Intestinally-Derived Preproglucagon Peptides Mediate Nutrient Absorption and Gut Adaptation with Exposure to Cold

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Abstract

Cold exposure impacts intestinal remodelling and metabolism. Signaling by glucagon-like peptide 1 (GLP-1), GLP-2 and glucose-dependent insulinotropic polypeptide (GIP) are tightly linked to nutrient intake and absorption. However, these peptide hormones' necessity to mediate gut adaptation and metabolic alternations during cold exposure has been incompletely explored. We hypothesize that GLP-1, GIP, and GLP-2 are released in proportion to required energy needs during cold exposure to enable efficient nutrient absorption and gut adaptation and subsequently impact nutrient handling. We evaluated morphological changes in the intestinal in wildtype, *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ and *Glp1r*$^{-/-}$*Gipr*$^{-/-}$ mice exposed to chronic cold or thermoneutral conditions for four weeks. Food intake and gut hormone secretion were significantly increased in all mice housed at 4-6˚C compared to those housed at thermoneutrality. Concomitantly, we observed increased remodeling measured by crypt to villus height (increased villi length) and intestinal circumference (increased circumference) in cold-exposed wildtype and *Glp1r*$^{-/-}$*Gipr*$^{-/-}$ mice housed. In contrast, intestinal morphology in *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ mice was unchanged in response to cold. Associated with these morphometric changes, we observed significant increases in fasting concentrations of GLP-1. These data suggest that GLP-1 and GLP-2 are key signaling molecules secreted from the gut in response to chronic cold exposure to enable intestinal remodeling.

Co-Authorship Statement

Antonio Hanson performed the design, experiments, and analysis of data with the following exceptions:

Dr. Erin E. Mulvihill provided intellectual input and scientific expertise with the project scope, hypothesis, experimental design, and other additional experimental details.

Iryna Abramchuk and Natasha Trzaskalski aided with the histological analysis of intestinal sections.

Natasha Trzaskalski, Nadya Morrow, Cassandra Locatelli, and Evgenia Fadzeyeva assisted with metabolic tests and measurement of plasma GLP-1.

Dr. Ilka Lorenzen-Schmidt assisted with breeding mouse colonies, genotyping, animal sacrifices, and tissue processing.

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AGPAT	1-acylglycerol-3-phosphate O acyltransferase
α-cell	Alpha Cell
AMPK	AMP-Activated Protein Kinase
ANOVA	Analysis of Variance
APC	Antigen Presenting Cell
ApoB48	Apolipoprotein B-48
ApoB100	Apolipoprotein B-100
ATP	Adenosine Triphosphate
β-cell	Beta Cell
BAT	Brown Adipose Tissue
Ca2+	Calcium
cAMP	Cyclic Adenosine Monophosphate
CD36	Cluster of Differentiation 36
CNS	Central Nervous System
DGAT	Diacylglycerol Acyltransferase
DIO	Diet-Induced Obesity
DIRKO	Double Incretin Receptor Knockout
DPP4	Dipeptidyl Peptidase-4
EDTA	Ethylenediaminetetraacetic Acid
EGF	Epidermal Growth Factor
EPAC	Exchange Protein Activated by cAMP

ER	Endoplasmic Reticulum
FA	Fatty Acid
FABP	Fatty Acid-Binding Protein
FATP	Fatty Acid Transport Protein
FFA	Free Fatty Acid
FLT1	FMS related receptor tyrosine kinase 1
FGF21	Fibroblast growth factor 21
GAPs	Goblet Cell-Associated Antigen Passages
GC	Goblet Cell
GI	Gastrointestinal
GIP	Glucose-Dependent Insulinotropic Polypeptide
GIPR	GIP Receptor
GLP-1	Glucagon-Like Peptide-1
GLP-1R	Glucagon-Like Peptide-1 Receptor
GLP-2	Glucagon-Like Peptide-2
GLP-2R	Glucagon-Like Peptide-2 Receptor
GLPDRKO	Glucagon-Like Peptide Double Receptor Knockout
GCGR	Glucagon Receptor
GLUT2	Glucose Transporter-2
GLUT5	Glucose Transporter-5
GPAT	Glycerol-3-Phosphate Acyltransferase
GPCR	G-Protein-Coupled Receptor
GPR/FFAR	G-Protein-Coupled Receptor/Free-Fatty Acid Receptors

GTP	Guanosine-5’-Triphosphate
HDL	High-Density Lipoproteins
H&E	Hematoxylin and Eosin
HFD	High-Fat Diet
HSL	Hormone Sensitive Lipase
iBAT	Intrascapular BAT
IGF	Insulin Growth-Like Factor 1
IL-6	Interleukin-6
IMM	Inner Mitochondrial Membrane
ingSAT	Inguinal Subcutaneous Adipose Tissue
IP_3R	Inositol Triphosphate Receptor
ISEMF	Intestinal Subepithelial Myofibroblasts
KGF	Keratinocyte Growth Factor
LCFA	Long-Chain Fatty Acids
LDL	Low-Density Lipoproteins
LEC	Lymphatic Endothelial Cells
LPL	Lipoprotein Lipase
MAPK	Mitogen-Activated Protein Kinase
mRNA	Messenger Ribonucleic Acid
MTP	Microsomal Triglyceride Transfer Protein
MUC2	Mucin-2
myf5	Myogenic Factor-5
Na^+	Sodium

Na^+ K^+ ATPase	Sodium-Potassium Pump
NE	Norepinephrine
NOD	Non-Obese Diabetic
NRP1	Neuropilin-1
Pax6	Paired Box-6
PC1/3	Prohormone Convertase-1/3
Pdx1	Duodenum Homeobox-1
PEPT1	Peptide Transporter-1
PFA	Paraformaldehyde
pgVAT	Perigonadal Visceral Adipose Tissue
PI3K	Phosphatidylinositol-3 Kinase
PKA	Protein Kinase A
PlagL2	Pleomorphic Adenoma Gene-Like-2
PH-LDL	Postheparin LpL
POMC	Proopiomelanocortin
PVN	Paraventricular Nucleus
Rfx6	Regulatory Factor-X6
RYR	Ryanodine Receptor
SBS	Short Bowel Syndrome
SCFA	Short-Chain Fatty Acids
SGLT1	Sodium-Glucose Transporter-1
SLC8A2	Solute Carrier Family-8-MemberA2
T1D	Type I Diabetes

T2D	Type II Diabetes
TG	Triglyceride
TNZ	Thermoneutral Zone
UCP1	Uncoupling Protein-1
Val-Pyr	Valine Pyrrolidide
VEGF	Vascular Endothelial Growth Factor
VLDL	Very Low-Density Lipoproteins
VMH	Hypothalamic Ventromedial Nucleus
WAT	White Adipose Tissue

1. Introduction

1.1 Energy Homeostasis in Mammals

Energy homeostasis is balanced by food intake and energy expenditure, contributing to optimal health in all organisms (Galgani & Ravussin, 2008). Dietary nutrient absorption and utilization are highly regulated, given their fundamental role in mediating many mechanisms involved in energy homeostasis (Li et al., 2019). Nutrient deficiency is linked to disturbed metabolism, thereby imposing cellular stress, resulting in tissue damage and metabolic dysregulation (Chen et al., 2018). Extrinsic factors, including the nutrient content of the diet, frequency of feeding and temperature of the external environment, significantly influence metabolism by altering the balance of nutrient intake and energy expenditure (Senn et al., 2018). Therefore, many levels of regulation exist for the body to adapt to regulate the assimilation of nutrients for both utilization and storage.

1.1.1 Post-Prandial Glucose Metabolism

Regulation of blood glucose levels is essential during the postprandial state. Following a meal, blood glucose levels rise, and in response, insulin is secreted by the beta cells (β-cells) of the pancreas (Fu et al., 2014). β-cells are stimulated by glucose when facilitative diffusion glucose transporters (GLUT) allow cellular entry of glucose into β-cells (Navale & Paranjape, 2016). When glucose levels are elevated intracellularly, they are metabolized to produce adenosine triphosphate (ATP) and depolarization of the β-cell follows. In response, voltage-dependent calcium channels open, vesicles fuse to the plasma membrane, and insulin release occurs via exocytosis (Meloni et al., 2013). Insulin is a hormone produced in the pancreas and stored in dense core vesicles, eventually becoming secretory granules (Meloni et al., 2013). Granules exist in two types of pools: a readily releasable pool, where release is in response to Ca^{2+}, or in a reserve pool (Meloni et al., 2013). Insulin release is then further characterized by two distinct phases (Meloni et al., 2013; Wilcox, 2005). Secretion is rapid and transient in the first

phase, while the second phase is slower and sustained (Meloni et al., 2013). β-cells are stimulated to secrete insulin by the glucose metabolism, but this response can be further tailored through the action of many different hormones, including those secreted from the gastrointestinal tract in response to food intake. The incretin hormones, glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide induce directly binding to their respective receptors on the pancreatic β-cells, increasing glucose-stimulated insulin secretion (Fu et al., 2013) to tailor the response to ingested nutrients. Also within the islet are alpha (α)-cells which are complementary to the β-cell, secreting the peptide hormone glucagon to regulate glucose in the fasted state (Fu et al., 2013; Rix et al., 2019).

1.1.2 Post-Prandial Lipid Metabolism

Dietary lipid digestion begins in the oral cavity, as lingual lipases are secreted and begin lipid degradation. Once in the stomach, lingual lipases and gastric lipases hydrolyze approximately 30% of triglycerides (Liao et al., 1984). Bile produced in the liver and stored in the gallbladder enters the duodenum, where the hydrophobic/hydrophilic bile salts and phospholipids emulsify the large lipid droplets into smaller droplets (Iqbal & Hussain, 2009). Emulsification contributes by increasing surface area and allowing lipolysis (Iqbal & Hussain, 2009).

Triglycerides are broken down into fatty acids, monoglycerides, and glycerol, as digested by pancreatic lipases, typically in the upper segment of the jejunum (Iqbal & Hussain, 2009). Interestingly, high concentrations of bile salts result in an inhibitory effect on pancreatic lipase. Colipase prohibits this effect from occurring; thus, ensuring the hydrolysis of lipids by pancreatic lipases (Tso & Fujimoto, 1991; van Tilbeurgh et al., 1999). The absence of colipase in both humans are mice results in steatorrhea, a condition associated with appreciable amounts of undigested lipids in the feces (D'Agostino et al., 2002; Hildebrand et al., 1982; Iqbal & Hussain, 2009). Pancreatic lipase functions to hydrolyze triglycerides into mainly 2-monoglyceride, and fatty acids (Tso & Fujimoto, 1991). Micellization occurs as bile salts assemble the broken-down products (including cholesterols and fat-soluble vitamins), which are presented to the apical membrane of enterocytes, where they

pass through the membrane dependent on their physical properties. Enterocytes have specific transport mechanisms. Longer, more complex fatty acids and steroids assemble into chylomicrons (Wahbeh & Christie, 2011). Chylomicrons are composed of a hydrophobic interior (triglycerides and cholesterol ester), and of a hydrophilic exterior (phospholipids, apolipoproteins, and cholesterol), allowing them to enter aqueous environments (lacteal) (Mansbach & Siddiqi, 2010).

Once in circulation, chylomicrons can obtain ApoC2 (co-factor for triglyceride hydrolysis) and ApoE (recognized by low-density lipoprotein (LDL) receptor, mediating the uptake of chylomicron remnants) (Feingold, 2021). Adipocytes come in contact with ApoC2 and in turn, activate lipoprotein lipase (embedded within chylomicron), hydrolyzing triglycerides (TG) to free fatty acids (FFA) (Feingold, 2021). Chylomicron remnants are returned to the liver and taken up through the LDL receptor (Feingold, 2021) where the cholesterol can be stored, secreted in very low-density lipoproteins (VLDL) or converted to bile acids (Feingold, 2021).

1.2 Role of the Liver in the Regulation of Energy Homeostasis

The liver is a fundamental organ involved in numerous physiological processes throughout the body (Kalra et al., 2021). In the fasted state, the liver can release glucose in the form of liberated glycogen and produce glucose in response to glucagon signaling through the stimulation of the gluconeogenic pathway (Trefts at al., 2017). Insulin stimulates glucose trapping through the enzyme glucokinase and utilization through cellular respiration in the fed state. In addition, it stimulates the synthesis of glycogen (Jensen et al., 2011).

