canada</td>
<td>-</td>
</tr>
<tr>
<td>Gt(ROSA)26Stm4(XC:1B: tdTomato,-Egfp)Luo/J</td>
<td>mT/mG</td>
<td>Jackson Laboratory, Bar Harbor, ME, USA</td>
<td>stock #007576</td>
</tr>
<tr>
<td>Gcg-Cre</td>
<td>Gcg-Cre</td>
<td>Dr. Pedro Herrera, University of Geneva, Switzerland</td>
<td>-</td>
</tr>
<tr>
<td>gp130fl/fl</td>
<td>gp130fl/fl</td>
<td>Dr. Werner Müller, University of Manchester, United Kingdom</td>
<td>-</td>
</tr>
<tr>
<td>Gcg-Cre+/-gp130fl/fl</td>
<td>agp130KO</td>
<td>In house</td>
<td>-</td>
</tr>
</tbody>
</table>

2.1.2 Genotyping

Ear notches were taken from newly weaned animals (3 weeks old) and digested in PCR strip tubes at 55°C for 45 minutes followed by 95°C for 15 minutes in 20% chelex 100 solution (cat. #142-1253, BioRad, Hercules, USA) containing proteinase K (cat. #BP1700, Fisher Scientific, Fair Lawn, USA). The extracted DNA within the supernatants was amplified by polymerase chain reaction (PCR) using the primers listed in Table 2.2. The PCR mixture contained isolated DNA, Taq buffer (Buffer E; gift by Dr. Francis Lynn, University of British
Columbia, Canada) dNTPs, forward and reverse primers, DMSO, Taq polymerase (gift by Dr. Francis Lynn) and ultrapure water. Reactions were run for 30-40 cycles following Invitrogen Taq DNA Polymerase protocol. For amplification of Cre recombinase, denaturation was carried out at 94°C for 4.5 minutes, annealing at 60°C for 0.5 minutes, and extension at 67°C for 1 minute for 30 cycles. For the gp130 floxed gene, denaturation was carried out at 94°C for 1.5 minutes, annealing at 56°C for 1 minute, and extension at 72°C for 2 minutes for 40 cycles. For amplification of the mT/mG gene, denaturation was carried out at 94°C for 3.5 minutes, annealing at 61°C for 1 minute, and extension at 72°C for 1 minute for 36 cycles. Amplified DNA was run on a 1.25% agarose gel containing SYBR Safe DNA Gel Stain (Invitrogen, Carlsbad, USA) and visualized using a Gel Doc System (Figure 2.1).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cre</td>
<td>Forward</td>
<td>5'-GAGAAATTTATATTGTCAGCG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-TCCATGGTGATACAAGGGAC-3'</td>
</tr>
<tr>
<td>gp130</td>
<td>Forward</td>
<td>5'-TGGCTTGAGCCTACAGCTGGCTAG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-GTGAACAGTCACCAGTGTCATCTGTACGC-3'</td>
</tr>
<tr>
<td>mT/mG</td>
<td>Forward</td>
<td>5'-CTCTGCTGCCTGCAGGGCTCTCT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse wildtype</td>
<td>5'-CGAGGCGGATCACAAGGAATA-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse mutant</td>
<td>5'-TCAATGGGCCGGGTCGTT-3'</td>
</tr>
</tbody>
</table>
2.1.3 Streptozotocin, high-fat diet, and streptozotocin/high-fat diet mouse models

Streptozotocin (STZ; cat. #S0130, Sigma-Aldrich, Oakville, Canada) was prepared in acetate buffer (pH 4.5), and administered (25 mg/kg) via intra-peritoneal (i.p.) injections for five consecutive days at 7-9 weeks of age. Control mice were administered an i.p. injection of acetate buffer. Some mice were put on high-fat, high-sucrose Surwit Diet (HFD; 58 kcal% fat w/sucrose; cat. #D12331, Research Diets, New Brunswick, USA), 3 weeks following first i.p. injection of acetate buffer or STZ.

2.1.4 Physiological measurements

For blood sampling, aprotinin (250 KIU/ml plasma; cat. #A6279, Sigma-Aldrich, Oakville, Canada) and DPP-IV inhibitor (50 µM; cat. #DPP4, Millipore, St. Charles, USA) were added to all collection tubes. Mice were fasted 12 hours and injected i.p. with 1.5g/kg body weight glucose for glucose tolerance tests (IPGTT). Blood glucose was measured via the
saphenous vein at 0, 15, 30, 60, 90, and 120 min using a glucometer (One Touch, LifeScan, Canada). Plasma insulin and glucagon were assayed from blood samples taken at 0 and 30 min by ELISA (Alpco, Salem, USA; Mercodia AB, Uppsala, Sweden).

For insulin tolerance testing (ITT), mice were fasted 2 hours and injected i.p. with 1 U/kg insulin (Novolin Ge, Novo Nordisk, Toronto, Canada). Blood glucose was measured via the saphenous vein at 0, 15, 30, 60, 90, 120 minutes using a glucometer. Plasma glucagon was assayed in blood samples taken at 0 and 15 min by ELISA (Mercodia AB).

For GLP-1 levels, mice were fasted 12 hours and blood was collected from the saphenous vein. Total GLP-1 was assayed using the MSD Total GLP-1 Kit (cat. #K150JVC-1; Meso Scale Discovery, Gaithersburg, USA) together with a Sector Imager 2400A instrument, following the manufacturer’s instructions. Fed (non-fasted) blood samples were obtained from the saphenous vein for measurement of plasma insulin (Alpco) and IL-6 levels were measured using a Perkin Elmer CS 1000 Autoplex Analyzer machine (Luminex; cat. #MMHMAC-44K, Millipore, St. Charles, USA) following the manufacturer’s instructions.

2.1.5 Hyperinsulinemic-euglycemic clamps

Hyperinsulinemic-euglycemic clamps were performed as previously described. Mice were fasted overnight and anesthetized with acepromazine (0.625 mg/ml; DIN 00053023, Wyeth, Guelph, Canada), midazolam (0.625 mg/ml; cat. #5270, Sandoz, Boucherville, Canada), and fentanyl (0.0313 mg/ml; cat. #2520, Sandoz), with an initial dose (8 ml/kg) by i.p. injection and top-up doses (2 ml/kg) by subcutaneous injection. Once fully immobilized from anesthesia, the tail vein was cannulated, and a 1 hour basal infusion of $^3$H-D-glucose (1.2 $\mu$Ci/h) was started to determine steady-state tracer. Duplicate blood samples were taken from the saphenous vein at the end of the basal period to measure fasting blood glucose levels, for determination of
endogenous glucose production (EGP), and for insulin measurements. Hyperinsulinemia was induced with a constant infusion of insulin (3.5 mU/kg/min). To maintain euglycemia (~5.0 mM), a variable infusion of 12.5% D-glucose was started simultaneously. Once euglycemia had been achieved, steady state was maintained for 60 minutes, after which triplicate blood samples were taken for further analysis. Ten microlitres of plasma was diluted with 90 µl of saline, then 20 µl of the diluted plasma was counted for total radioactivity using a 1450 MicroBeta TriLux LSC and Luminescence Counter (Perkin Elmer). The remaining 80 µl of diluted plasma was added to 80 µl of 20% trichloroacetic acid to precipitate proteins/lipids and centrifuged at 13,000 rpm for 10 minutes. One hundred fifty microlitres of supernatant was transferred to 1.5 ml tubes and incubated at 70°C to dry overnight. The following day, 100 µl of distilled water was added to pellets, and 90 µl was taken to count radioactivity. Insulin concentrations were measured by ELISA (Alpco). Whole-body glucose use (µmolkg⁻¹min⁻¹) was determined as the ratio of the specific activity of glucose to the rate of ³H-D-glucose appearance (see example calculation below). Subsequently, EGP (µmolkg⁻¹min⁻¹) could be calculated as the difference between whole-body glucose uptake and exogenous glucose infusion.

Sample calculation:

a) Specific activity for glucose:

Specific activity (SA) = plasma radioactivity × blood glucose

\[
\text{Specific activity (SA)} = \frac{12500 \text{ cpm}}{\text{ml plasma}} \times \frac{\text{L}}{4.8 \text{ mmol}} = 2604.1 \text{ cpm/µmol}
\]

b) Rate of radioactive glucose appearance:

Radioactivity counts in infusion solutions/min = Vin

\[
\text{Vin} = 884497.5 \text{ cpm} \div 60 \text{ minutes} = 14741.6 \text{ cpm/min}
\]
c) Glucose turnover = body glucose uptake = $V_{in}/SA$

$$\frac{14741.6 \text{ cpm}}{\text{min}} \times \frac{\mu\text{mol}}{2604.1 \text{ cpm}} = 5.834 \mu\text{mol/min}$$

d) Whole body glucose use normalized to weight

$$5.834 \mu\text{mol/min} \div 0.033 \text{ kg} = 176.78 \mu\text{mol/kg min}$$

2.2 In Vitro Studies

2.2.1 Pancreatic islet isolation

Mouse and rat islets were isolated as described\textsuperscript{106}. Briefly, the bile duct was clamped at the duodenum and the pancreas was perfused via the pancreatic duct with 2 ml of collagenase type XI (1000 U/ml; Sigma-Aldrich, Oakville, Canada) dissolved in 1x Hank’s Balanced Salt Solution (HBSS; cat. #14185, Gibco, Grand Island, USA). Excised pancreata were placed in a 50 ml conical tube containing 2 ml of collagenase in 1x HBSS on ice. Next, conical tubes containing pancreata were incubated in a 37°C waterbath for 14 minutes. Pancreata were further digested by manual shaking by hand until a homogenous solution was achieved (approximately 5 min). HBSS containing 1 mM CaCl\textsubscript{2} was added to stop the digestion and tubes were centrifuged for 1 min at 1000 rpm. Supernatants were discarded and pancreata rinsed with HBSS containing 1 mM CaCl\textsubscript{2}. Centrifugation and rinsing steps were repeated once more. Isolated islets were filtered through a 70 µm nylon filter. Islets were hand-picked to remove exocrine tissue and cultured in 11.1 mM glucose RPMI 1640 (cat. #11875, Gibco) with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM GlutaMAX (cat. #35050, Gibco), and 10% heat-inactivated FBS (hereafter referred to as islet culture media) overnight at 37°C with 5% CO\textsubscript{2} unless otherwise stated.
Pancreatic islets from \textit{db/db} mice were isolated by Mette Ladefoged and Ann Maria Hansen at the Diabetes Research Unit, Novo Nordisk A/S, Måløv, Denmark, as described\textsuperscript{118}. Human islets were isolated from pancreata of eight organ donors at the University of Alberta and Prodo Laboratories (Irvine, USA). All human islet preparations had \textbf{>80\%} purity of islets, were hand-picked and plated to ensure purity and were cultured in CMRL-1066 medium (cat. \#11530, Gibco, Grand Island, USA) containing 5.5 mmol/l glucose, 100 U/ml penicillin, 100 µg/ml streptomycin, 40 µg/ml gentamicin (cat. \#15710-064, Gibco, Grand Island, USA) and 10\% heat-inactivated FBS overnight (hereafter called human islet media) at 37°C with 5\% CO\textsubscript{2}. Human islet studies were approved by the Clinical Research Ethics Board.