In addition to glucose, insulin also regulates the uptake and synthesis of lipids. Given their hydrophobic nature, lipids cannot circulate in the blood and must be assembled into lipoproteins. The four forms of lipoproteins are chylomicrons, VLDL, LDL, and high-density lipoproteins (HDL). Chylomicrons have the lowest density amongst the lipoproteins; they are the largest and are produced exclusively by the enterocytes within the gastrointestinal tract. There are various apolipoproteins associated with lipoproteins, but the two which do not exchange and form the structural scaffold are apolipoprotein B-100 (ApoB100), and apolipoprotein

B-48 (ApoB48) (Dash et al., 2014). Within the enterocytes, lipids including triglyceride, cholesterol ester, phospholipids and free cholesterol are assembled on ApoB48 through the actions of microsomal triglyceride transfer protein with the endoplasmic reticulum (further described in section 1.4.2.1.1.3). In contrast, apoB100 is synthesized in the liver and is the backbone of VLDL and LDL. Lipogenesis is the process of fatty acid synthesis, which can increase triglyceride stores (Kersten, 2001). Triglyceride synthesis occurs through the acetylation of fatty acids to acyl-CoA. Acyl-CoA synthetase is a fatty acid activating enzyme, catalyzing FFA activation to Acyl-CoA esters (Ahmadian et al., 2007). Acyl-CoA esters participate in the glycerol 3-phosphate pathway, leading to the storage and synthesis of triglycerides. Acyl-CoA is first esterified on a molecule of glycerol 3-phosphate (catalyzed by glycerol-3-phosphate acyltransferase (GPAT)), leading to the production of lysophosphatic acid. A second Acyl-CoA participates in forming phosphatic acid (done by 1-acylglycerol-3-phosphate O acyltransferase (AGPAT)). Phosphatidic acid is phosphorylated to create diacylglycerol (activity by phosphatidic acid phosphatase), where a third acyl-CoA is added to finally form a triglyceride molecule (catalyzed by diacylglycerol acyltransferase (DGAT)) (Ahmadian et al., 2007). The synthesis of triglycerides and their assembly onto lipoproteins is regulated by insulin in the liver. Insulin stimulates lipogenesis to facilitate storage of nutrients in the post-prandial state (Wilcox, 2005) and hepatic VLDL secretion is reduced in the post-prandial state (Kamagate et al., 2008).

1.3 Role of Adipose Tissue in the Regulation of Energy Homeostasis

1.3.1 White Adipose Tissue

Adipose tissue is an essential organ involved in storage and synthesis of triglycerides (Ahmadian et al., 2007). Lipoprotein lipase (LPL) is synthesized in a variety of tissue types, including adipose tissue (Feingold, 2021). LPL plays a role in hydrolysis of triglycerides carried in chylomicrons and VLDL, allowing FFA to be taken up by adipocytes (Feingold, 2021). Delivery of triglycerides from the liver occurs through VLDL (Foufelle & Ferré, 2013). Fatty acid transport proteins (FATP), as well the cluster of differentiation 36 (CD36) transporter, allow FFA to pass

through the adipocyte's plasma membrane (Feingold, 2021). FATP function by translocation from an intracellular location to the membrane of adipocytes in response to insulin (Foufelle & Ferré, 2013). Once fatty acids enter the adipocyte, they are transported to the cytoplasm by fatty acid-binding proteins (FABP); generally, FABP4 in adipocytes (FABP5 can compensate when FABP4 is deleted) (Foufelle & Ferré, 2013). They are then re-esterified to triglyceride and stored in lipid droplets within the cytoplasm of adipocytes, which may occupy most of the cell volume of white adipose tissue (Berg et al., 2002). LPL expression is highly regulated by insulin (Feingold, 2021), where its expression increases in the post-prandial state and is reduced in the fasting state. In addition to the storage of triglycerides, white adipose tissue also secretes hormones (leptin, adiponectin, and adipsin) to influence food intake, insulin sensitivity, and insulin secretion (Cohen & Spiegelman, 2016). Leptin is an adipokine involved in regulating energy balance, mainly produced in adipose tissue. Interestingly, leptin levels positively correlate with adipose tissue mass (Clarke, 2007; Moller et al., 1998). Furthermore, fasting influences decreases plasma leptin levels (Kersten, 2001) consistent with a role in suppressing food intake (Klok et al., 2007). Leptin-deficient mice experience increased food intake, as well as increased body weight (Pelleymounter et al., 1998).

1.3.2 Brown Adipose Tissue

Brown adipose tissue (BAT) is responsible for the dissipation of energy through the production of heat (Contreras et al., 2016) by burning fuels such as FFA and glucose (Tabuchi & Sul, 2021). WAT can adopt a similar phenotype as BAT, allowing for increased thermogenesis in response to various stressors (Cuevas-Ramos et al., 2019). Interestingly, subcutaneous WAT depots contain beige or brite cells, which increase uncoupling protein 1 (UCP1) expression in response to various stimuli, such as cold (Herz & Kiefer, 2019; Tabuchi & Sul, 2021). Non-shivering thermogenesis is mediated by UCP1, a protein transporter in the inner mitochondrial membrane (IMM) (Tabuchi & Sul, 2021). Adrenergic stimulation of BAT leads to the lipolysis of cytoplasmic lipid droplets, resulting in the formation of FA, activating

UCP1 (Fedorenko et al., 2012). As protons leak through UCP1 from the IMM into the mitochondrial matrix, the H^+ gradient is uncoupled from ATP synthase, resulting in the proton gradient being dissipated as heat, rather than the production of ATP (Fedorenko et al., 2012; Tabuchi & Sul, 2021).

Beige adipocytes have been observed to differentiate from progenitor cells present in WAT. Brown adipocytes arise from the myogenic factor 5^+ ($myf5^+$) precursor while beige and white adipocytes arise from the $myf5^-$ preadipocyte; thus, there is potential for transdifferentiation between beige and white to occur (Brestoff & Artis, 2015). Fibroblast growth factor 21 (FGF21) expression is increased with cold exposure, where production leads to upregulation of peroxisome proliferator-activated receptor gamma co-activator (PCC)-1-alpha, increasing thermogenesis and browning (Cuevas-Ramos et al., 2019). Differentiation and activation occur when exposed to cold temperatures, as norepinephrine (NE) acts on β-3 adrenergic receptors on the surface of adipocytes in mice (Christian, 2017). Cold is sensed by the hypothalamus, stimulating the release of NE, which then binds and activates β-3 adrenergic receptors. Activation leads to the production of cyclic adenosine monophosphate (cAMP) from ATP, allowing secondary messengers to phosphorylate hormone sensitive lipase (HSL). HSL functions by converting triglycerides (TG) to fatty acids (FA), used for the electron transport chain to create a proton gradient for UCP-1 (Tabuchi & Sul, 2021).

1.4 The Role of the Intestine in Energy Homeostasis

Most nutrient breakdown and absorption occur within the small intestine (Collins & Bhimji, 2018). The small intestine is divided proximally to distally into the duodenum, jejunum, and the ileum. The proximal region is the main site of nutrient absorption (Collins & Bhimji, 2018). A single layer of specialized cells within the intestine called the intestinal epithelium (Ali et al., 2020), provides a dynamic lining between the external and internal environment (Allaire et al., 2018). The intestinal epithelium regulates the uptake of nutrients and fluids while preserving barrier function (Ma et al., 2018). The surface of the epithelial lining is found in the form of finger-like protrusions, known as villi (Kim & Kim, 2020), which maximize surface

area by 30-fold and regulate nutrient absorption (Kiela & Ghishan, 2016). Located at the base of each villus are intestinal crypts, where progenitor cells are found and give rise to the lineages of specialized cells, including enterocytes, goblet cells, Paneth cells and enteroendocrine cells, which form the intestinal epithelium (van der Flier & Clevers, 2009). Cell turnover occurs every 4-5 days, allowing for cell proliferation and differentiation (Park et al., 2016).

The most abundant cell, the enterocyte, absorbs water and nutrients to circulation (Noah et al., 2011). After absorption, carbohydrates and proteins enter the hepatic portal vein (De Iuliis & Pulerà, 2011), and lipids enter the lymphatic system (Dixon, 2010).

Goblet cells synthesize and secrete mucins, which form a protective layer (Birchenough et al., 2015). They also protect the body as they regulate the innate immune function (Dao & Le, 2021), and Paneth cells regulate the intestinal flora population (Lueschow & McElroy, 2020) (Fig.1.1).

At least 15 different cell types make up the enteroendocrine lineage, which produces various peptide hormones (Shroyer & Kocoshis, 2011). L-cells are one of the primary members of enteroendocrine lineage, responsible for the secretion of glucagon-like peptide-1 (GLP-1)/glucagon-like peptide-2 (GLP-2) (Jorsal et al., 2018). Another important cell, the K-cell, is responsible for the secretion of glucose-dependent insulinotropic polypeptide (GIP) (Jorsal et al., 2018). In the presence of nutrients, enteroendocrine cells secrete the incretin hormones GLP-1 and GIP, which bind to their receptors to potentiate insulin secretion from pancreatic β-cells (Holst & Gromada, 2004). These aspects are discussed further below in sections 1.4.2.3.2. and 1.4.2.3.3.

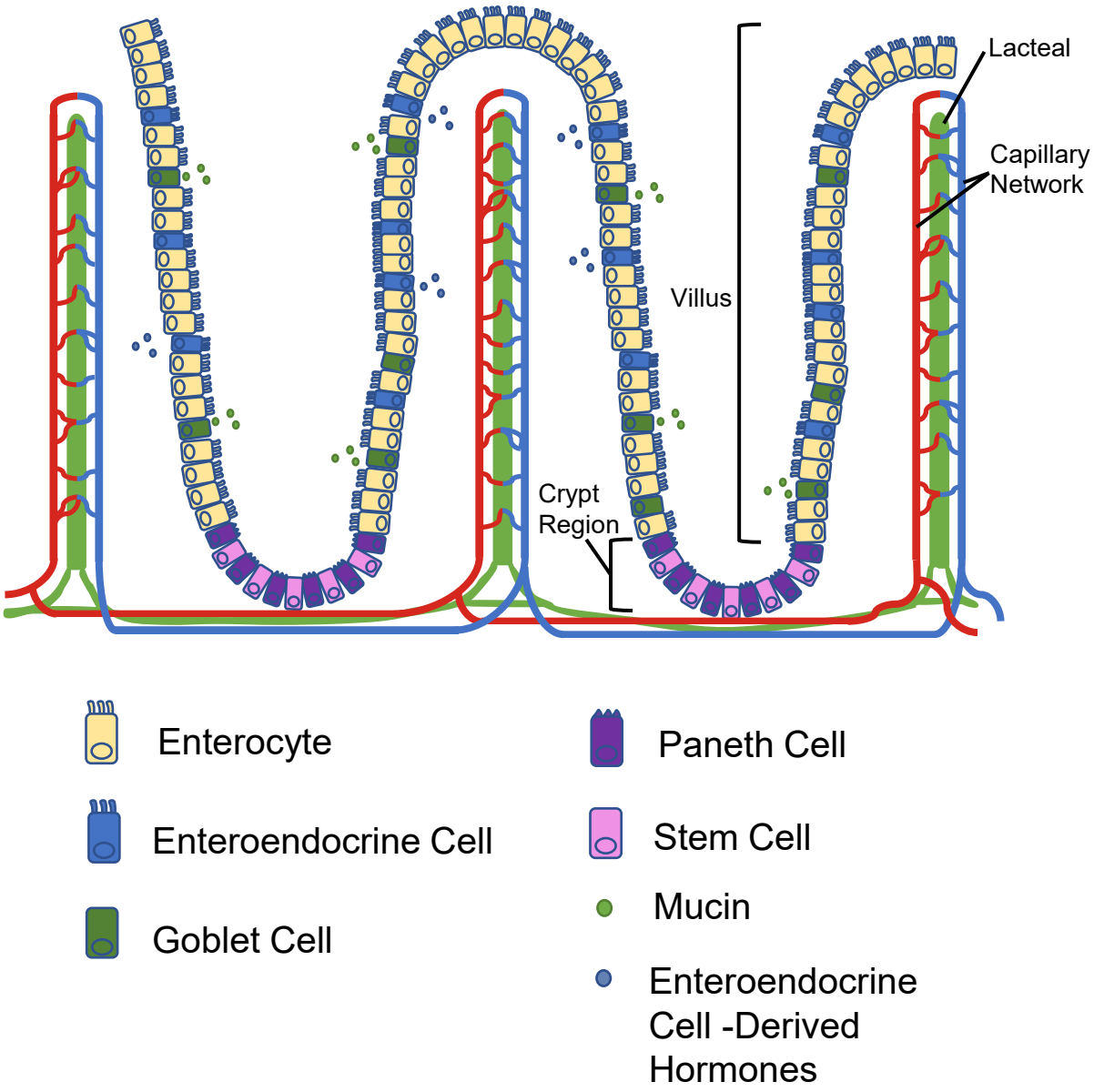


Figure 1.1. Anatomy of the Intestinal Epithelium.
Nutrients enter through enterocytes into the capillary network or are assembled into chylomicrons to enter the lacteal. Enteroendocrine cells sense nutrients and secrete hormones glucagon-like peptide 1 (GLP-1), glucagon-like peptide 2 (GLP-2) and glucose-dependent insulinotropic polypeptide (GIP). Goblet cells secrete mucins to form a protective layer. The crypt region contains Paneth cells which regulate immune function, and stem cells, which give rise to the specialized cells found further up the intestinal villus.

1.4.1 Intestinal Remodelling

The intestine is a highly dynamic organ and possesses significant plasticity. It allows organisms to respond to their surrounding environment by altering its size or thickness, along with cell content. The effect of this response ultimately allows for modulation of nutrient absorption and secretory responses (Le Gall et al., 2019). Modulation is typically dependent on the environment, and availability of nutrients, sufficing for tissue damage, stress conditions, and inflammation (de Sousa e Melo & de Sauvage, 2019). The intestinal epithelium is sensitive to DNA damage and ionizing radiation, particularly, the crypt stem cells (Blanpain et al., 2011). Cold exposure results in increased food intake and (Irwin et al., 2011; Ravussin et al., 2014) the gut can respond and adapt by increasing size; ultimately, increasing absorptive capacity (Price et al., 2013). The maintenance and growth of the gut is dependent on the quantity of luminal nutrients present (Dailey, 2014), such that whole body energy deficit leads to increased intestinal size (Nilaweera & Speakman, 2018). In hyperphagic *db/db* obese mice and in high-fat diet-induced, obese mice, β-catenin levels were increased in both models, a protein essential for maintaining intestinal integrity and homeostasis. The proliferation of cryptic stem cells, villi length and nutrient absorption were all increased; however, reversed with JW55 (inhibitor of the β-catenin pathway), as well as caloric restriction (Mao et al., 2013). High concentration glucose and FFA (nutrient overload) activate the GSK-3β/β-catenin pathway and increase β-catenin accumulation and induce increased intestinal cell proliferation.

1.4.2 Role of the Cells of the Intestinal Epithelium

1.4.2.1 Enterocytes

The enterocyte is the most abundant cell within the intestinal epithelium (Hooper, 2015). Enterocytes function as a physical barrier, preventing pathogens within the intestinal lumen from entering the bloodstream. While serving barrier function, they are also highly ordered absorptive cells (McConnell et al., 2009). Crypt stem cells give rise to enterocytes and typically reside towards the apical tips

of the villus (Engevik & Goldenring, 2018). A brush border, made up of microvilli, exists on the apical end of enterocytes and functions by further increasing surface area for nutrient absorption (McConnell et al., 2009). Along the brush border, selective transporters allow nutrient passage through the apical end of enterocytes and exit the basolateral membrane (McConnell et al., 2009).

1.4.2.1.1 Nutrient Transport in Enterocytes

1.4.2.1.1.1 Carbohydrate Transport

Glucose and galactose enter enterocytes by sodium-glucose transporter 1 (SGLT1) (Kiela & Ghishan, 2016). SGLT1 uses a Na^+ gradient for transport, permitting one sugar molecule's entry and two sodium ions into the transporter (Kiela & Ghishan, 2016). Following the entry into the enterocyte, sodium ions along the basolateral membrane undergo active transport through the sodium-potassium pump (Na+ K+ ATPase). Unlike glucose and galactose, fructose is transported through GLUT5 transporter, facilitating entry into the enterocyte and enteroendocrine cells. These carbohydrates enter into the blood stream via facilitated diffusion utilizing glucose transporter 2 (GLUT2) in the basolateral membrane of enterocytes, (Wright et al., 2011) and enter systemic circulation through the portal vein.

1.4.2.1.1.2 Protein Transport

Proteins entering the intestine are hydrolyzed, forming oligopeptides and amino acids, where most amino acids are absorbed through sodium co-transporters (Na^+ and H^+ linked amino acid transporters) (Chen et al., 2010; McCauley et al., 2020). Peptide transporter 1 (PEPT1) is another common transporter present in the brush border (A. Chen et al., 2010; McCauley et al., 2020). There are at least 6 unique amino acid transporters in the basal membrane of the cell; however, the most common form is solute carrier family 8 memberA2 (SLC8A2), where sodium dependent transport for all amino acids occurs (Kiela & Ghishan, 2016). Other

transporters are specialized for glycine (SLC6A9), cationic amino acids (SLC7A1), neutral amino acids (SLC7A8), along with a variety of others (Kiela & Ghishan, 2016). These also enter circulation through the portal vein.

1.4.2.1.1.3 Lipid Transport

Unlike carbohydrates and proteins, lipids cannot enter the bloodstream as easily due to their hydrophobic nature (Feingold, 2021). Lipids freely diffuse into enterocytes through CD36 receptors (Dixon, 2010; Iqbal & Hussain, 2009). Short and medium-chain FA can diffuse directly into capillaries for portal circulation via simple diffusion, while longer FA, monoglycerides, fat-soluble vitamins, and cholesterol are assembled into chylomicrons (Wahbeh & Christie, 2011). For these lipids unable to simply diffuse, fatty acid binding proteins within the cytosol aid with transportation to the endoplasmic reticulum (ER), where fatty acids are transported through the fatty acid transfer protein. Embedded within the endoplasmic reticulum are Acyl-CoA: cholesterol acyltransferases, diacylglycerol acyltransferases and lecithin:retinol acyltransferases, which serve the purpose of esterifying the cholesterol, monoacylglycerols, and retinol, to be packed into pre-chylomicrons (Iqbal & Hussain, 2009).

A single apolipoprotein B gene allows for the synthesis of both apoB48 and apoB100 proteins. Due to messenger ribonucleic acid (mRNA) editing, ApoB48 is 48% of the length of ApoB100, where a cytidine is replaced by uracil, creating a stop codon (Nakajima et al., 2014). This phenomenon occurs with the APOBEC1 editing complex being found only in the intestine of humans but in the liver and intestine of mice (Davidson & Shelness, 2000; Nakajima et al., 2014). Microsomal triglyceride transfer protein (MTP) is a cellular protein present in the ER, chaperoning the neutral lipids (triglycerides and cholesterol esters) onto the ApoB48 backbone by a shuttle mechanism (Hussain et al., 2012), and by binding to ApoB48 with high affinity (Hussain et al., 2003). Pre-chylomicrons are transported to the Golgi, further processed (Hesse et al., 2013), and fused into another transport vesicle, transported to the basolateral membrane (Giammanco et al., 2015). Chylomicrons are

transported to lacteals through lymph vessels and eventually enter the bloodstream at the thoracic duct.

1.4.2.2 Goblet Cells

The intestine is central to both nutrient absorption and maintaining a protective barrier. Goblet cells (GC) are specialized cells embedded within the intestinal epithelium and have a principal role in secreting mucins, which form a layer over the intestinal epithelium (Hooper, 2015). The formation of a mucus gel aid in fortifying the mucosal barrier (Camilleri et al., 2019) and limiting bacterial penetration (Hansson & Johansson, 2010). Much like other specialized intestinal epithelial cells, pluripotent stem cells give rise to goblet cells, and are renewed continuously (Dao & Le, 2021).

Goblet cells in the small intestine secrete glycoproteins, a key mucin component. Mucin is secreted upon stimulation by endocytosis or acetylcholine (Dao & Le, 2021). Goblet cells also play a role in immune function by taking up antigens (Hooper, 2015). Mucin 2 (MUC2) (predominantly secreted from intestinal GC's) mucin is secreted from goblet cells, a critical structural component of the outer mucin layer. MUC2 deficiency renders goblet cells unable to produce mucin properly, and they are unable to protect the epithelium from bacteria invasion; thus, leading to intestinal inflammation (Hansson & Johansson, 2010). Goblet cells are in a lesser abundance than the absorptive cells population. These specialized cells increase in abundance distally such that they are most abundant towards the ileum and in the colon (Shroyer & Kocoshis, 2011). Interestingly, recent data suggest that GC contains goblet cell-associated antigen passages (GAPs), which allow luminal antigens to enter the cell. The antigens are then delivered to antigen-presenting cells (APCs). Based on the signal received, secretion of chemokines and cytokines also occurs for recruitment of immune cells to regulate an immune response (Knoop & Newberry, 2018). APCs present the antigen to T-cells for removal.

1.4.2.3 Enteroendocrine Cells

Enteroendocrine cells are specialized cells that reside within the intestinal epithelium and secrete peptides that regulate satiety, energy metabolism and gastrointestinal (GI) motility in response to nutrient stimulation (Lee & Owyang, 2019). Various enteroendocrine cell types exist, with specific receptors to synthesize and secrete their respective hormone (Lee & Owyang, 2019). Hormones are secreted from their storage granules into circulation, and where they can rapidly activate their receptors to initiate signaling (de Lartigue & Raybould, 2012).

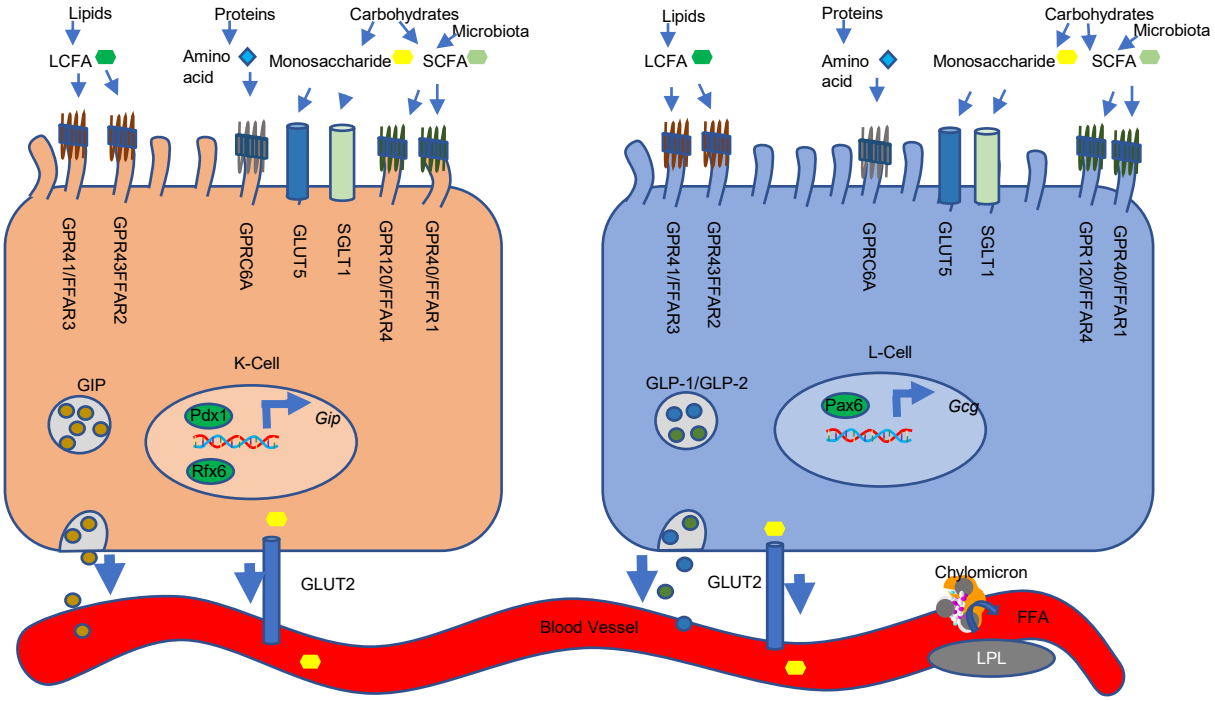


Figure 1.2 : Receptors and Signalling Components Present on Enteroendocrine K and L-Cell, Trigger Secretion of GLP-1, GLP-2, and GIP.