\subsection*{2.2.2 Islet dispersion}

Fifty islets were transferred to a 1.5 ml microcentrifuge tube containing islet media. Islets were centrifuged at 300x g for 4 minutes and rinsed with 1 ml Ca\textsuperscript{2+}- and Mg\textsuperscript{2+}-free PBS (cat. \#70013, Gibco, Grand Island, USA) with 0.5 mM EDTA. Islets were dispersed in Ca\textsuperscript{2+}- and Mg\textsuperscript{2+}-free PBS with a final concentration of 0.02\% trypsin-EDTA for 6 minutes at 37°C. Islets were dispersed mechanically by pipetting at 3 minutes during the incubation period. The reaction was stopped by adding the single cell suspension to 7 ml of islet media in a 15 ml conical tube. Dispersed islets were centrifuged for 5 minutes at 300x g and resuspended in an appropriate volume of islet media (50 µl/50 islets).

\subsection*{2.2.3 \textit{αTC1-9} cells}

A clonal \textit{α} cell line, \textit{αTC1} clone 9 (gift from Dr. Timothy Kieffer, UBC, passages 12-25), was maintained in high-glucose DMEM (cat. \#11960, Gibco, Grand Island, USA) containing 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM GlutaMAX and 10\% heat-inactivated FBS.
(hereafter called cell culture media). For passaging, cells were removed from flasks by 10 minutes with 0.05% trypsin-EDTA at 37°C.

2.2.4 Glucagon, GLP-1, and insulin secretion assays

Extra-cellular matrix (ECM) was made by incubating 804G cells in low glucose DMEM (cat. #11885, Gibco, Grand Island, USA) supplemented with 10% heat-inactivated FBS, 100 µg/ml streptomycin at 37°C with 5% CO₂. After cells were 75% confluent (approximately 3 days), cells were rinsed with low glucose DMEM without FBS. Cells are then incubated at 37°C with 5% CO₂ in low glucose DMEM without FBS (12 ml for a 75 cm² flask). After 2 days, conditioned medium was collected, centrifuged for 5 minutes at 400x g, and supernatants filtered through a 0.2 µm filter. Supernatants of 804G ECM were frozen until further use.

For secretion assays, 6-well plates were incubated with 800 µl/well of 804G ECM overnight, prior to plating islets the following day. The next day, wells were washed three times with distilled water for 5 minutes before plating islets in islet culture media.

For glucagon and GLP-1 secretion, fifty medium sized islets were plated on 804G ECM-coated 6-well plates. Islets were allowed to adhere overnight in 1 ml islet culture media at 37°C with 5% CO₂. The following day, fresh islet culture media was added containing aprotinin (250 KIU/ml; Sigma-Aldrich) and DPP-IV inhibitor (50 µM; Millipore). Islets were then treated with IL-6 or hyper (H)IL-6 (1, 10, 100 ng/ml) for 1, 24, and 48 hours. Conditioned media were collected, centrifuged at 1200 rpm to remove cell debris, and 1 ml of 70% acid ethanol was added to islets overnight (12 hours) for assessment of total hormone content. Glucagon and GLP-1 were measured by radioimmunoassay (cat. #GL-32K and #GLP1T-36HK, Millipore) using a PerkinElmer 1470 Automatic Gamma Counter instrument and following the manufacturer’s instructions.
Glucose-stimulated insulin secretion from islets was performed as previously published\textsuperscript{106}. Briefly, twenty medium sized islets were plated on 804G ECM-coated 6-well plates. Islets were allowed to adhere overnight in 1 ml islet culture media at 37°C with 5% CO\textsubscript{2}. Islets were washed and pre-incubated for 1 hour in Krebs Ringer Buffer (KRB; 129 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO\textsubscript{4}, 1.2 mM KH\textsubscript{2}PO\textsubscript{4}, 2.5 mM CaCl\textsubscript{2}, 5 mM NaHCO\textsubscript{3}, 10 mM HEPES, 0.5% BSA) containing 2.8 mM glucose. Next, islets were incubated for 1 hour in 2.8 mM glucose KRB at 37°C with 5% CO\textsubscript{2}. Conditioned buffer was collected, centrifuged at 1200 rpm to remove cell debris, and 16.7 mM glucose KRB was added to islets for an additional 1 hour. Conditioned buffer was collected, centrifuged at 1200 rpm to remove cell debris and 1 ml of 70% acid ethanol was added to the islets overnight (12 hours) to determine total insulin content. Insulin in conditioned buffer was assayed by ELISA (Alpco).

For total pancreatic insulin, glucagon and GLP-1 content, hormones were extracted from a small piece of the tail of the pancreas using 70% acid ethanol and determined using Luminex technology (cat. #MMHMAG-44K, Millipore). Total pancreatic protein concentration was determined using the Pierce BCA Protein Assay kit (Thermo Scientific, Rockford, USA) following manufacturers instructions.

2.2.5 Islet secretion of IL-6, LIF, and sIL-6R

One hundred freshly isolated islets were plated in 1 ml islet culture media for 24 hours in suspension in a 24-well plate. Conditioned media were collected, centrifuged at 1200 rpm, and IL-6 and LIF were assayed using Luminex technology (cat. #MMHMAG-44K, Millipore). Soluble IL-6 receptor (sIL-6R) secretion was assayed using an internal ELISA in the laboratory of Professor S. Rose-John (Christian Albrechts University of Kiel, Germany)\textsuperscript{119}. 
2.2.6 Gp130-mediated gene expression in islet and αTC1.9 cells

To determine effects of gp130 activation on gene expression in α cells, 50 islets or 10^6 αTC1.9 cells were plated in a 6-well plate in 1 ml islet or cell culture media. Plates were incubated overnight at 37°C. The next day, plates were briefly washed with media and 1 ml of islet or cell culture media was added. A time course of up to 6 days was conducted with IL-6 and HIL6 (100 ng/ml) added to islets and αTC1.9 cells respectively.

2.2.7 Gene expression analysis

Total RNA was extracted using the MN NucleoSpin RNA II kit (cat. #740955, Macherey-Nagel, Düren, Germany) according to the manufacturer’s instructions. Following islet isolation, islets were allowed to recover for 4 hours prior to RNA extraction.

Following RNA isolation, cDNA was synthesized using 400 ng RNA and SuperScript II Reverse Transcriptase (cat. #18064, Invitrogen), using random hexamers and following the manufacturers instructions. Synthesized cDNA was diluted 1:12 with ultrapure water. Quantitative-PCR was performed using PrimeTime primers/probes (Integrated DNA Technologies, Coralville, USA) shown in Table 2.3 (probe sequence not shown) and TaqMan Universal Master Mix II, no UNG (cat. #4440040, Applied Biosystems, Foster City, USA). Reactions were run for 40 cycles in a ViiA7 real-time PCR system (ABI). Differential expression was determined using the 2^-ΔΔCT method with both Rplp0 and 18S used as reference genes.
Table 2.3 Mouse RT-qPCR forward and reverse primers.