Microvilli protrude from the apical side of the cell, extending towards the lumen. Incoming macronutrients from the lumen are detected and sensed by receptors present on the apical border. Carbohydrates are divided into monosaccharides and short-chain fatty-acids (SCFA). Glucose is transported through the sodium-glucose linked transporter (SGLT1). Glucose passes through the glucose transporter 2 (GLUT2) present on the basolateral membrane, and into the bloodstream. GLUT5 is responsible for sensing fructose. Short-chain fatty acids (SCFA) are derived from microbial fermentation, and are sensed by the free fatty acid receptors (GPR40/FFAR1 and GPR120/FFAR4). Long-chain fatty-acids are sensed by GPR43/FFAR2 and GPR41/FFAR3, along with G-protein coupled receptor 119 (GPR119). Amino acids are detected by GPR9C6A. Lipoprotein lipase is involved in the production of LCFA and monoacylglycerols. Regulatory factors x6 (Rfx6) and insulin promoter factor 1 (Pdx1) influence GIP expression and secretion. Sensing by the majority of listed receptors allow for secretion of GLP-1/GLP-2 from L-cells and GIP secretion from K-cells into circulation, allowing for various endocrine, paracrine, and neuronal function.

Adapted from Figure 2, Morrow, N. M., Hanson, A. A., & Mulvihill, E. E. (2021). Distinct Identity of GLP-1R, GLP-2R, and GIPR Expressing Cells and Signaling Circuits Within the Gastrointestinal Tract. *Frontiers in Cell and Developmental Biology*, 9. https://doi.org/10.3389/fcell.2021.703966

1.4.2.3.1 Regulation of the Synthesis and Secretion of Gut Hormones

Luminal contents (mainly macronutrients) contact microvilli located on the apical end of enteroendocrine cells and are sensed by the chemosensors (Spreckley & Murphy, 2015). There are a variety of chemosensors, including the G-protein-coupled receptor/free-fatty acid receptors (GPR/FFAR) examples include: GPR40/FFAR1 and GPR120/FFAR4, which sense short-chain fatty acids (SCFA), and GPR43/FFAR2, GPR41/FFAR3, which sense long-chain fatty acids (LCFA). Further, amino acids are sensed by GPRC6A (Spreckley & Murphy, 2015), and monosaccharides are sensed by SGLT-1 (Gribble et al., 2003; Moriya et al., 2009; Ritzel et al., 1997; Wu et al., 2010). Sensing of macronutrients leads to the secretion and control of the physiological concentrations of GLP-1, GLP-2, and GIP into circulation (Spreckley & Murphy, 2015). The intestinal derived, preproglucagon derived peptides are processed and secreted from enteroendocrine L-cells. GLP-1 and GLP-2 are modification products which arise from a prohormone, proglucagon (Sandoval & D'Alessio, 2015). Proglucagon is a post-translational product from the preproglucagon gene expressed in L-cells. Proglucagon is cleaved by prohormone convertase 1/3 (PC1/3) to form four active proteins (Fig.1.3), including GLP-1 and GLP-2 (Hruby, 1997). Interestingly, GLP-1 and GLP-2 are secreted in a 1:1 molar ratio (Matikainen et al., 2016). The GIP precursor has been less well-defined; however, immunohistochemistry reveals PC1/3 expression in GIP immunoreactive cells, and it was later determined that PC1/3 is also essential for the cleavage of proGIP to GIP in K-cells (Ugleholdt et al., 2006) (Fig.1.4). Regulatory factor X6 (Rfx6) and pancreatic and duodenum homeobox 1 (Pdx1) are transcription factors that regulate *Gip* messenger ribonucleic acid (mRNA) expression by binding to the *Gip* promoter (Suzuki et al., 2013). Similarly, paired box 6 (Pax6) is an essential transcription factor in enteroendocrine cells that binds to the *Gcg* promoter, activating proglucagon gene expression (Hill et al., 1999). The half-lives of many peptides are generally short (minutes) due to the presence of dipeptidyl-peptidase-4 (DPP4) (Singh, 2014). DPP4 is a serine protease that cleaves at position-2 alanine or proline and renders the hormones unable to engage their respective receptors

(Mulvihill & Drucker, 2014) (Fig. 1.5). GLP-1 and GIP (incretins) are the main contributors to the incretin effect as they are linked to insulinotropic effects, following nutrient stimulation (Nauck & Meier, 2016). The incretin effect is characterized by the potentiation effect glucose administered orally has on insulin secretion, compared to achieving similar glycemia with intravenous glucose in healthy individuals (Holst & Ørskov, 2004). Subjects with type 2 diabetes (T2D) almost lose their entire ability to undergo this phenomenon (Holst & Ørskov, 2004), suggesting impaired incretin function.

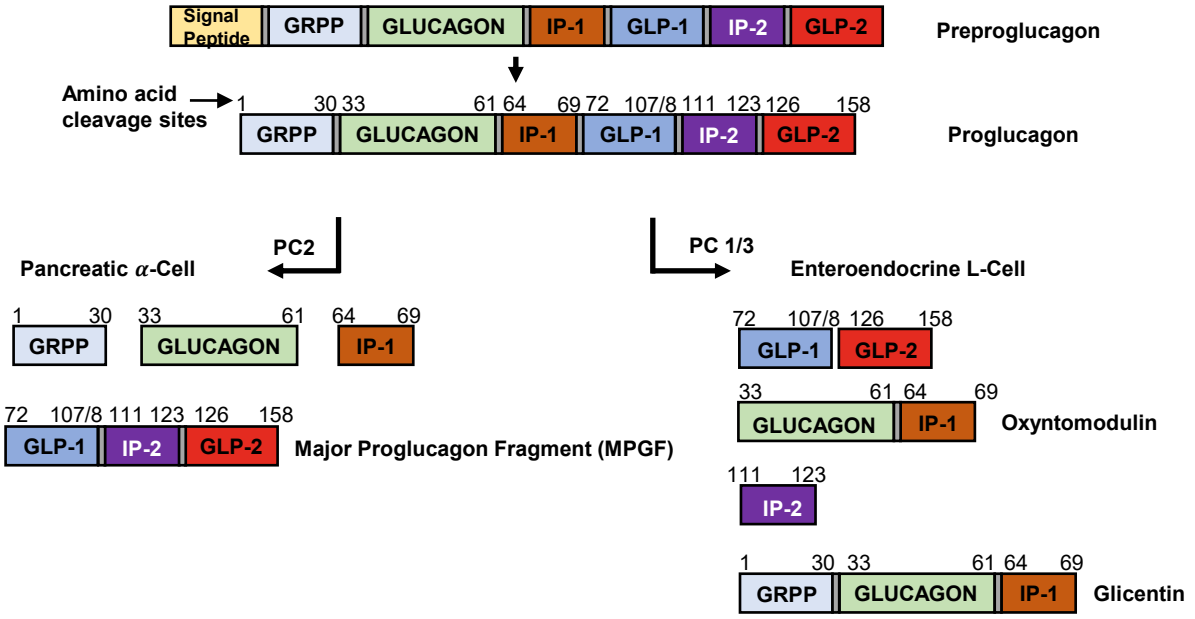


Figure 1.3. Glucagon-Like Peptide 1 (GLP-1) and Glucagon-Like Peptide 2 (GLP-2) are Cleaved by Prohormone Convertase 1/3 (PC1/3) and Prohormone Convertase 2 (PC2) from the Proglucagon Protein.
PC1/3 cleaves proglucagon in enteroendocrine L-cells, resulting in GLP-1, GLP-2, oxyntomodulin, and glicentin. PC2 cleaves proglucagon in pancreatic α-cells, resulting in GRPP, glucagon, and MPGF.

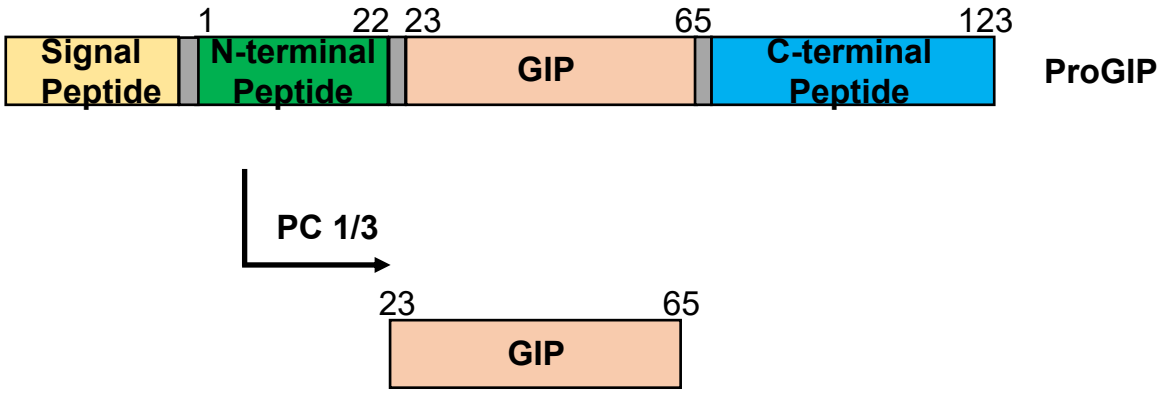


Figure 1.4. Glucose-Dependent Insulinotropic Polypeptide (GIP) is Cleaved by PC1/3 from the ProGIP Protein.
ProGIP is cleaved by PC1/3 in enteroendocrine K-cells, resulting in a mature 42-amino acid GIP.

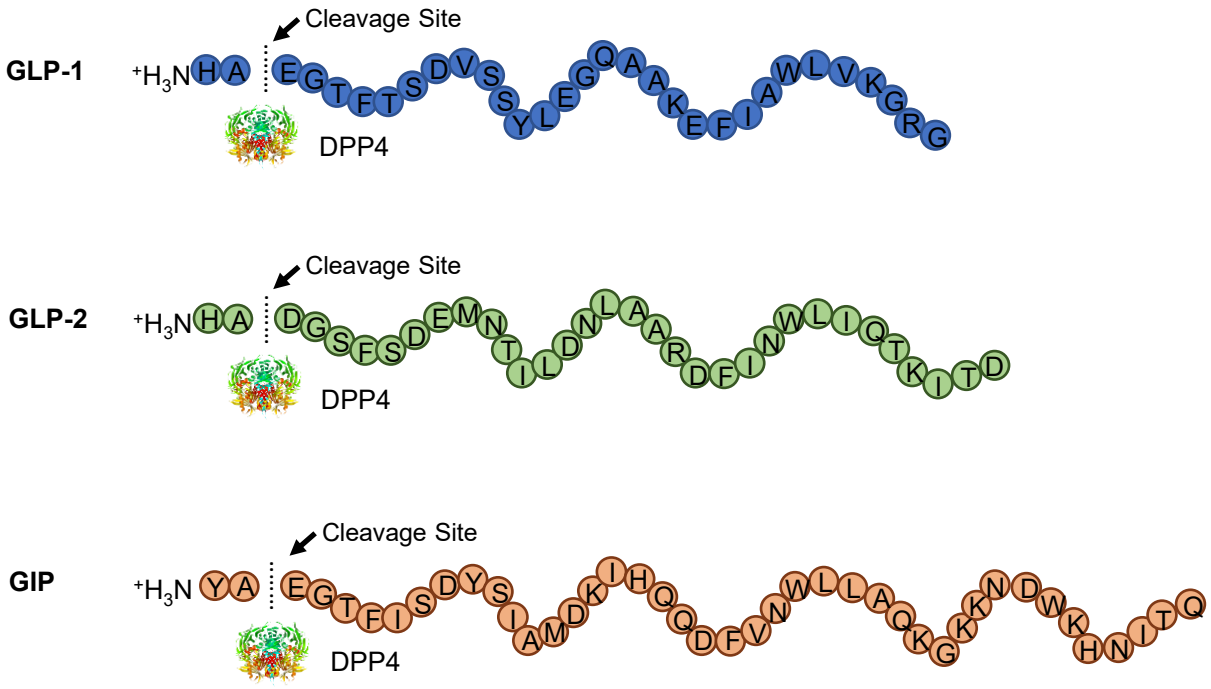


Figure 1.5. Glucagon-like peptide 1 (GLP-1), Glucagon-like peptide 2 (GLP-2), and Glucose-Dependent Insulinotropic Polypeptide (GIP) are Cleaved and Inactivated by DPP4.
Incretin hormones GLP-1 and GIP, and gut-derived peptide GLP-2 are cleaved at position-2 alanine by serine protease DPP4, rendering them inactive for canonical receptor signalling.

1.4.2.3.2 Glucagon-Like Pepide-1 (GLP-1)

GLP-1 is one of the two incretin hormones, most well-known for its ability to potentiate nutrient-stimulated insulin secretion. GLP-1 also plays many roles in glucose and lipid metabolism, energy balance, and food intake (please see sections 1.4.2.3.5.1.1 and 1.4.2.3.5.1.2). Synthesis of GLP-1 results in a peptide with a length of 30-31 amino acids (Mapelli et al., 2009). Once L-cells of the intestine secrete GLP-1, the hormone signals receptors in the pancreas, intestine, neurons, and stomach (Andersen et al., 2021; Richards et al., 2014). GLP-1 receptor (GLP-1R) is a 7- transmembrane protein, a class of receptors known as a G-protein-coupled receptor (GPCR) (Graaf et al., 2016; Usdin et al., 1993; Yang et al., 2015). Upon GLP-1 binding, G-protein coupled receptor trimeric subunits undergo a conformational change. The alpha subunit is activated by guanosine-5'-triphosphate (GTP) binding, allowing release from the beta and gamma subunits (Lodish et al., 2000). The alpha subunit further stimulates adenylyl cyclase, catalyzing the conversion of ATP to cyclic adenosine monophosphate (cAMP), an essential second messenger. Protein kinase A (PKA) and exchange protein activated by cAMP (Epac) are downstream effectors that inhibit the K^{+}_{ATP} channel; and in turn, repolarization does not occur (Müller et al., 2019). Inositol triphosphate receptor (IP_3R) and ryanodine receptor (RYR) are found on the endoplasmic reticulum, responsible for mediating the release of calcium, influencing the secretion of insulin (Müller et al., 2019). An increase in glucose concentration induces GLP-1-meditated insulin secretion following a meal (potentiates secretion by increasing the inward calcium current, which initiates exocytosis) (Müller et al., 2019) (Fig.1.6). Ultimately, GLP-1 is one of the more potent potentiators insulin release, surpassing the ability of GIP (Holst & Ørskov, 2004). Additionally, GLP-1 must rapidly stimulate insulin secretion due to its short half-life of 2-3 minutes following release from L-cells (Seino et al., 2010).

1.4.2.3.3 Glucose-Dependent Insulinotropic Polypeptide (GIP)

GIP is also an incretin hormone, which plays a role in potentiating insulin secretion from pancreatic β-cells and increasing glucagon secretion, along with regulating postprandial metabolic homeostasis (Campbell, 2021). GIP is secreted from up the upper region of the small intestine (Seino et al., 2010) due to higher K-cell density (Holst & Ørskov, 2004; Khan et al., 2020). mRNA isolated from various intestinal tissue indicated that more significant expression of the GIP gene is present in the duodenum and the jejunum, while lower expression was identified in the ileum (Tseng et al., 1993). GIP was the first incretin to be discovered after being observed in pigs, functioning to inhibit gastric acid secretion. Receptors for GIP are in the proximal small intestine (particularly in the duodenum), adipose tissue, heart, and several brain regions such as the cerebral cortex, hippocampus, and adrenal cortex (Usdin et al., 1993). GIP receptor (GIPR) is also a GPCR, the same protein class as the GLP-1R and interestingly, shares 25-50% amino acid sequence similarities (Usdin et al., 1993). Like GLP-1, GIP is quickly cleaved and inactivated by DPP4; with a half-life of 4-5 minutes (Seino et al., 2010). Importantly, GIP promotes an insulinotropic effect by inducing signaling through its receptor on the pancreatic β-cell (Khan et al., 2020).

Binding to the receptor allows for an increase in intracellular cAMP due to activation of adenylate cyclase. The increase in cAMP activates protein kinase A (PKA) (closes K_{ATP} channels and membrane depolarization) and EPAC2/cAMP-GEFII. Membrane depolarization allows for calcium intake, which further mobilizes calcium from intracellular stores (through PKA and EPAC2), ultimately triggering the release of insulin granules (Yabe & Seino, 2011). As mentioned, increased calcium concentrations result in increased exocytosis of insulin (Holst & Ørskov, 2004). While known for its incretin action in β-cells, receptor activation also stimulates adipogenesis (Park et al., 2016) and modulates metabolic function through the gut-brain axis (Higgins et al., 2016).

1.4.2.3.4 Glucagon-Like Peptide-2 (GLP-2)

GLP-2 (1-33) is a 33 amino acid-long peptide involved in nutrient assimilation and energy homeostasis (Sherwood & Adams, 2004). Unlike the incretin hormones, activation of the GLP-2 receptor (GLP-2R) does not facilitate insulin secretion; but promotes intestinal growth and proliferation through downstream mediators (Fesler et al., 2020). GLP-2 is most known for its intestinotrophic capacity, increasing intestinal proliferation, inhibiting apoptosis, improving barrier function, increasing absorption, as well as increasing blood flow (Markovic & Brubaker, 2019). Treatment with a GLP-2R agonist often leads to increases in intestinal villus length, small bowel weight and crypt depth (Sigalet et al., 2006), while opposite effects are observed in mice lacking the *Glp2r* (Bahrami et al., 2010). Receptors for GLP-2 are localized to the hypothalamus, jejunum, and in stromal cells of the lamina propria. Receptors localized to the stromal cells enforce the link of GLP-2 with other intestinal growth factors, including keratinocyte growth factor (KGF), and IGF-1 (Yusta et al., 2019). GLP-2 receptor activation increased cAMP concentrations in various transfected cells (Drucker & Yusta, 2014), which consistently activated PKA (Shin et al., 2005). Caco-2 cells, an *in vitro* model of the intestinal epithelium, requires activation of the phosphatidylinositol-3 kinase (PI3K) and the mitogen-activated protein kinase (MAPK) pathways for increasing cell proliferation in response to GLP-2 treatment (Jasleen et al., 2000; Shin et al., 2005.; Walsh et al., 2003).

1.4.2.3.5 Gut Hormone Receptors and Signaling Circuits

1.4.2.3.5.1 GLP-1- Mediated Regulation of Post-Prandial Lipid Metabolism

As previously stated, activation of the GLP-1R potentiates nutrient stimulated insulin secretion and facilitates glucose uptake into cells (Abdulla et al., 2014). Insulin can also regulate carbohydrate, lipid, and protein metabolism (Wilcox, 2005). Potentiation of endogenous GLP-1 has been shown to regulate lipid metabolism as treatment with the DPP4 inhibitor sitagliptin attenuated dyslipidemia in mice with diet-induced insulin resistance (Hsieh et al., 2010).

To further demonstrate the metabolic effects of the incretin hormones, studies have generated and characterized *Glpr1*$^{-/-}$ mice. Unsurprisingly, *Glp1r*$^{-/-}$ demonstrate increased blood glucose levels during an oral glucose tolerance test, compared to wildtype mice (Scrocchi, Brown, MacLusky, et al., 1996).

GLP-1 action has been recognized to decrease post-prandial triglyceride secretion and ApoB48 levels in rodents (Hsieh et al., 2010). This is relevant for patients with T2D who take GLP-1R agonists, as dyslipidemia in T2D is characterized by increased plasma TG levels and decreased high-density lipoprotein (HDL) cholesterol (Patel et al., 2014). Treatment with either native GLP-1 or exendin-4 (GLP-1R agonist) on HepG2 cells increases the apoA1 gene, a component of HDL (Chehade et al., 2013). In patients with T2D, serum TG levels were significantly lower with Exenatide (GLP-1R agonist) than with treatment with a placebo (Schwartz et al., 2010). Furthermore, post-prandial plasma TG levels were markedly greater in *Glp1r*$^{-/-}$ mice compared to wildtype mice (Hsieh et al., 2010); thus, demonstrating GLP-1's important role in the uptake and distribution of dietary lipids.

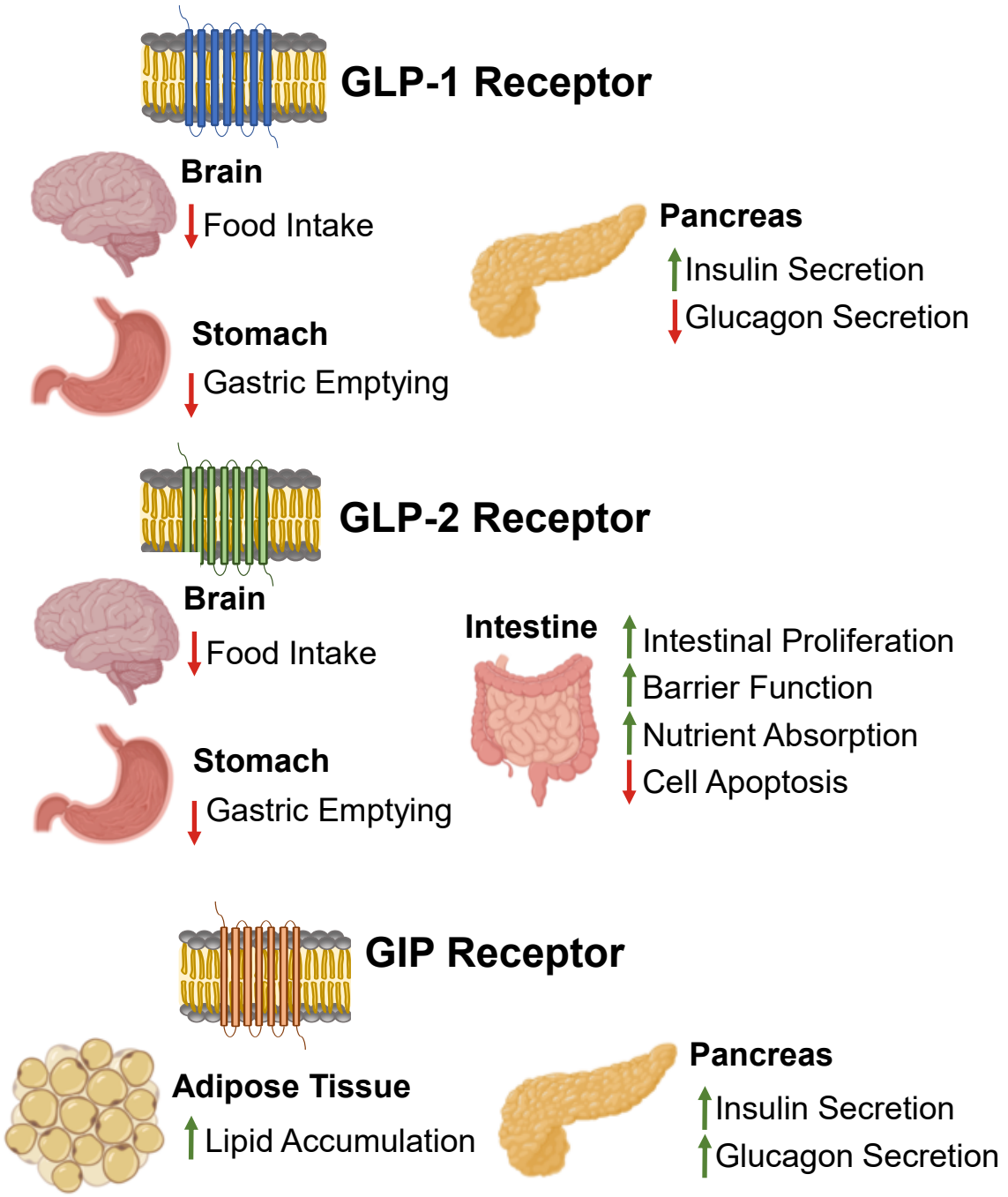


Figure 1.6. Hormone Action for Binding of Glucagon-Like Peptide 1 (GLP-1), Glucagon-Like Peptide 2 (GLP-2) and Glucose-Dependent Insulinotropic Polypeptide (GIP) to its Respective Receptor in Various Organs.
GLP-1 receptor activation leads to decreased food intake (brain), gastric emptying (stomach), glucagon secretion (pancreas), and an increase in insulin secretion (pancreas). GLP-2 receptor activation allows for decreased food intake, gastric emptying, decreased cell apoptosis (intestine), and increased intestinal proliferation, barrier function, and nutrient absorption (intestine). GIP receptor activation leads to increased lipid accumulation (adipose tissue), and increased insulin and glucagon secretion (pancreas). Created with BioRender.com

1.4.2.3.5.2 GLP-1 and Food Intake

Energy balance is another key factor of homeostasis linked to the action of GLP-1. GLP-1 action has also been associated with reduced food intake in both humans and rodents and increased energy expenditure in rodents (Boguszewski, 2015) (Fig.1.6). Rats were treated with exendin-9 (GLP-1R antagonist) for three days, displayed hyperphagia and increased body weight. Oppositely, injection of GLP-1 (3nmol) in rats for six days led to decreased food intake and body weight (Meeran et al., 1999).