<table>
<thead>
<tr>
<th>Group</th>
<th>Gene</th>
<th>Sequence (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp130 family cytokines</td>
<td>Il6</td>
<td>F: CAAAGCCAGAGTCCCTTCAGAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GTCTTAGCCACTCCCTCTG</td>
</tr>
<tr>
<td></td>
<td>Lif</td>
<td>F: TCCCCATCACCCTGTAAAATG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GAAACGGCTCCCCTTGAG</td>
</tr>
<tr>
<td></td>
<td>Il11</td>
<td>F: TGGGACATTGGAGATCTTTTG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CATTGTACATGCCGGAGGTAG</td>
</tr>
<tr>
<td></td>
<td>Clefl</td>
<td>F: AGCTCTTAATGCCACAGAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: AGTCAAGGCTGGTAAAAAGG</td>
</tr>
<tr>
<td></td>
<td>Cntf</td>
<td>F: CAGACCTGACTGCTTTATGG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GTTCTCTGGAGTCCCTCTG</td>
</tr>
<tr>
<td></td>
<td>Ctf1</td>
<td>F: CCTCAATCTCATCCTACCCCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GGCTCTCCCTGGTCTG</td>
</tr>
<tr>
<td></td>
<td>Ctf2</td>
<td>F: AGAAGACGCAGCAGCTGC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TCTGCACTCTCCATGGGCAGG</td>
</tr>
<tr>
<td></td>
<td>Il27</td>
<td>F: TCGATTGCCAGGAGTGAAC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GAAGTGTTGAGTCCAGAGAG</td>
</tr>
<tr>
<td></td>
<td>Osm</td>
<td>F: AACCTCTCTCTCAGCCTCCT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TGTTTTAGGTTTGGAGG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α cell specific genes</td>
<td>Arx</td>
<td>F: GGGTCTGAGCACCTTTTCTAGG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TGTTGGGTGTCTCAGGAG</td>
</tr>
<tr>
<td></td>
<td>Proglucagon</td>
<td>F: AGCGACTACAGCAAAATACCTG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CTCAATCTCACTGGTAAAAAGTGGCC</td>
</tr>
<tr>
<td></td>
<td>Irxl</td>
<td>F: AGTTAAGTGCACCCTTCAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CCCCAGCAAAATCCCTGAGATGAC</td>
</tr>
<tr>
<td></td>
<td>Irx2</td>
<td>F: CATTCATCGAGTCCACCTAC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CAATTGATCACTCTGTGCCAC</td>
</tr>
<tr>
<td></td>
<td>Brn4</td>
<td>F: TGCAGTGCTCGTTCTCTATAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: ACCGTTGAGAAATACCTC</td>
</tr>
<tr>
<td></td>
<td>Gata4</td>
<td>F: CTGTCATCTCATTGGGCCAC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GAGTGACAGGAGATGGCATAGC</td>
</tr>
<tr>
<td></td>
<td>Pax6</td>
<td>F: CCGCTCCCAACAGTACAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TCATAACTCCGAGCTCAGAC</td>
</tr>
<tr>
<td></td>
<td>MafB</td>
<td>F: AGAAAACTACCTCGAGAAACG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TTCTCGACCTGCAGTCAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gp130 signalling pathway</td>
<td>Socs3</td>
<td>F: GAAGATCTCGTGATCTGACG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GCTTGGGTCTTTCTCAGAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housekeeping</td>
<td>Rplp0</td>
<td>F: TGGACATCGTCTTTAAACCCCG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TGTCATGCTCCACATGAAG</td>
</tr>
<tr>
<td></td>
<td>18S</td>
<td>F: TTTGCGATCATCGCCATTAGA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: ATCACAGTTCCACTCATC</td>
</tr>
</tbody>
</table>
Table 2.4 Rat RT-qPCR forward and reverse primers.

<table>
<thead>
<tr>
<th>Group</th>
<th>Gene</th>
<th>Sequence (5'→3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp130 family cytokines</td>
<td>Il6</td>
<td>F: AAGCCAGAGTCATTCAGAGC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GTCTTACGCTACGCCCTCCTCTCTG</td>
</tr>
<tr>
<td></td>
<td>Lif</td>
<td>F: TTCCACACCCTCTGATAATG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: AATGTCCACCTCTGAGCCTGC</td>
</tr>
<tr>
<td></td>
<td>Il11</td>
<td>F: CGGACAGGGAAGGGTTAAGAAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GGGCAACGACTCTATCTG</td>
</tr>
<tr>
<td></td>
<td>Clec1</td>
<td>F: TGCCAGCTCTTAATCGAACAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: AGTCAGGCTCGTGAAGAGG</td>
</tr>
<tr>
<td></td>
<td>Cntf</td>
<td>F: CAGACCTGACTGCTCTTATGG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GTTCTCTTGAGTCGCTCTG</td>
</tr>
<tr>
<td></td>
<td>Ctf1</td>
<td>F: TGAAGGAAACAGGATCACGCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: ATATTTGGTGACAGAGGCGTG</td>
</tr>
<tr>
<td></td>
<td>Ctf2</td>
<td>F: CATGCCAGGCTCTGAAAGGAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CATGCCAGGCTCTGAAAGGAG</td>
</tr>
<tr>
<td></td>
<td>Il27</td>
<td>F: CAAGCAGGACAGAAAGTGAAAC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GCAAAGGTCAGGCAGCAAAC</td>
</tr>
<tr>
<td></td>
<td>Osm</td>
<td>F: CTCAGCCAGTTGAAAGGTCAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GCCGTGGTTCCAGGTTTGTTG</td>
</tr>
</tbody>
</table>

Table 2.5 Human RT-qPCR forward and reverse primers.

<table>
<thead>
<tr>
<th>Group</th>
<th>Gene</th>
<th>Sequence (5'→3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>α cell specific genes</td>
<td>ARX</td>
<td>F: CTGAGCACTTTTCTCCTCGGAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TGTTGCCGCTCGTGACG</td>
</tr>
<tr>
<td></td>
<td>PROGLUCAGON</td>
<td>F: ACGTTCCCTCAAGACACAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GTCCAGGACTTCTGAGTACG</td>
</tr>
<tr>
<td></td>
<td>IRX1</td>
<td>F: AGTTAAAACTCGCCCTTCCAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CCCGCAAAAGTAAAAAGAAGAC</td>
</tr>
<tr>
<td></td>
<td>IRX2</td>
<td>F: GAGAAACAAAAAGCGAGACTGAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CGAGTTGATCCCGTGACG</td>
</tr>
<tr>
<td></td>
<td>BRN4</td>
<td>F: TGCGTGTCTGTTCTGTAATC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GTGGTGCAATAAACCTCATGC</td>
</tr>
<tr>
<td></td>
<td>GATA4</td>
<td>F: AGATGGGACCGGTACTTAC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CAGTTGCAACAGGAGAG</td>
</tr>
<tr>
<td></td>
<td>PAX6</td>
<td>F: GCCCTCACAACACCTACAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TCATAACTCCGCACATCCAC作</td>
</tr>
<tr>
<td></td>
<td>MAFB</td>
<td>F: CCCAGTCTGAGGTATAAAAGC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TTCTCGTCACAGCTCTGAGT</td>
</tr>
<tr>
<td>Housekeeping</td>
<td>RPLP0</td>
<td>F: TCGTCTTTAACCCCTGCGT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TGCTGCTCCACAAATGAAAC</td>
</tr>
<tr>
<td></td>
<td>18S</td>
<td>F: CTTGGCCATCAGGTTGACATTAAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: ATCACACGTTCCACCTCATC</td>
</tr>
</tbody>
</table>
2.2.8 Western blotting

For analysis of gp130 receptor activation in α cells, 2 x 10⁶ αTC1.9 cells were plated in a 6-well plate and incubated for 24 hours overnight at 37°C. The next day, cells were rinsed with cell culture media and serum starved in 1 ml of high-glucose DMEM (cat. #11960, Gibco) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM GlutaMAX and 0.5% heat-inactivated FBS (hereafter called serum-starved media) overnight at 37°C. The following morning, cells were rinsed with KRB and 1 ml KRB was added to each well. IL-6 and HIL-6 (100 ng/µl) were added to the cells and incubated at 37°C for 30 minutes. After incubation, cells were aspirated and protein was extracted with lysis buffer (0.5% Triton X100, 60 mM β-glycerophosphate, 20 mM MOPS pH 7.2, 5 mM EDTA, 5 mM EGTA, 1mM Na₃VO₄, 20 mM NaF, 1% Trasylol, and 1 mM PMSF). Thereafter, samples were sonicated (30 s), centrifuged (12 000 rpm for 30 minutes at 4°C), and protein content was quantified using Pierce BCA Protein Assay Kit (Thermo Scientific) in order to ensure equal loading of gels for subsequent Western blotting.

Lysates (50 µg) were diluted in Laemmli buffer and heated at 95°C for 5 minutes, chilled briefly on ice, and loaded into 12% Mini-PROTEAN TGX Precast Gels (cat. #456-1044, BioRad, USA) and run at 100 V for approximately 90 minutes. Protein was then transferred to nitrocellulose using Trans-Blot Turbo Transfer Pack (cat. #170-4159, BioRad, USA). Blots were then briefly washed in TBS, and blocked with 5% skim milk in TBS containing 0.1% Tween (hereafter called TBS/T) for 1 hour at room temperature on shaking platform. Next, the blot was washed three times with TBS/T for 5 minutes each, and incubated with rabbit anti-pSTAT3 antibody (1:2000, cat. #9145, Cell Signaling) with TBS/T containing 5% BSA overnight at 4°C. The next day, the blot was washed with TBS/T three times for 5 minutes each and incubated with
HRP-conjugated secondary antibodies (1:2000) for 1 hour at room temperature. Blots were washed three times for 5 minutes and incubated with Amersham ECL Prime Western Blotting Detection Reagent (cat. #RPN2232, GE Healthcare, Buckinghamshire, UK) following manufacturer’s instructions, and exposed to X-ray films for 30 s.

2.2.9 Immunostaining

2.2.9.1 Immunohistochemistry of paraffin-embedded pancreatic sections

Pancreata were excised and fixed in 4% paraformaldehyde (PFA; cat. #O4042500, Fisher Scientific) overnight. Next, pancreata were dehydrated through a series of ethanol steps. Dehydrated pancreata were embedded in paraffin and 5 µm sections from 3 regions of the pancreas were acquired. Sections were deparaffinized and rehydrated through a series of ethanol steps. Antigen retrieval was performed using 10 mM citrate buffer (cat. # S279, Fisher Scientific) for 10 min in a vegetable steamer. Next, sections were blocked with 1% goat serum and donkey/goat anti-mouse IgG (1:30, eBioscience). Sections were incubated with guinea pig anti-insulin (1:1000, cat. #4011-01F, Millipore) and mouse anti-glucagon (1:2000, clone K79bB10, cat. #G 2654, Sigma-Aldrich) at 4°C overnight on a plate shaker in blocking solution. The next day, sections were incubated with Alexa 488 donkey anti-guinea pig (1:250, cat. #706-545-148, Jackson ImmunoResearch, USA) and Alexa 594 donkey anti-mouse (1:450, cat. #715-516-150, Jackson ImmunoResearch) for 1.5 hours at room temperature. Sections were mounted using Vectashield with DAPI (cat. #H-1200, Vector Laboratories, CA, USA) and imaged on a BX61 microscope (Olympus, PA, USA).