GLP-1Rs are widely expressed in neurons and control appetite (Dailey & Moran, 2013). GLP-1 can be derived from preproglucagon-expressing neurons in the brain stem function to signal locally to restrict food intake (Katsurada & Yada, 2016). Additionally, GLP-1 has been reported to cross the blood-brain barrier. GLP-1 ensues action potentials, which increase cytosolic calcium concentrations in the neurons of nodose ganglia. GLP-1 neurons from the nucleus tractus solitarius (NTS) project in the central nervous system and include the paraventricular nucleus (PVN). Endogenous GLP-1 in the brain was reported to target the PVN, leading to the restricting of feeding. Additionally, neurons are activated by central and peripheral signals from the GLP-1R+ vagal afferent (Katsurada & Yada, 2016). Furthermore, GLP-1 interacts with leptin; a hormone linked to satiety. Interestingly, leptin receptors are present on intestinal L-cells and on GLP-1-secreting neurons, where leptin was demonstrated to stimulate GLP-1 secretion from L-cells (Anini & Brubaker, 2003; Ronveaux et al., 2015). However despite GLP-1 is known to be associated with inhibition of food intake (Dailey & Moran, 2013; Hansotia et al., 2004); however, food intake (Scrocchi, Brown, MacLusky, et al., 1996) and body weight was similar between *Glp1r*$^{-/-}$ and *Glp1r*$^{+/+}$ mice (Ayala et al., 2010; Scrocchi, Brown, MacLusky, et al., 1996) suggesting other hormones may compensate in its absence.

1.4.2.3.5.3 GLP-1 and Intestinal Growth

In addition to the actions of GLP-2, GLP-1 also promotes nutrient absorption and contributes to intestinal growth (Drucker & Yusta, 2014). Koehler *et al.* used glucagon receptor knockout (*Gcgr*$^{-/-}$) mice to determine the influence of GLP-1 on bowel growth. GLP-1 levels were significantly elevated in *Gcgr*$^{-/-}$ mice, along with GLP-2, as GLP-1, GLP-2, and Glucagon are all derived from preproglucagon (Ali et al., 2011). Native GLP-1 did not significantly increase small bowel weight and length in *Glp1r*$^{-/-}$ mice; however, *Gcgr*$^{-/-}$ mice did experience increased large bowel weight and length. Treatment with exendin-4 resulted in increased weight and small bowel length. Chronic treatment (twice daily for one month) results in increased small bowel weight and length, intestinal circumference, and crypt number in the duodenum and jejunum but not in the ileum. Interestingly, villus height was not increased with treatment (Koehler et al., 2015). These studies demonstrate the influence of GLP-1 on intestinal growth, specifically, increasing bowel weight and length (Koehler et al., 2015).

1.4.2.3.5.4 GIP Action on Energy Homeostasis and Adipose Tissue

GIP has been described to have roles in glucose and lipid metabolism, energy balance, and food intake (Barrera et al., 2011). Interestingly, native GIP itself may not contribute to these effects as significantly as GLP-1, as food intake or body weight was unaffected in global germ-line deletion of the *Gipr* mice in chow-fed mice (Miyawaki et al., 1999). However *Gipr*$^{-/-}$ mice are resistant to diet-induced obesity (DIO) (Killion et al., 2018). Administration of long-acting fatty-acylated GIP (GIP analog) in wildtype mice with diet-induced obesity (DIO) resulted in increased cFOS neuronal activity in the hypothalamic feeding centers, decreased food intake and body weight and improved glucose handling (Zhang et al., 2021). Humans treated with GIP (3-30) NH_2, a GIPR antagonist, demonstrated decreased total insulin, blood glucose and subcutaneous abdominal adipose tissue blood flow. Further, adipose tissue TG and glucose uptake were decreased, emphasizing GIP's effect on glucose and lipid metabolism (Asmar et al., 2017). GIP(3-30)NH2 treatment resulted in increased body weight, fat mass, TG levels, LPL, and leptin in rats, which

illustrates GIP's role in lipid metabolism (Baldassano et al., 2019). GIP also demonstrates an anabolic effect on adipose tissue and regulates adipokine secretion (Baldassano et al., 2019). Signaling through its receptor leads to an increase in fat accumulation by effectively increasing LPL, along with a variety of other pathways (Yamane & Harada, 2019). GIP is capable of increasing fat storage, however, it is unclear whether this is entirely mediated by binding to its receptor on adipocytes or by the secretion of insulin or both, allowing a switch from lipolysis to lipogenesis in white adipose tissue (Martin et al., 2020) (Fig.1.6). GIP has also been suggested to play a role in insulin resistance as *Gipr*$^{-/-}$ mice were protected from diet-induced insulin resistance (Miyawaki et al., 2002).

1.4.2.3.5.5 Metabolic Role of Endogenous Incretin Hormone Receptor Signalling

Metabolic processes have additionally been investigated in double incretin receptor knockout mice (DIRKO), (*Glp1r*$^{-/-}$*Gipr*$^{-/-}$). Despite lacking both the *Glp1r* and *Gipr*, male and female DIRKO mice exhibit normal food intake and body weight (Hansotia et al., 2004). Treatment with either GIP or exendin-4 DIRKO improved glucose tolerance in wildtype mice, but not in DIRKO mice (Hansotia et al., 2004). Interestingly, the islets of DIRKO mice were perfused with basal medium (1mmol/L glucose) to determine insulin-releasing capacity, where insulin secretion was preserved in these mice, suggesting that the incretin signaling through potentiating pathways are not required for basal insulin secretion (Hansotia et al., 2004).

1.4.2.3.5.6 GLP-2R Signaling and Glucose Metabolism

Unlike GLP-1 and GIP, GLP-2 does not play a role in insulin secretion (Janssen et al., 2013) and conversely to GLP-1, GLP-2 increases glucagon secretion (Janssen et al., 2013; Sørensen et al., 2003). Although not influencing insulin secretion, GLP-2 does play a role in glycaemic control and insulin sensitivity (Amato et al., 2016). *Glp2r*$^{-/-}$ mice exhibit modest deterioration in glucose tolerance (Bahrami et al., 2010; Fuchs et al., 2020). Interestingly, tissue specific knockout of the *Glp2r* in the proopiomelanocortin (POMC) hypothalamic neurons (region for

energy balance) demonstrates impaired postprandial glucose tolerance and hepatic insulin resistance (Amato et al., 2016; Shi et al., 2013). HFD is known to be associated with glucose intolerance and insulin resistance; therefore, a GLP-2 antagonist (GLP-2 (3-33)) was used to block GLP-2 signaling (Baldassano et al., 2015). Treatment with a GLP-2R antagonist resulted in increased glucose intolerance, fasting and glucose-induced insulin levels, as well as insulin resistance (Baldassano et al., 2015). These results suggest that endogenous GLP-2 is protective against dysregulation of glucose metabolism under HFD feeding.

1.4.2.3.5.7 GLP-2 Signaling and the Regulation of Lipid Metabolism

GLP-2 is involved in lipid homeostasis (Matikainen et al., 2016), and facilitates intestinal absorption of lipids (Hsieh et al., 2009). It is thought that GLP-2 mediates CD36/fatty acid translocase, and also influences chylomicron secretion (Hein et al., 2013). Administration of GLP-2 was able to demonstrate increased TG and ApoB48 levels while feeding (Dash et al., 2014), further implying GLP-2 effect on intestinal lipid metabolism. As the *Glp2r* is not expressed in enterocytes (Markovic & Brubaker, 2019), GLP-2's effect on intestinal lipid metabolism is not completely understood. Many studies use exogenous GLP-1 and GLP-2; thus, it is suggested that the effect on the pathways differ compared to endogenously released peptides (Matikainen et al., 2016).

1.4.2.3.5.8 GLP-2 and Food Intake

Far less is known about GLP-2's role in energy homeostasis than the extensively studied GLP-1; however, results have shown reduced food intake and increased energy expenditure (Burrin et al., 2001; Guan, 2014) (Fig.1.6). Similarly to mice infused with GLP-1 (Boguszewski, 2015), GLP-2 infusion in rats suppresses food intake (Burrin et al., 2001). Compared to vehicle treated mice, mice treated with [Gly^2]GLP-2 displayed significantly reduced food intake in both lean and DIO mice. There is very little known about the pathways in which GLP-2 influence food intake; however, it has been speculated that GLP-2R activation leads to acetylcholine release on post-ganglionic neurons to elicit control (Guan, 2014).

1.4.2.3.5.9 GLP-2 and Intestinal Growth

GLP-2's role in intestinal growth has been well established pharmacologically, adapting to maximize surface area when the nutrient requirement is increased (Sherwood & Adams, 2004) (Fig.1.6). Studies involving native GLP-2 injection have further shown the contribution the hormone makes to intestinal growth. Injection of GLP-2 twice daily for 10 days in 8 week old females, resulted in significantly greater bowel weight and crypt-villus height than control mice; while glicentin, GLP-1, and IP-2 did not influence significant growth (Drucker et al., 1996). Greater small bowel weight with GLP-2 administration was seen after six days of treatment, while one-, two-, and four-day treatment did not demonstrate significantly greater weight compared to control mice (Drucker et al., 1996). Crypt-villus height was measured in the proximal and distal jejunum, along with the ileum, with all sections demonstrating greater height compared to vehicle treated mice (Drucker et al., 1996). These results imply that GLP-2 functions as a small bowel growth factor (Drucker et al., 1996). Villus height increases were also observed in Fischer rats treated with GLP-2; however, crypt depth was not increased. DPP4 mutant Fischer rats demonstrated significantly increased villus height and crypt depth, indicating that DPP4 is a determinant of GLP-2 bioactivity (Drucker et al., 1997).

The intestinotrophic potential of GLP-2, growth is not direct and occurs through its downstream mediators (Markovic & Brubaker, 2019). GLP-2 is involved in inducing growth by signaling through the GLP-2R on intestinal subepithelial myofibroblasts (ISEMF) (Dubé et al., 2006), which induces secretion of IGF-1 (Markovic & Brubaker, 2019). The necessity for IGF-1 action to increase growth was determined by knocking out the *Igf1r*; thus, in these conditions, GLP-2 administration alone was insufficient towards increasing microvillus growth (Markovic & Brubaker, 2019). IGF-1 has also been demonstrated to influence intestinal bowel growth and improve barrier function after intestinal resection (Drucker et al., 1996). Alongside IGF-1, activation of the GLP-2R on subepithelial myofibroblasts results in the release of IGF-2, epidermal growth factor (EGF), KGF, and transforming growth factor-β (Connor et al., 2016). Interestingly, EGF

synergistically enhances IGF-1's proliferative properties, and both are critical for GLP-2's intestinotrophic capabilities (Fesler et al., 2020). IGF-1 binds to its receptor, inducing β-catenin activation on intestinal epithelial cells, resulting in intestinal growth (Rowland et al., 2011). Mice lacking the intestinal epithelial IGF-1 receptor were treated with either EGF, hGly^{2}GLP-2, or a combination to determine if the growth factor influence GLP-2's intestinotrophic abilities in vivo (Fesler et al., 2020). Treatment of hGly^{2}GLP-2 alone resulted in increased crypt cell proliferation; however, co-administration of EGF and hGly^{2}GLP-2 impressively resulted in increased small bowel weight and villus height. Although growth was observed, far greater growth was observed in wildtype mice compared to mice lacking the IGF-1 receptor. These results underline the synergistic effects of intestinal growth factors and GLP-2 (Fesler et al., 2020).

1.4.2.3.6 Development of GLP-2R agonists for the Treatment of Short Bowel Syndrome

Short bowel syndrome (SBS) is the shortening of the small bowel due to intestinal resection, such that less than 200 cm of the small intestine remains (Donohoe & Reynolds, 2010). Due to the shortening of the bowel, there is a need for increased nutrient absorption, as malabsorption is a very profound outcome. Patients experience malabsorption, diarrhea, steatorrhea, dehydration, malnutrition, as well as electrolyte imbalance with accompanying weight loss (Guillen & Atherton, 2020). Management includes maintaining good nutritional status and maximizing absorptive surface area (Guillen & Atherton, 2020). There exist a few therapeutic approaches, one namely being a DPP4 resistant recombinant human GLP-2 analogue: teduglutide. This drug replaces alanine with position 2 glycine of the GLP-2 peptide, allowing for resistance to DPP4 cleavage, enhancing GLP-2 activity and increasing its half-life to 0.9-2.3 hours (Jeppesen et al., 2005). Teduglutide treatment for 21 days in patients with SBS resulted in decreased fecal wet weight, fecal energy excretion. Further, patients experience increased urine output, sodium absorption, villus height and crypt depth compared to baseline measurements, and, importantly, body weight. Wet weight absorption (difference in diet wet weight and

fecal wet weight) was increased with treatment, while fecal output was decreased in 15/16 SBS patients (Jeppesen et al., 2005). Compared to native GLP-2, teduglutide increased wet weight absorption threefold, and absolute energy absorption twofold, allowing several patients to be removed from parenteral nutrition and demonstrating its ability to drastically improve intestinal function in patients who suffer from SBS (Jeppesen et al., 2005).

1.4.2.3.7 Incretin Hormones and Thermogenesis

While the action of GIP in WAT is more well-defined, its role in BAT is just beginning to be explored**.** In HFD-fed *Gipr*$^{BAT-/-}$ mice, body weight and interscapular BAT (iBAT) weight were decreased, along with increased iBAT oxygen consumption at 4°C (Beaudry et al., 2019). These results were not seen in mice exposed to 21°C and 30°C; as body weight, energy expenditure, glucose and insulin tolerance were normal (Beaudry et al., 2019). Thermogenic and inflammatory-related gene expression significantly differed between the control and *Gipr*$^{BAT-/-}$ mice. Interleukin-6 (IL-6) mRNA expression (a proinflammatory cytokine responsible for the regulation of core body temperature) (Egecioglu et al., 2018) was markedly decreased in *Gipr*$^{BAT-/-}$ mice. Administration of GIP directly increased IL6 mRNA expression and IL-6 protein secretion in BAT cells; thus, linking BAT GIPR to the control of IL-6 (Beaudry et al., 2019; Timper et al., 2013). These results further define GIP's functional role in adipose tissue (Beaudry et al., 2019)**.**

Interestingly, GLP-1 is observed to stimulate BAT (Beiroa et al., 2014). As central nervous system (CNS) GLP-1 activates the GLP-1R in the hypothalamic ventromedial nucleus (VMH), dephosphorylation of AMP-activated protein kinase (AMPK) occurs, ultimately simulating BAT thermogenesis (Beiroa et al., 2014). Very few studies have observed the role in GLP-2 on adipose tissue; however, it is known that activation of its receptor facilitates anti-inflammatory effects on visceral adipose tissue (Ejarque et al., 2020), which may be beneficial under cold stress.

1.5 Scope of the Project

The gut has an important role in nutrient absorption and hormonal regulation in metabolic homeostasis, including energy balance, food intake, and glycemia and insulin secretion (Ussar et al., 2017). Many factors can positively modulate these functions; however, homeostatic imbalances can lead to various complications and gastrointestinal disorders. Stress is an acute threat to homeostasis (Konturek, 2011). Housing at 4-6˚C is a stress on whole-body homeostasis as large amounts of energy must be expended to maintain body temperature (Hylander & Repasky, 2016). The gut relies on digestion and absorption of nutrients to fuel the homeostatic processes (Vaanholt et al., 2009). In long-term stress conditions, the intestine can remodel or modify its morphology to regulate absorption and secretion function (Le Gall et al., 2019). Intimately linked to nutrient absorption and gut adaptation are the incretin hormones GLP-1 and GIP, along with GLP-2 (Koehler et al., 2015; Shin et al., 2005). However, how the secretion and subsequent receptor activation of GLP-1, GIP, and GLP-2 in response to housing at cold temperatures to mediate intestinal adaptation has not been determined. Using whole-body knockout mice, this project aims to identify the requirement of intact GLP-1R, GLP-2R and GIPR signaling in intestinal remodelling and overall metabolic changes in response to cold exposure. Given the overlapping roles and potential for compensation observed in single receptor knockout as described above, we chose to characterize models where both the GLP-1R and GIPR (DIRKO) and the GLP-1R and GLP-2R (GLPDRKO) are genetically eliminated.

1.6 Hypothesis

This thesis will address the following hypothesis: GLP-1, GIP and GLP-2 are released in proportion to required energy needs during cold exposure to enable efficient nutrient absorption and gut adaptation.

1.7 Specific Aims

1. Characterize incretin hormone secretion in response to chronic housing in the cold and assess differences in intestinal morphology to determine whether GLP1R/GLP2R/GIPR signaling contribute to intestinal growth (assessed by intestinal length and weight, histological analysis of intestinal circumference, villi length, and crypt depth).

2. Characterize the contribution of GLP1R/GLP2R and GIPR to the differences in glucose and lipid metabolism observed in cold-exposed mice during the fasting period and post prandial state.

3. Identify differences in metabolic demand with cold exposure by observing changes adipose tissue weight and plasma leptin levels.

2. Methods

2.1 Animals and Housing

Wildtype C57BL/6J were purchased from Jackson Laboratory. Double incretin receptor knockout (DIRKO) have been previously described (Hansotia et al., 2004), and glucagon-like peptide double receptor knockout (GLPRDKO) mice were generously provided by Dr. Daniel Drucker (Toronto, CAN). *Glp1r*$^{-/-}$ mice (Scrocchi et al., 1996) were crossed with *Glp2r*$^{-/-}$ mice to generate *Glp1r*$^{+/-}$*Glp2r*$^{+/-}$, then bred to generate *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ and wildtype littermate controls. Male and female wildtype, DIRKO, GLPDRKO mice were age, sex, weight, and fat matched (n=3-6) 20 weeks old (pilot study) (Table 1) or between 7-10 weeks old and 28-35 weeks old (genotype study). Mice were individually housed in temperature-controlled chambers set to 4-6˚C (cold) or 27˚C (thermoneutral), under a 12-hour light/dark cycle at the University of Ottawa Heart Institute. The weight of food consumed was calculated daily by the change in food mass from the day prior. All experiments undertaken were approved by the University of Ottawa Animal Care Committee (AUP 2909).

Table 1. Study summaries and structures used to assess gut remodeling and the associated incretin hormones in wildtype, DIRKO and GLPDRKO mice

Study	Study 1 Pilot Study	Study 2 Genotype Study
Genotype	Wildtype	Wildtype DIRKO (*Glp1r*$^{-/-}$*Gipr*$^{-/-}$) GLPDRKO (*Glp1r*$^{-/-}$*Glp2r*$^{-/-}$)
Age/Sex	Male	Male and female
Purpose	Evaluate plasma GLP-1, GIP levels and gut remodeling, comparing mice housed in 4ºC or 27ºC.	Fully characterize the gut remodeling and metabolic responses in males and females by disrupting the GLP-1R, GLP-2R, GIPR signalling.
Anticipated Outcomes	Mice housed at 4ºC will demonstrate greater villi length and intestinal circumference, along with greater total plasma GLP-1 and GIP levels.	• DIRKO mice (disruption of the GLP-1R/GIPR) will remain capable of increasing villi length and intestinal circumference in response to cold exposure. • GLPDRKO mice (disruption of the GLP-1R/GLP-2 receptor) will fail to increase villi length and intestinal circumference in response to cold exposure.

2.2 Mouse Genotyping

For genotype confirmation, genomic DNA was extracted from either ear-notch or tail samples and analyzed by polymerase chain reaction (PCR). The resulting bands were separated on a 1% agarose gel for 30 minutes at 150V and visualized using UV transillumination (Fig. 2.1).

Table 2**.** Primer Sequences Used to Genotype Wildtype, DIRKO and GLPDRKO Mice

	Forward	Reverse
GIPR Wildtype	AGT GTG AGA ATC CAG AGA AGA ATG G	CCA CGG TAT ACA TGA TCT GCA GGC G (COMMON)
GIPR Mutant	TAA AGC GCA TGC TCC AGA CTG CCT T	CCA CGG TAT ACA TGA TCT GCA GGC G (COMMON)
GLP-2R Wildtype	CTT AGC AGG GCA TGG TAC CAC (COMMON)	AGG GGG CAT CTC TCT ATT CTC
GLP-2R Mutant	CTT AGC AGG GCA TGG TAC CAC (COMMON)	TGC GCG GCC AGA GGC CAC TTG TGT AGC
GLP-1R Wildtype	GGG CAG GGC ATC AGA GAT TT	GCA GCC GTG CTA TAC ATC CA
Neomycin Resistance Cassette	GTC TTG TCG ATC AGG ATG ATC TG	CAA TAT CAC GGG TAG CCA ACG C