2.2.9.2 Immunohistochemistry of frozen pancreatic sections

Pancreatic Gcg-cre X mT/mG and mT/mG tissues were fixed in 4% PFA overnight. Next, tissues were washed three times for 10 minutes with PBS and equilibrated in 20% sucrose
in PBS at 4°C overnight in a 15 ml conical tube on a shaker platform. Once the tissues had settled to the bottom of the conical tube, the tissues were transferred to a 30% sucrose solution at 4°C overnight. After settling at the bottom of the conical tube once more, tissues were frozen in O.C.T. compound (cat. #4583, Tissue-Tek, Torrance, USA) and cryosectioned. Next, sections were permeabilized with 0.1% Triton X-100 (Fisher Scientific) in PBS (0.1% PBS/T) for 30 minutes at room temperature and then blocked in 5% goat serum and 1% BSA (Fisher Scientific) in PBS for 1 hour at room temperature. Next, sections were incubated in 1% BSA and 0.1% PBS/T (hereafter called antibody buffer) with mouse anti-glucagon (1:2000; clone K79bB10, cat. #G2654, Sigma-Aldrich) at 4°C overnight. The next day, sections were washed three times in 0.1% PBS/T for 5 minutes. Sections were then incubated with Alexa 647 goat anti-mouse (1:250; cat. #ab150107, Abcam) in antibody buffer for 1 hour at room temperature in the dark. Sections were washed three times in 0.1% PBS/T for 10 minutes in the dark and mounted with Vectashield with DAPI (Vector Laboratories). Images were taken on a Leica SP5 II confocal imaging system.

2.2.9.3 Pancreatic β and α cell mass analysis

For pancreatic β and α cell mass analysis, all islets in three sections (spaced 200 µm apart) were analyzed using Image-Pro Analyzer (Media Cybernetics, Rockville, USA) as previously described. Briefly, insulin-positive, glucagon-positive, and total pancreatic cell area were counted using the area/count function. Fraction of total endocrine cell area/total pancreatic section area was multiplied by pancreatic weight to determine β and α cell mass.

2.2.9.4 Immunocytochemistry of dispersed islets

For pSTAT3 experiments, islets were dispersed and plated (250 µl/well) on 804G ECM-coated 8-chamber slides (cat. #154534, Lab-Tek, Naperville, USA) and left overnight to adhere.
Islet cells were serum starved in 1640 RPMI with 0.5% heat-inactivated FBS for 2 hours at 37°C with 5% CO₂. Next, islet cells were rinsed with KRB, incubated with 250 µl KRB, and treated with IL-6, HIL-6, LIF, or IL-27 (100 ng/ml was used for all cytokine treatments unless otherwise stated) for 30 minutes at 37°C with 5% CO₂. After treatment, islet cells were fixed in 4% PFA for 15 minutes at room temperature, rinsed three times in PBS for 5 minutes, and permeabilized with ice-cold methanol at -20°C for 10 minutes. After permeabilization, islet cells were rinsed with PBS for minutes at room temperature, and blocked with 5% goat serum and 0.3% Triton X-100 in PBS for 1 hour at room temperature. Next, islet cells were incubated with rabbit anti-PStat3 (1:100, cat. #9145 Cell Signalling) and mouse anti-glucagon (1:1000, Sigma-Aldrich) antibody dilution buffer (1% BSA, 0.3% Triton X-100 in PBS) at 4°C overnight. The following day, slides were washed three times with PBS for 5 minutes, and incubated with Alexa 488 donkey anti-rabbit (1:250, Jackson ImmunoResearch) and Alexa 594 donkey anti-mouse (1:450, Jackson ImmunoResearch) in antibody dilution buffer for 1-2 hours at room temperature, protected from light. Slides were then mounted using Vectashield with DAPI (Vector Laboratories), imaged using a BX61 microscope and Leica SP5 II confocal imaging system, and quantified using ImagePro Analyzer. An average of 1130 ± 261 glucagon positive α cells were counted per experimental condition.

Pancreatic α cell apoptosis was assessed by TUNEL staining (cat. #11684795919, Roche, Laval, Canada) as described following the manufacturer’s instructions, and imaged using a BX61 microscope. Glucagon-positive α cells were identified by co-staining with mouse anti-glucagon and Alexa 594 donkey anti-mouse antibodies as above. An average of 3164 ± 722 glucagon positive α cells were counted per experimental condition.
2.2.9.5 Flow cytometry

Isolated islets were dispersed, washed twice in azide-free, serum/protein-free PBS and incubated with the viability dye eFluor506 (1:1000, eBioscience, San Diego, CA, USA) for 30 minutes on ice protected from light. Next, cells were washed with FACS buffer (1% FBS, 1 mM EDTA, 11 mM glucose in PBS) and Fc block (1:100; Anti-Mouse CD16/CD32, clone 93, eBioscience) was added to islets for 10 min prior to staining them with CD45 eFluor450 (1:250, clone 30-F11, eBioscience), Ly-6c APC (1:1200, clone HK1.4, eBioscience), CD11b-PE (1:1200, clone M1/70, eBioscience), CD11c-PE-Cy7 (eBioscience, clone N418) and F4/80-FITC (eBioscience, clone BM8) for 30 min on ice, in dark. Cells were washed twice with FACS buffer and fixed in 4% PFA for 15 minutes, at 4°C. Unstained, single stains of splenocytes and fluorescence minus one (FMO) controls were used to set gates and compensation using a BD LSRII instrument (BD Biosciences). Data were analyzed using FlowJo vX.0.7, (Tree Star, Ashland, OR, USA) software. Cells were gated on live cells, CD45+ cells, followed by Ly6C+ CD11b+ and Ly6C-Cd11b+Cd11c+F4/80+ cells. Islets were pooled from 2 mice per treatment per time point.

2.2.10 Statistical Analysis

Data are expressed as means ± SEM/SD. All data were analyzed using the nonlinear regression analysis program PRISM (GraphPad, La Jolla, CA, USA), and significance was tested using Student's t-test or analysis of variance (ANOVA) with post hoc tests for multiple comparison analysis. Significance was set at p<0.05.
Chapter 3: Results

3.1 Gp130 family cytokines are elevated in pancreatic islets from rodent models of T2D

To investigate whether gp130 receptor signalling contributes to α cell dysfunction, we first assessed whether islet gp130 receptor family cytokines are elevated in genetic rodent models of T2D. We analyzed mRNA expression of gp130 receptor family cytokines in the islet in two rodent models, the GK rat and the db/db mouse.

Both the GK rat and the db/db mouse develop T2D spontaneously, and displayed elevated blood glucose and declining insulin levels at 12 and 15 wks of age respectively, suggestive of β cell failure (Figure 3.1A-B,D-E). Next, islets were isolated from 8-12 week old GK and Wistar rats, and 4, 8 and 15 week old db/db mice to analyze gene expression of gp130 receptor family cytokines. The GK rat had significantly increased levels of Il6, Lif, Clcf1, and Osm mRNA relative to Wistar controls (Figure 3.1C), while Il6, Lif, Il11, Clcf1, and Osm were increased in islets from 15-week old db/db mice (Figure 3.1F). Thus, elevated gp130 family cytokine expression accompanies hyperglycemia and declining insulin levels in rodent models of T2D.
Figure 3.1 IL-6 family cytokine expression is increased in rodents with T2D. Nonfasted glycemia (A) and insulin levels (B) in Wistar and GK rats. IL-6 family cytokine expression levels in islets from 8-12 week old Wistar and GK rats (C). Expression levels were assessed by qRT-PCR, normalized to the housekeeping gene Rplp0, and expressed as fold control (Wistar). Nonfasted glycemia (D) and insulin levels (E) in 4 to 15 wk old db/db mice. IL-6 family cytokine expression levels in islets from 4, 8, and 15 week old db/db mice (F). Expression levels were assessed by qRT-PCR, normalized to the housekeeping gene Rplp0, and expressed as fold control (4 weeks db/db). Data represent mean ± SEM from 3-4 Wistar and 3-4 GK rats (A-C). Data represent mean ± SEM from 5-20 db/db mice (D-E) and mean ± SD from islets pooled from ≥3 mice/age (F). White bars = Wistar/4 wk db/db; gray bars = 8 week db/db; black bars = GK or 15 week db/db. *p<0.05 as tested by Student’s t-test.
3.2 Gp130 receptor activation stimulates STAT3 phosphorylation in and glucagon secretion from α cells

After establishing that gp130 receptor family cytokine mRNA expression is elevated in the islet in T2D, we evaluated whether gp130 receptor signalling can be activated in α cells. Using IL-6 or HIL-6 (IL-6 fused to the sIL-6R) as gp130 receptor ligands, dispersed islets were stimulated and immunostained for a downstream phosphorylated mediator of gp130 receptor signalling, pSTAT3. IL-6 induced phosphorylation of STAT3 in 70.4% of α cells to a similar degree as HIL-6 (Figure 3.2A) indicating that gp130 and IL-6 receptors are functional in α cells.

Next, we assessed the effect of different IL-6 doses and time of exposure on glucagon secretion in islets. In the presence of 100 ng/ml of IL-6, glucagon secretion (expressed as percent of total glucagon content) was significantly increased after 24 and 48 hours (Figure 3.2B). Furthermore, glucagon secretion was only increased significantly after 48 hours upon stimulation with 10 ng/ml of IL-6 (Figure 3.2B), with no significant differences in glucagon content (Figure 3.2C). Consistent with our pSTAT3 data, IL-6 stimulated glucagon secretion to a similar degree as HIL-6 (Figure 3.2D-E), suggesting that α cell gp130 receptor signalling is maximally activated by IL-6, and supporting previous observations that pancreatic α cells express the IL-6 transmembrane receptor.

To ensure that gp130 receptor signalling did not induce α cell death, causing glucagon release into the incubation medium and complicating the interpretation of the data, islets were treated with IL-6 and apoptosis visualized by TUNEL staining. IL-6 protected from cell death induced by a mixture of cytokines (IL-1β, TNFα, and IFNγ; Figure 3.2F), consistent with previous observations under nutrient stress. Collectively, these data indicate that activation of
gp130 receptor signalling within α cells leads to phosphorylation of STAT3 and stimulation of glucagon secretion.

Figure 3.2 IL-6 and HIL-6 activate α cell STAT3 signaling and stimulate glucagon secretion from islets. STAT3 activation in primary mouse α cells following 30 min cytokine treatment (A). Representative image indicates staining for glucagon (red), pSTAT3 (green), and DAPI (blue). Glucagon secretion (B) and glucagon content (C) following IL-6 treatment of mouse islets at the indicated doses and times. Glucagon secretion (D) and glucagon content (E) from IL-6 and HIL-6 treated mouse islets following 48 h. TUNEL positive α cells were analyzed following 24 h exposure to IL-6, cytokine mix (200pg/ml IL-1b, 1ng/ml TNFα, 5ng/ml IFNγ), or cytokine mix plus IL-6 (F). Data represent mean ± SEM from n=3 mice performed in three independent experiments (A), n=3-6 mice performed in two independent experiments (B-C), n=6 mice performed in three independent experiments (D-E), n=4 mice in 2 independent experiments (F). *p<0.05, ***p<0.001 vs untreated control, # p<0.05 vs CM as tested by ANOVA with Dunnett’s (A-E) or Newman Kuel’s post test (F).
3.3 Generation and validation of α cell specific gp130 receptor KO mice

3.3.1 Analysis of Cre recombinase expression in Gcg-Cre mice

To study the role of gp130 receptor signalling in pancreatic α cells, we established a line of mice with α cell specific knockout of the gp130 receptor (see Chapter 2.1.1). Studies have reported different efficiencies for generating α cell specific deletion of genes using the Gcg-Cre mice, ranging from 13% to 80%.\textsuperscript{122,123} Therefore, we investigated Cre-mediated recombination efficiency by crossing our Gcg-Cre mice with a line of mT/mG reporter mice\textsuperscript{124} (see chapter 2.1.1). Upon Cre-mediated recombination, the tdTomato (mT) cassette is excised, resulting in the expression of eGFP (mG) localized to the cell membrane. Frozen pancreatic sections revealed expression of eGFP in a proportion of α cells stained with glucagon, demonstrating active Cre recombinase in 44.1 ± 1.5% of α cells (Figure 3.3A-B), similar to a previous report using this line of Gcg-Cre mice\textsuperscript{125}.

3.3.2 Partial knockout of the α cell gp130 receptor impairs pSTAT3 activation and glucagon secretion

Next, we assessed pSTAT3 levels in response to gp130 receptor activation in gp130\textsuperscript{fl/fl} control and αgp130KO islets. Stimulation of pSTAT3 with IL-6 and LIF was significantly decreased in α cells of αgp130KO islets (Figure 3.3C). IL-27 failed to induce pSTAT3 within control islets suggesting lack of IL27 receptors on α cells.

We also assessed glucagon secretion in response to gp130 receptor activation in gp130\textsuperscript{fl/fl} control and αgp130KO islets. As previously demonstrated, IL-6 stimulated chronic secretion of glucagon from gp130\textsuperscript{fl/fl} islets\textsuperscript{90}, however IL-6 stimulated glucagon secretion was reduced by ~50% in αgp130KO islets (Figure 3.3D). As previous work demonstrated that IL-6 stimulated GLP-1 secretion from purified human α cells\textsuperscript{91}, we investigated the effect of IL-6 on GLP-1
secretion in mouse islets. IL-6 failed to stimulate GLP-1 secretion from islets over 48 hours (Figure 3.3E). Collectively, αgp130KO islets have Cre-mediated recombination occurring in a proportion of α cells, and reduced gp130 receptor signalling coinciding with a 50% reduction in gp130 receptor-mediated glucagon secretion.

3.3.3 Partial knockout of the α cell gp130 receptor has no effect on normal α cell function and fasting GLP-1 levels

As the proglucagon promoter is constitutively expressed throughout development, we sought to rule out any developmental abnormalities in αgp130KO mice by assessing several physiological parameters. αgp130KO mice displayed no difference in body weight, glucose tolerance, glucose-stimulated insulin secretion, or insulin sensitivity at 16-18 wks of age (Figure 3.3F-I), suggesting no developmental defects impacting glucose homeostasis.

The proglucagon promoter is active in pancreatic α cells and intestinal L cells\textsuperscript{126,127} and previous work has demonstrated IL-6 stimulated GLP-1 secretion from L cells\textsuperscript{91}. Therefore, to rule out any intestinal L cell developmental abnormalities in αgp130KO mice, we attempted to use the mT/mG reporter mice to assess Cre-mediated recombination of eEGF in L cells. However, immunostaining for GLP-1 in the gut in Gcg-Cre x mT/mG mice was unsuccessful (data not shown). Akiyama et al. have shown <5% expression of Cre recombinase in L cells using the same line of Gcg-Cre mice\textsuperscript{123}. As an alternative approach, we assessed fasting GLP-1 levels in gp130\textsuperscript{fl/fl} and αgp130KO mice. Fasting GLP-1 levels were unchanged between genotypes (Figure 3.3J).

To assess pancreatic α cell function, we first investigated the ability of the α cell to suppress glucagon secretion during an IPGTT. Thereafter, we examined the response of the α cell to secrete glucagon in a hypoglycemic setting during an ITT. Glucose-mediated glucagon
suppression during an IPGTT and hypoglycemia-mediated glucagon secretion during an ITT did not differ between genotypes (Figure 3.3J-K). Lastly, β cell and α cell mass were similar between genotypes (Figure 3.3L-M). Thus, αgp130 receptor signalling does not appear to affect α cell development or function under normal chow fed conditions.
Figure 3.3 agp130KO mice exhibit partial loss of α cell gp130 receptor function and normal glucose homeostasis and α cell function under chow fed conditions. Gcg-Cre mice were bred with mT/mG mice to assess activity of α cell Cre recombinase (A-B). Images indicate non-recombinant cells (tdTomato), recombinant cells (eGFP) and glucagon positive cells (purple). eGFP was expressed in 44.1 ± 1.5% of α cells (B). STAT3 activation in primary mouse α cells from fl/fl and agp130KO mice following 30 min cytokine treatment (C). Glucagon (D) and GLP-1 (E) secretion from fl/fl and agp130KO mouse islets following IL-6 treatment. Basal glucagon secretion was 2.26 ± 0.54 for fl/fl and 2.35 ± 0.53 for KO as % glucagon content. Basal GLP-1 secretion was 1.97 ± 0.39 for fl/fl and 1.31 ± 0.17 for KO as % GLP-1 content. Body weight of fl/fl and agp130KO mice at 16-18 weeks of age (F). Intraperitoneal glucose tolerance test (IPGTT, G; 1.5g/kg), insulin levels during IPGTT (H), and insulin tolerance test (ITT, I; 1 U/kg). Fasting GLP-1 levels (J) and glucagon levels during IPGTT and ITT (K). Pancreatic β cell and α cell mass (L-M). Gray bars = fl/fl ctrl; light red bars = agp130KO. Scale bars, 50µm. Data represent 3-5 mice per...
genotype (A-B), n=3-4 mice per genotype performed in three independent experiments (C), n=6 mice per genotype performed in two independent experiments (D, E), and 3-12 mice per genotype (F-M). *p<0.05, #p<0.05 as tested by ANOVA and Newman Keul’s post test.

3.4 Pancreatic α cell dysfunction and islet inflammation in the STZ/HFD mouse model of T2D

3.4.1 The STZ/HFD mouse: a non-genetic mouse model of T2D

The db/db mouse model of T2D is an ideal rodent to study islet inflammation and β cell failure (Figure 3.1D-F), however, breeding of the gp130^{fl/fl} and Gcg-Cre mice with db/db mice requires several generations of backcrossing due to their different genetic backgrounds (BKS and C57BL/6J). Therefore, to investigate the role of the α cell gp130 receptor in a mouse model of human T2D, we generated a non-genetic model displaying features of T2D using a combination of STZ and HFD. STZ is transported into β cells via the GLUT2 transporter, inducing DNA damage by reactive oxygen species, and thereby creating a localized milieu of islet inflammation via necrotic β cells^{128,129}. Moreover, mice were administered HFD to stress the remaining β cells to maintain glucose homeostasis in the face of insulin resistance. To control for all treatments, mice were administered STZ, HFD, or STZ in combination with HFD as depicted in Figure 3.4A.

To determine the effect of STZ and HFD treatment on glucose homeostasis, mice were subjected to several physiological experiments. STZ alone caused glucose intolerance, tended to reduce insulin secretion in response to glucose in vivo, had minimal effects on glucose-mediated glucagon suppression and hypoglycemia-induced glucagon secretion in vivo, and tended to reduce β cell mass and increase α cell mass (Figure 3.4B-L). Thus, STZ alone induced slight impairments in α cell and β cell function, but not sufficient to be used as a model on its own.
Next, HFD alone increased body weight, caused glucose intolerance, increased insulin secretion in response to glucose in vivo, impaired glucose-mediated glucagon suppression, had no effect on hypoglycemia-induced glucagon secretion in vivo, and had minimal effects on β cell and α cell mass (Figure 3.4B-L). Thus, while HFD alone had more exaggerated effects on α cell and β cell function, β cells were still able to adapt by increasing insulin secretion and α cell function was only partially impaired.

Lastly, STZ in combination with HFD caused increased body weight, fed hyperglycemia, glucose intolerance, and marked α cell dysfunction (Figure 3.4B-H). In addition, β cell mass was reduced by ~50%, in parallel with impaired glucose-stimulated insulin secretion from islets ex vivo and reduced islet insulin content (Figure 3.4J-N). α cell mass was also significantly increased in STZ/HFD mice (Figure 3.4K). Thus, treatment with STZ plus HFD exacerbated several of the α cell and β cell parameters tested and induced a more pronounced state of hyperglycemia reminiscent of the human disease.

### 3.4.2 Characterization of islet inflammation in the STZ/HFD mouse model

To characterize islet inflammation post-STZ, islet infiltrating monocytes (CD11b+Ly6c+ cells) were analyzed by flow cytometry. The number of islet monocytes tended to increase at week 0.5 and 1 post-STZ, and were significantly increased at week 2 post-STZ (Figure 3.4O). To determine if islet gp130 family cytokine expression was increased, we analyzed islet gene expression at multiple times post-STZ and during subsequent HFD feeding (Figure 3.4P). IL-27 mRNA was increased ~1 week post-STZ, Osm mRNA was increased 2 weeks post-STZ, and IL-6 and Lif mRNA were increased 3 weeks post-STZ. Other IL-6 family cytokines were unchanged (Figure 3.4P and not shown). Consistent with these mRNA data, IL-6 and LIF protein secretion were increased in islets isolated 3 weeks post-STZ, with no change in soluble IL-6 receptor (sIL-
6R) secretion (Figure 3.4Q). Islet IL-6 secretion was not significantly increased at week 9 in our treatment groups (Figure 3.4R), indicating that gp130 cytokines are only transiently increased following STZ and not further increased by HFD. However, plasma IL-6 levels were significantly increased in our STZ/HFD group at the end of our study, with adipose tissue being the likely source of IL-6\textsuperscript{92} (Figure 3.4S). Taken together, these data show that STZ/HFD treated mice have many hallmark characteristics of T2D, including pancreatic α cell dysfunction and increased expression of pancreatic islet IL-6 family cytokines.
Figure 3.4 Pancreatic α cell dysfunction and islet inflammation in STZ, HFD, and STZ/HFD treated mice. Schematic of chow, STZ (25mg/kg), HFD, and STZ/HFD treatment groups (A) with weekly fed blood glucose monitoring (B). IPGTT (C; 1.5g/kg glucose), ITT (D; 1U/kg insulin) and body weight (E) of chow, STZ, HFD, and STZ/HFD mice. Plasma insulin (F) and glucagon (G-H) during IPGTT and ITT, and fed insulin (I) after 9 weeks. Pancreatic β and α cell mass (J-K) in treated mice with representative images (L). Glucose-stimulated insulin secretion (M) and insulin content (N) of islets ex vivo after 9 weeks. Pancreatic islet infiltrating monocytes (CD11b+Ly6c+ cells) in chow and STZ treated mice (O). Expression levels of IL-6 family cytokines post-STZ injection and during HFD relative to chow mice (P). IL-6, sIL-6R, and LIF protein secretion from 100 islets/well isolated 3 weeks post-STZ (Q). Islet-derived IL-6 (R) from 100 islets/well and systemic IL-6 (S) during fed after 9 weeks. White bars/circles = Chow ctrl; horizontally-striped bars/white squares = STZ; vertically-striped bars/white triangles = HFD; black bars/triangles = STZ/HFD. Scale bars, 50µm. Data represent mean ± SEM from 5 mice (B-D), n=3-11 mice (J-L) per experimental group, n=3-5 mice per experimental group (M-N), n=4-5 mice per time point (O), n=5-6 mice per time point (P), n=4-5 mice for each experimental group (Q-S). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 as tested by 2-way ANOVA with Bonferroni’s post test (B-D), ANOVA with Dunnett’s post test (E-H), or Student’s t-test (M-Q) compared to chow controls.
3.5 Characterization of αgp130KO mice in a non-genetic model of T2D

3.5.1 Partial knockout of the α cell gp130 receptor protects from hyperglycemia following STZ and STZ/HFD treatment

Having established a non-genetic model of T2D, we next assessed the role of α cell gp130 receptor signalling in this model by subjecting our gp130fl/fl control and αgp130KO mice to STZ alone, HFD alone, or STZ followed by HFD feeding. αgp130KO mice displayed no difference in body weight following STZ or STZ/HFD (Figure 3.5A). When subjected to STZ alone, αgp130KO mice showed mildly improved glucose tolerance, with no differences in fasting glycemia, insulin sensitivity, insulin secretion, or α cell function in vivo (Figure 3.5B-I). However, following STZ/HFD, αgp130KO mice had decreased fasting glycemia, improved glucose tolerance, decreased fasting insulin, and showed improvements in glucose modulated glucagon secretion in vivo (Figure 3.5B-I). Paradoxically, this was despite decreased fasting GLP-1 levels in αgp130KO mice (Figure 3.5E). Insulin tolerance tests indicated no difference in insulin sensitivity in αgp130KO mice following both treatments (Figure 3.5F).

No differences in pancreatic glucagon, insulin, or GLP-1 content (Figure 3.5J-L) were observed between genotypes, along with no differences in β cell and α cell mass (Figure 3.5M-O). These observations exclude the possibility that the effects seen in our αgp130KO mice are due to changes in total hormone levels or differences in endocrine cell mass. Lastly, STZ/HFD treated Gcg-Cre mice did not show any glucose intolerance compared to wild type mice excluding any phenotypic effect of Cre recombinase expression in α cells (Figure 3.5P). Lastly, αgp130KO mice on HFD alone showed no phenotypic differences in glucose tolerance or α cell and β cell function (Figure 3.6).
3.5.2 STZ/HFD treated αgp130KO mice display no changes in insulin sensitivity

Finally, to determine if changes in insulin sensitivity contributed to the protective phenotype in STZ/HFD treated αgp130KO mice, we performed hyperinsulinemic-euglycemic clamps. There were no differences in glucose infusion rate, whole body insulin-stimulated glucose uptake, or hepatic insulin sensitivity between genotypes (Figure 3.7). Interestingly, hepatic glucose production under fasting conditions tended to be reduced in αgp130KO mice (Figure 3.7D), consistent with reduced fasting glycemia in these mice. Taken together, these data show that gp130 receptor signalling contributes to α cell dysfunction in a non-genetic model of T2D, and partial inhibition of gp130 receptor signalling improves glucose tolerance and protects from hyperglycemia through improved α cell function.
Figure 5

A) Body weight (g)

B) Blood glucose (mM)

C) Blood glucose (mM) over time (min)

D) IPGTT AUC (mM x min)

E) GLP-1 (pg/ml)

F) Blood glucose (mM) over time (min)

G) Insulin (pM)

H) Glucagon (pM)

I) Glucagon (pM) over time (min)

J) Insulin/Protein (mmol/g)

K) Glucagon/Protein (mmol/g)

L) GLP-1/Protein (pmol/g)

M) β cell mass (mg)

N) α cell mass (mg)
Figure 3.5 *agp130KO* mice display improved glucose homeostasis and α cell function following STZ/HFD. Body weight (A) and fasting glycemia (B) of fl/fl and *agp130KO* mice at 16-18 weeks of age following STZ or STZ/HFD. IPGTT (C; 1.5g/kg) with quantification of area under curve above basal (AUC; D), fasting GLP-1 (E), and ITT (F; 1U/kg) with corresponding plasma insulin (G) and glucagon levels (H-I). Insulin (J), glucagon (K), and GLP-1 (L) pancreatic content of fl/fl and *agp130KO* mice following STZ/HFD. Pancreatic β cell and α cell mass (M-N), and representative islet images (O) of STZ/HFD fl/fl and KO mice. An IPGTT (P; 1.5g/kg) was performed in wild type (WT) and Gcg-cre (Cre) mice at 16-18 weeks of age following STZ/HFD. Representative image indicates glucagon (red), insulin (green), and nuclei (blue; DAPI). Scale bars, 50µm. Data represent mean ± SEM from n=4-6 mice per genotype treated with STZ (A-I), n=22-23 mice per genotype treated with STZ/HFD (A-D), n=4 mice per genotype (E), n=4-8 mice per genotype treated with STZ/HFD (F), n=11-14 mice per genotype treated with STZ/HFD (G-I), n=4-8 mice per genotype (J-O) from 1-4 independent cohorts of mice, n=3-4 mice per genotype (P). Black border white bars/triangles = STZ fl/fl; Red border white bars/triangles = STZ *agp130KO*; Black border grey bars/circles = STZ/HFD fl/fl; Red border red bars/squares = STZ/HFD *agp130KO*; Black border white squares = STZ/HFD WT; Black squares = Cre STZ/HFD Cre; n.s. = not significant. *p<0.05, **p<0.01 as tested by Student’s t-test. In (C), *p<0.05 and #p<0.05 as tested by Student’s t-test compared to fl/fl controls.
Figure 3.6 *agp130KO* mice display no difference in glucose homeostasis or α cell function on HFD. Body weight (A), IPGTT (B; 1.5g/kg), and ITT (C; 1U/kg) of fl/fl and *agp130KO* mice following 6 weeks HFD. Plasma insulin (D) and glucagon levels (E-F) during the IPGTT and ITT. Data represent mean ± SEM from n=4-6 mice per genotype.
Figure 3.7 No difference in insulin sensitivity in agp130KO mice following STZ/HFD. Hyperinsulinemic–euglycemic clamps were performed after STZ plus 6 weeks of HFD feeding in fl/fl and agp130KO littermates. Blood glucose during clamping (A) and glucose infusion rate (B). Glucose turnover (C), suppression of hepatic glucose production (D), and insulin levels (E) during clamp. Data represent mean ± SEM from n=7 fl/fl and n=6 agp130KO mice from 2 independent cohorts. P value determined using student’s t-test.

3.6 Mechanism of action of gp130 receptor signalling in α cells

3.6.1 Gp130 receptor signalling may influence α cell identity.

Data in Figure 3.2C above suggest that gp130 receptor signalling does not increase glucagon secretion via effects on glucagon content. To rule out effects of gp130 receptor activation on the proglucagon gene, we assessed its transcriptional regulation. Using IL-6 as a tool to activate gp130 receptor signalling, isolated islets from C57BL/6 mice were stimulated in two independent time-course experiments terminating at 24 and 144 hours. We assessed mRNA expression of proglucagon and several α cell specific genes known to regulate the proglucagon gene and maintain α cell identity\textsuperscript{130-134}. Interestingly, IL-6 significantly reduced Arx mRNA expression after 8, 12, and 24 hours with proglucagon mRNA expression also tending to decrease during this time (Figure 3.8A). Indeed, over 1 to 6 days, numerous α cell-specific genes
tended to decrease following IL-6 treatment (Figure 3.8B). Consistent with these rodent data, IL-6 also tended to reduce ARX, GCG, and IRX2 mRNA expression in human islets (Figure 3.8C), perhaps suggesting that gp130 receptor signalling influences α cell identity or the state of α cell differentiation. In agreement with this hypothesis, Arx and Irx1 mRNA expression tended to be increased in αgp130KO mouse islets following STZ/HFD (Figure 3.8D). Taken together, these data suggest that gp130 receptor signalling regulates glucagon secretion independently of effects on proglucagon gene expression, and perhaps via effects on α cell identity.
Figure 3.8 Gp130 receptor signalling may influence α cell identity. Expression levels of genes specific to α cells were assessed by qRT-PCR in IL-6 treated mouse (A-B) and human islets (C), normalized to untreated control. IL-6 reduced *Arx* gene expression after 8-24 hours in mouse islets (A). αgp130KO islets from chow and STZ/HFD treated mice were also assessed for α cell genes that regulate α cell identity (D), normalized to fl/fl chow controls. Data expressed as fold control, and represent mean ± SEM from 3-9 mice performed in 3 independent experiments (A-B), n=8 human subjects (C), and n=3-9 mice per genotype. **p<0.01 as tested by ANOVA with Dunnett’s post-test.

3.6.2 Gp130 receptor signalling induces SOCS3 mRNA expression

Activation of the gp130 receptor induces a negative feedback loop, orchestrated by the SOCS3 protein. In humans with T2D, SOCS3 is increased in skeletal muscle\textsuperscript{135}, and deletion of SOCS3 in skeletal muscle and adipose tissue of mice prevents insulin resistance\textsuperscript{136,137}. Thus, we assessed the induction of *Socs3* mRNA transcript by IL-6 stimulation in islets. IL-6 stimulated
C57BL/6 mouse islets significantly increased $Socs3$ mRNA expression after 4, 8, 12, and 24 hours, remained elevated over 2 days, and tended to remain elevated over 6 days (Figure 3.9A-B). Thus, stimulation with IL-6 has prolonged effects on $Socs3$ gene expression in mouse islets.

![Figure 3.9 IL-6 induced $Socs3$ mRNA expression](image)

*Figure 3.9 IL-6 induced $Socs3$ mRNA expression.* Expression levels of $Socs3$ mRNA were assessed by qRT-PCR and normalized to untreated controls after IL-6 stimulation for 24 hours (A) and over 1-6 days (B) in mouse islets. Data represent mean ± SEM from 3-9 mice from 3 independent experiments. *p<0.05, **p<0.01, ***p<0.001 as tested by ANOVA with Dunnett’s post-test.

Due to the heterogeneous composition of islets, we investigated mRNA levels of $Soc3$ in response to gp130 receptor activation in an immortalized murine $\alpha$ cell line, $\alpha$TC1 clone 9. First, we assessed pSTAT3 protein levels upon gp130 receptor activation to ensure functional receptor expression. IL-6 failed to induce STAT3 phosphorylation, whereas HIL6 robustly induced STAT3 phosphorylation (Figure 3.10A). Thus, $\alpha$TC1.9 cells do not possess the IL-6 transmembrane receptor, but activation of gp130 receptor signalling via HIL6 induces phosphorylation of STAT3. HIL6 was used to activate gp130 receptor signalling in subsequent experiments using $\alpha$TC1.9 cells. HIL-6 significantly increased $Socs3$ mRNA expression within 4-8 hours, and this was sustained for 24 hours (Figure 3.10B). Thus, activation of gp130 receptor
signalling induces Soce3 mRNA specifically in α cells. Further experiments are required to access the role of SOCS3 in gp130 receptor-mediated effects on glucagon secretion.

Figure 3.10 Gp130 receptor activation induces STAT3 phosphorylation and Soce3 mRNA expression in αTC1.9 cells. Representative western blot of pSTAT3 levels in αTC1.9 cells upon IL-6 and HIL6 (A). IL-6 failed to activate the gp130 receptor and activate pSTAT3 whereas HIL6 induced phosphorylation of STAT3 (79 kDa). HIL6 also induced expressed of Soce3 mRNA expression levels (B). Data expressed as fold of untreated control, and represent mean ± SEM from 3 independent experiments. *p<0.05 as tested by ANOVA with Dunnett’s post-test.
Chapter 4: Discussion

Normally, glucagon secretion is increased during fasting and suppressed as blood glucose rises following a meal in a counter-regulatory fashion to insulin. However, during T2D, glucagon secretion is inappropriately elevated, contributing to hyperglycemia\textsuperscript{11,39,138}. Pioneering studies by Müller\textsuperscript{139} and elegant clamp studies by Shah and colleagues have demonstrated that lack of glucagon suppression contributes to postprandial hyperglycemia in people with T2D\textsuperscript{140}. The main mechanism underlying this hyperglucagonemia is thought to be β cell dysfunction and a decrease in β cell-derived secretory products that are known to have inhibitory effects on α cell secretion, including insulin, GABA, and zinc ions\textsuperscript{11,39,138}. All of these factors inhibit glucagon secretion, and their secretion is likely decreased in T2D. In our study, we investigated the role of gp130 receptor signalling in α cell function. We expanded on previous work that IL-6, a gp130 family cytokine, is increased in rodent models of T2D\textsuperscript{106,110}, and found that several other gp130 family cytokines are elevated in rodent models of T2D. Together, these family cytokines signal via the gp130 receptor to modulate α cell function, and promote disease progression. Using a non-genetic model of T2D, mice with partial gp130 receptor deficiency showed improved glucose tolerance, decreased fasting insulin and glycemia, and improved α cell function. Thus, our data demonstrate that islet inflammation also contributes to α cell dysfunction, with a central role for gp130 receptor signalling in this process, and with implications for T2D.

Inflammation normally acts to defend the body from foreign antigens and injury. However, unsuccessful resolution of inflammation and chronic secretion of harmful by-products contributes to the pathology of disease\textsuperscript{121}. For example, gp130 receptor signalling has been implicated in various chronic inflammatory diseases including atherosclerosis, rheumatoid arthritis, and cancer\textsuperscript{121,140}. Indeed, STAT3 activation via gp130 is increased in experimental
models of arthritis and cancer\textsuperscript{141,142}, with reduced inflammation observed in STAT3\textsuperscript{+/-} mice\textsuperscript{141}. T2D is likewise associated with chronic inflammation in numerous organs including the pancreatic islet, where elevated IL-1β levels lead to impaired β cell insulin secretion\textsuperscript{75}. Inhibition of IL-1β signalling results in improved HbA1c levels and insulin secretion in diabetic individuals\textsuperscript{103}. In addition, islet IL-6 mRNA expression and protein secretion is highly elevated in rodent models of T2D\textsuperscript{106,110,118,143}, consistent with published human data\textsuperscript{102}. We found that other gp130 family cytokines are also expressed at elevated levels in islets of the db/db mouse, GK rat, and the STZ/HFD mouse. Thus, in addition to the disease pathologies listed above, gp130 receptor signalling may also contribute to T2D via effects on α cell function.

αgp130KO mice were protected from glucose intolerance following STZ alone, but not following HFD alone. This was consistent with STZ treatment causing a transient increase in IL-6 and LIF secretion from islets, while HFD alone did not increase islet IL-6 family cytokine expression. Our data suggest that local islet IL-6 may be necessary for the phenotype observed in our STZ- and STZ/HFD-treated αgp130KO mice, rather than increased systemic IL-6 levels most likely derived from the adipose tissue. One caveat in our study is that isolating islets induces IL-6 mRNA expression and secretion, increasing baseline expression and adding background noise to our assay\textsuperscript{144}. It is therefore likely that islet IL-6 is elevated for a more prolonged period of time than depicted in Figure 4P. Interestingly, islet IL-6 cytokine mRNA levels correlated very well with macrophage infiltration following STZ. Initial reports hypothesized that increased macrophage infiltration in the islet of T2D individuals would contribute to islet cytokine production\textsuperscript{106,145}. Indeed, a recent study found that depletion of islet macrophages reduced islet amyloid polypeptide-induced islet IL-6 mRNA expression\textsuperscript{146}. 
Whether islet macrophages are the cellular source of gp130 family cytokines causing α cell dysfunction should be addressed in future studies.

Interestingly, the combination of STZ plus HFD did not prolong or increase islet gp130 family cytokine expression, but it did result in further impairments in α cell function compared to STZ or HFD treatments alone in αgp130KO mice. One possible explanation for this synergism is that HFD mediates α cell dysfunction by causing α cell insulin resistance, and that STZ exacerbates these effects by decreasing insulin and inducing inflammation. This might explain the synergistic effect of STZ plus HFD on glucose-mediated glucagon suppression in vivo during an IPGTT. With respect to ITT responses, hypoglycemia induced glucagon secretion is strongly driven by the parasympathetic nervous system. Thus, STZ acts synergistically with HFD to impair this response. Regardless of the mechanism of action, these hypotheses are consistent with effects of gp130 receptor signalling on α cell function and glycemic control being more pronounced in the STZ/HFD model, compared to STZ or HFD treatments alone. Thus, gp130 receptor signalling acts as a modifier of α cell function in a synergistic manner with HFD induced effects on α cell function.

Improved α cell function in αgp130KO mice treated with STZ/HFD resulted in reduced fasting hyperglycemia, likely due to reduced hepatic glucose output. Although glucagon is a main driver of hepatic glucose production, we did not observe noticeable differences in fasting glucagon levels. However, hyperinsulinemic-euglycemic clamps demonstrated a trend towards decreased hepatic glucose production in αgp130KO mice treated with STZ/HFD. A recent study by Wang and colleagues demonstrated reduced hepatic glucagon signalling and similar improvements in α cell function in mice with α cell specific PTEN deletion. Deletion of α cell PTEN, a negative regulator of PI3K, decreased circulating glucagon levels in mice fed HFD for
7 months. Reduced expression of hepatic glycogenolytic and gluconeogenic genes accompanied improved α cell function, but fasted glycemia only trended to be decreased in α cell PTEN deficient mice. Wang et al. went on to suggest that exploitation of the PI3K pathway in α cells could improve T2D\textsuperscript{149}. These findings may seem at odds with our data as the PI3K/Akt pathway is activated by gp130 receptor signalling and should be partially inhibited in our αgp130KO mice. However, we have yet to elucidate the downstream mechanisms mediating the improved phenotype observed in our αgp130KO mice, and our effects may be independent of PI3K/Akt signalling.

Intriguingly, enhancing α cell function appears to improve β cell insulin secretion in both our study and that of Wang and colleagues\textsuperscript{149}. Reduced fasting glycemia was likely responsible for the decreased fasting insulin observed in αgp130KO mice. We ruled out any contribution of increased insulin sensitivity in αgp130KO treated with STZ/HFD by performing hyperinsulinemic-euglycemic clamps. However, reduced fasting glycemia may have improved β cell function in αgp130KO mice by reducing the effects of glucotoxicity on the β cell. Another possible mechanism of improved β cell function owing to improved α cell function was recently demonstrated by Song et al., where glucagon-stimulated kisspeptin1 from the liver impairs glucose-stimulated insulin secretion (GSIS)\textsuperscript{150}. Hyperglucagonemia in HFD-fed and db/db mice was associated with increased hepatic kisspeptin1, consistent with human data from subjects with T2D. Furthermore, deletion of the pancreatic kisspeptin1 receptor improved glucose tolerance due to improved GSIS\textsuperscript{150}. This sequence of events may also help explain how a transient increase in islet IL-6 cytokines can have long lasting effects on glycemic control. Although absolute glucagon values were not observed to be significantly elevated in our αgp130KO treated with STZ/HFD, we cannot rule out the possibility of a pancreatic-hepatic
feedback loop until we examine kisspeptin1 levels in our αgp130KO mice. Despite not knowing the exact mechanism, there is growing evidence that α cells regulate β cell function, with clear implications for T2D.

Two independent groups recently investigated the effect of gp130 receptor cytokines on the α cell. McGuinness and colleagues found that IL-6−/− mice had a blunted glucagon response to endotoxin that was restored by replacement of IL-6\textsuperscript{111}. They went on to show that IL-6 amplifies adrenergic-dependent glucagon secretion from mouse islets\textsuperscript{112}. In a separate study, Fernández-Millán and colleagues showed that α cell mass expansion and hyperglucagonemia during suckling in rats is partly IL-6-dependent, contributing to hyperglycemia post-weaning\textsuperscript{113}. These studies, previous work from the supervisor’s laboratory\textsuperscript{90}, and data presented here support the notion that gp130 cytokines are modulators of α cell glucagon secretion. Furthermore, our previous work demonstrated that IL-6 mediates α cell mass expansion, however we do not know by what mechanisms\textsuperscript{90}. We found that in our αgp130KO mice treated with STZ/HFD there was no effect on α cell mass, demonstrating that extra-islet IL-6 targets may mediate α cell mass expansion. Taking these findings into consideration, we propose that gp130 receptor actions are context-dependent and that gp130 cytokines modulate α cell glucagon secretion to allow for increased glucose output during normal physiology (e.g. during acute infection, or post-exercise), while also driving excessive α cell glucagon secretion and dysfunction during T2D.

Exposure of human islets to IL-6 for 4 days or enriched human pancreatic α cells to IL-6 enhances GLP-1 secretion in addition to glucagon secretion, perhaps via upregulation of PC1/3\textsuperscript{91}. Mouse islets exposed to IL-6 for 2 days did not have elevated GLP-1 secretion. Dedifferentiation of α cells to a pre-α cell state has been shown to induce GLP-1 secretion\textsuperscript{151}. In addition, prolonged culture of human islets in vitro results in increased numbers of glucagon+
cells due to conversion of β cells to α cells. We speculate that in the previous study done in purified α cells, loss of gap junctions and crosstalk with neighbouring β cells may have caused α cell de-differentiation or conversion of β cells to α cells. These dedifferentiated α cells may increase GLP-1 secretion in response to gp130 cytokines, however further studies will need to be conducted to clarify this mechanism of gp130-mediated GLP-1 secretion.

In addition to regulating GLP-1 secretion, a previous report demonstrated that IL-6 increased proglucagon mRNA expression in human islets. We investigated the effect of IL-6 on proglucagon mRNA and on additional genes known to regulate α cell identity in mouse and human islets. We observed a trend to decreased proglucagon mRNA levels following IL-6 treatment and significantly reduced Arx mRNA expression after 8 hours of IL-6 exposure in mouse islets, with similar trends observed in human islets. We are unsure why our data is inconsistent with previous data in human islets. Interestingly, a loss of the differentiated state of α cells may lead to uncontrolled glucagon secretion, however more work needs to be done to determine the mechanism of gp130 cytokine-stimulated glucagon secretion.

Various clinical trials targeting IL-6-mediated gp130 signalling in inflammatory diseases have been undertaken. Interestingly, the impact of an IL-6 receptor specific antibody, Tocilizumab, on glucose homeostasis was recently evaluated in rheumatoid arthritis patients. Tocilizumab decreased HbA1c in T2D patients and reduced fasting insulin in nondiabetic individuals. Despite these patients being treated with prednisolone, making the data interpretation complex, it would be interesting to know if glucagon levels were impacted by Tocilizumab, potentially accounting for some effects on glycemic control. Due to ubiquitous tissue expression of the gp130 receptor and possible redundancy of gp130 family cytokines, future studies on cell-specific mechanisms may provide a more targeted approach.
Despite decreased systemic GLP-1 levels in αgp130KO mice (likely due to receptor deletion in L cells), activation of α cell gp130 receptor signalling in a setting of reduced β cell mass and HFD-induced insulin resistance had detrimental effects on normal α cell function and glycemic control. Although one would presume that decreased GLP-1 levels in αgp130KO mice would further impair glucose tolerance, the surprisingly improved phenotype suggests that effects of gp130 receptor cytokines on α cell function were more important for glycemic control than effects on L cell GLP-1 secretion. While it remains to be determined what the mechanisms are that facilitate IL6/gp130 cytokine actions on glucagon secretion, insight into how IL-6/gp130 influences α cell secretory processes and/or α cell differentiation may allow us to identify druggable targets that modify the actions of the α cell and could thereby be used for the treatment of T2D.

**Future directions**

The mechanism by which gp130 receptor signalling mediates α cell dysfunction remains elusive. We initially attempted experiments aimed at inhibiting the individual pathways of gp130 receptor activation –PI3K, STAT3, and MAPK. However, this resulted in islet dispersion likely due to these canonical pathways being implicated in cell growth and survival.

Intriguingly, our preliminary mRNA data suggest that IL-6 may cause α cell de-differentiation. De-differentiated α cells may lack regulated glucagon secretion. To explore this further, we plan to assess Arx protein levels in IL-6 treated islets in addition to re-expressing Arx in IL-6 treated α cells in attempt to reverse effects on glucagon secretion. Indeed, these data bring up the intriguing possibility that α cells in T2D are de-differentiated, perhaps contributing to dysfunctional glucagon secretion in this disease.
An alternative hypothesis for how gp130 receptor signalling may dysregulate glucagon secretion implicates IL-6 upregulated SOCS3 expression. SOCS3 has also been demonstrated to inhibit insulin signalling in the liver and muscle, contributing to the insulin resistant state of these tissues\textsuperscript{135,136,155}. Because insulin resistance is mediated by SOCS3 in these tissues, gp130 signalling may also mediate similar effects in α cells. Consistent with our hypothesis, gp130 receptor activation in a clonal α cell line, αTC1-9, does not stimulate glucagon secretion due to the lack of insulin (and neighboring β cells) to constitutively suppress glucagon secretion in untreated control conditions. Furthermore, gp130 receptor activation increases SoCS3 mRNA expression in mouse islets and αTC1-9 cells. Unfortunately, experiments assessing insulin sensitivity in mouse islets and αTC1-9 cells have hit several roadblocks, however, other experiments will need to be conducted to test this hypothesis in the future, such as: 1) determining the effects of gp130 receptor signalling on SOCS3 protein levels in α cells; 2) determining the effects of gp130 receptor signalling on insulin-induced Akt phosphorylation in α cells; and 3) determining the effects of \textit{in vitro} knockdown of SoCS3 via siRNA on gp130-mediated glucagon secretion. Ultimately, it is hoped that uncovering the mechanism by which gp130 receptor signalling impairs α cell function may identify new therapeutic targets for T2D.
References

70. Unger, J. *Diabetes Management in Primary Care*. (Lippincott Williams & Wilkins, 2007).