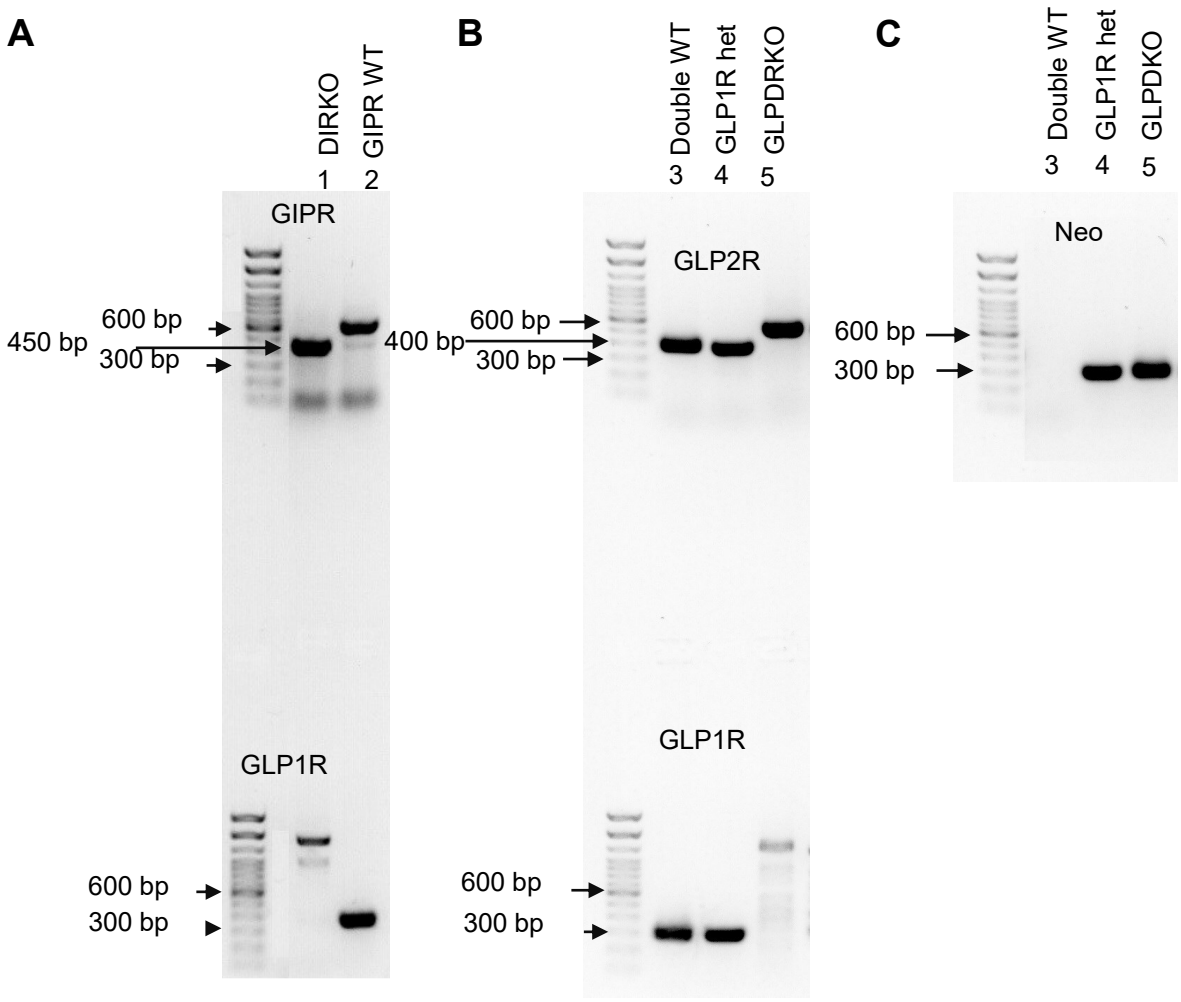


Figure 2.1. Genotyping of Double Incretin Receptor Knockout (DIRKO) and GLP1/2R Double Receptor Knockout (GLPDRKO) Mice.
(A-C): The genotype of the mice was confirmed by PCR. DIRKO mice (sample 1) were identified through the presence of the GIPR mutant allele at 450bp. Sample 2 was identified as a GIPR wildtype, as the wildtype allele was shown at 600bp. The GLP1R wildtype allele is shown at 300bp **(A)**. GLPDRKO mice were identified through the presence of the GLP2R wildtype allele at 400bp (sample 3), and mutant allele at 500bp (sample 4) and GLP1R wildtype allele at 300bp **(B)**. Genotyping of the double wildtype from both lines was confirmed by the absense of the neomycin resistance cassette (Neo), showing at 300bp, confirming sample 3 as a double wildtype, sample 4 a wildtype for GLP2R and heterozygous for GLP1R and sample 5 as a GLP1/2R double knockout **(C)**.

2.3 Body Composition

Body composition (adipose tissue, lean mass) was determined by EchoMRI scanning of individual mice (Fig. 2.2). Lean mass (g) consists of muscle tissue mass equivalent of all the body parts containing water, excluding fat, bone, hair, and claws.

2.4 Lipid Tolerance Test (LTT)

Mice were fasted for five hours and gavaged with 200µL of olive oil. Blood from the tail vein was collected from baseline (prior to gavage) and 1, 2, 3 hours after gavage in ethylenediaminetetraacetic acid (EDTA) coated capillary tubes, with the addition of 10% TED (vol/vol) (5000KIU/mL Trasylol,1.2mg/mL EDTA, and 0.1nmol/L Diprotin A) (DPP4 inhibitor) for GLP-1 and GIP measurements. Blood was collected at baseline, 1 hour, 2 hour, and 3 hours post olive oil gavage for measurement of triglycerides. Plasma triglyceride levels were measured following the manufacturer's protocol (TR22421, FisherScientific).

2.5 Incretin Hormone and Leptin Measurements

Total plasma GLP-1 levels (K15171C-1, MSD) and total plasma GIP levels (81527, Crystal Chem) were measured in plasma samples obtained prior to and 5-minutes post olive oil gavage, both following the manufacturer's protocol. Plasma leptin levels (90030, Crystal Chem) were measured in plasma samples from cardiac puncture at sacrifice.

2.6 Oral Glucose Tolerance Test (OGTT)

Mice were fasted for five hours fasted and gavaged with 2g/kg body weight glucose solution. Glucose concentrations from tail blood were measured at baseline (prior to gavage), 15, 30, 45, 60, 75 and 90 minutes post-gavage with a glucometer (Medisure). Blood was collected from baseline and 15 minutes in EDTA coated capillary tubes for insulin measurements. Plasma insulin levels (80-INSMSU-E10,

Alpco) were measured in plasma samples obtained before and 15 minutes after glucose administration.

2.7 Insulin Tolerance Test (ITT)

Five-hour fasted mice were injected with insulin intraperitoneally. Female mice received 0.4IU/kg and male mice received 0.5IU/kg. Glucose concentrations from tail vein blood were measured at baseline (prior to injection), 15, 30, 45, 60, 75 and 90 minutes post insulin injection.

2.8 Tissue Collection

Five-hour fasted mice were sacrificed by carbon dioxide asphyxiation, followed by cervical dislocation. Following cervical dislocation, blood was extracted by cardiac puncture. The liver, pancreas, inguinal subcutaneous adipose tissue (ingSAT), perigonadal visceral adipose tissue (pgVAT), and epididymal adipose tissue were weighed, divided, and placed in 4% paraformaldehyde (PFA) or flash frozen. The spleen was flash frozen. The small intestine was removed, cleaned, weighed, and sections of the duodenum, proximal jejunum, distal jejunum, and ileum were isolated (4 equal sections). Each section was flushed with ice-cold PBS, and further sectioned for RNA/protein isolation or histology. The large intestine was flushed with PBS, weighed, and the first centimeter was flash frozen. Tissue sections for histology were placed in PFA for 24 hours, then processed and embedded in paraffin blocks. Sections (10μm) were stained with H&E. Goblet cells present on villi were stained with Alcian blue (pH 2.5) and counterstained with Nuclear Fast Red, as previously described (Monk et al., 2017).

2.9 Software Analysis

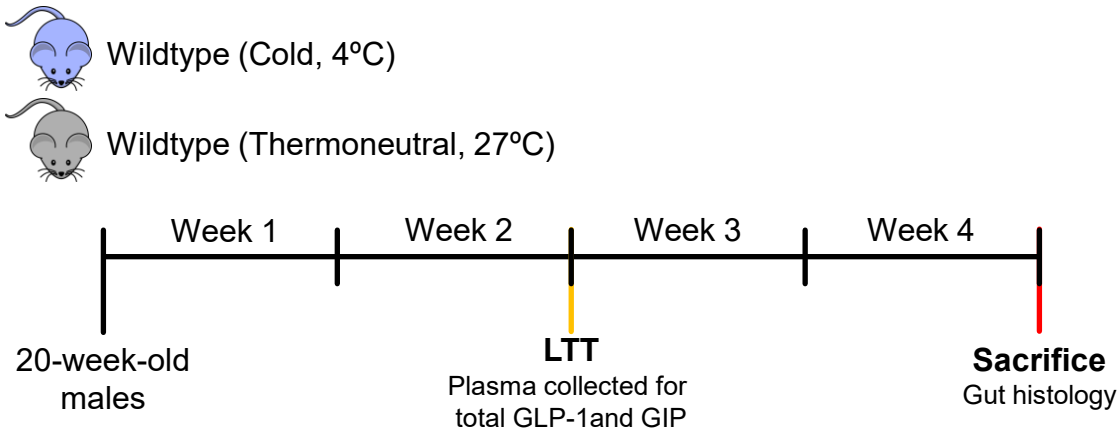
Histological measurements were performed using Aperio ImageScope 12.1. Each data point represents: the average intestinal circumferences of three sections per tissue, average length, and crypt depth from 40 villi per slide, and the average number of goblet cells per villus of 30 villi per slide. Each data point indicates each

mouse's value for plasma GLP-1/GIP, insulin, leptin, tissue weights/lengths, and EchoMRI data.

2.10 Statistical Analysis

Unless otherwise specified, results are expressed as the mean ± SEM. Statistical significance was calculated by two-way ANOVA with post-hoc Tukey test, or unpaired two-tailed Student’s T-test where appropriate using Graphpad Prism 9. Statistical significance was defined as $p<0.05$.

Study 1: Pilot Study



Study 2: Genotype Study

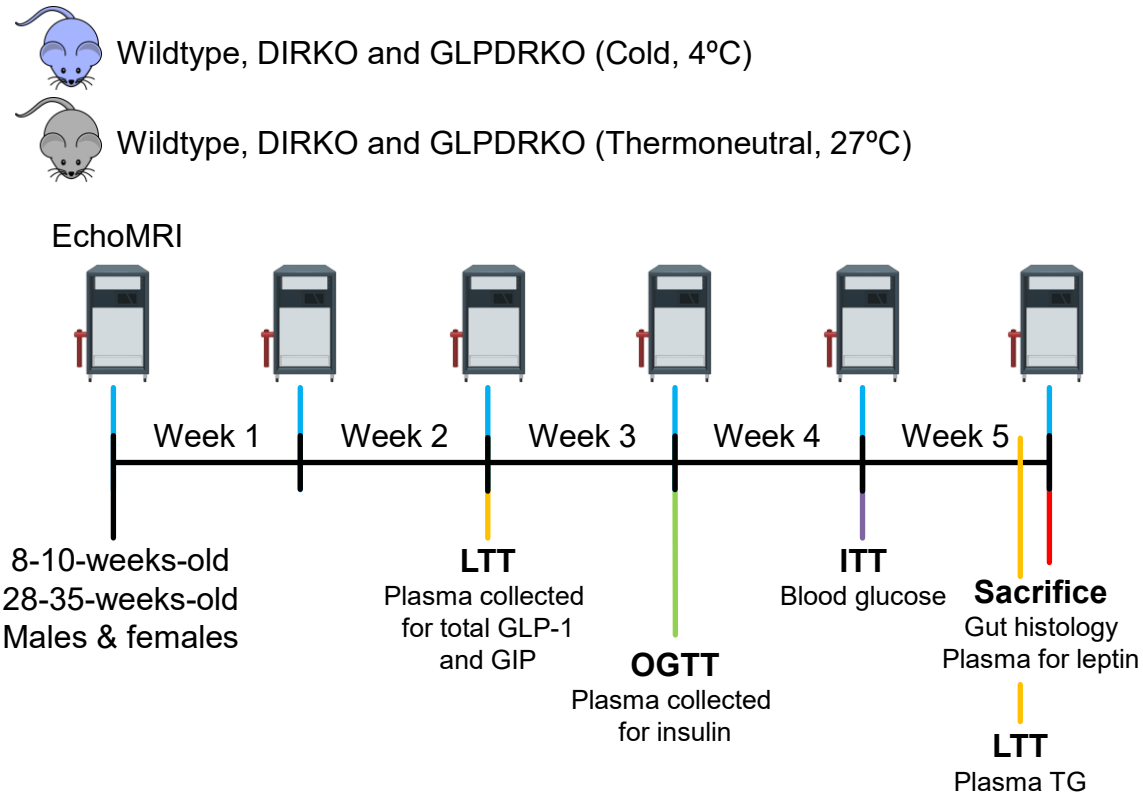


Figure 2.2. Male and Female Wildtype, DIRKO, GLPDRKO Mice are Exposed to either Cold (4ºC) or thermoneutral (27ºC) Housing Four or Five Weeks. Metabolic Tests are Conducted Once a Week in the Genotype Study.
Study 1: Pilot Study: Male mice are housed at 4ºC or 27ºC for four weeks, measuring total GLP-1 and GIP, along with analyzing intestinal histology.
Study 2: Genotype Study: Male and female mice are housed at 4ºC or 27ºC for five weeks, introducing various metabolic experiments.

3. Results

3.1. Intestinal Circumference and Villi Length Were Increased in 20-Week-Old Cold-Exposed Male Wildtype Mice

We performed a pilot study to reproduce previous reports of increased villi length and intestinal circumference in cold-exposed mice (Chevalier et al., 2015). Consistent with previous studies, we observed that intestinal circumference and villi length are increased with cold exposure (Fig.3.1e-g), while crypt depth remains similar between housing temperatures (Fig.3.1h). We additionally evaluated the concentrations of the incretin hormones under chronic cold stress. Plasma total GLP-1 and GIP levels are more significant at baseline and 5 minutes post-olive oil gavage in 20-week-old cold exposed male mice (Fig.3.1a-d).

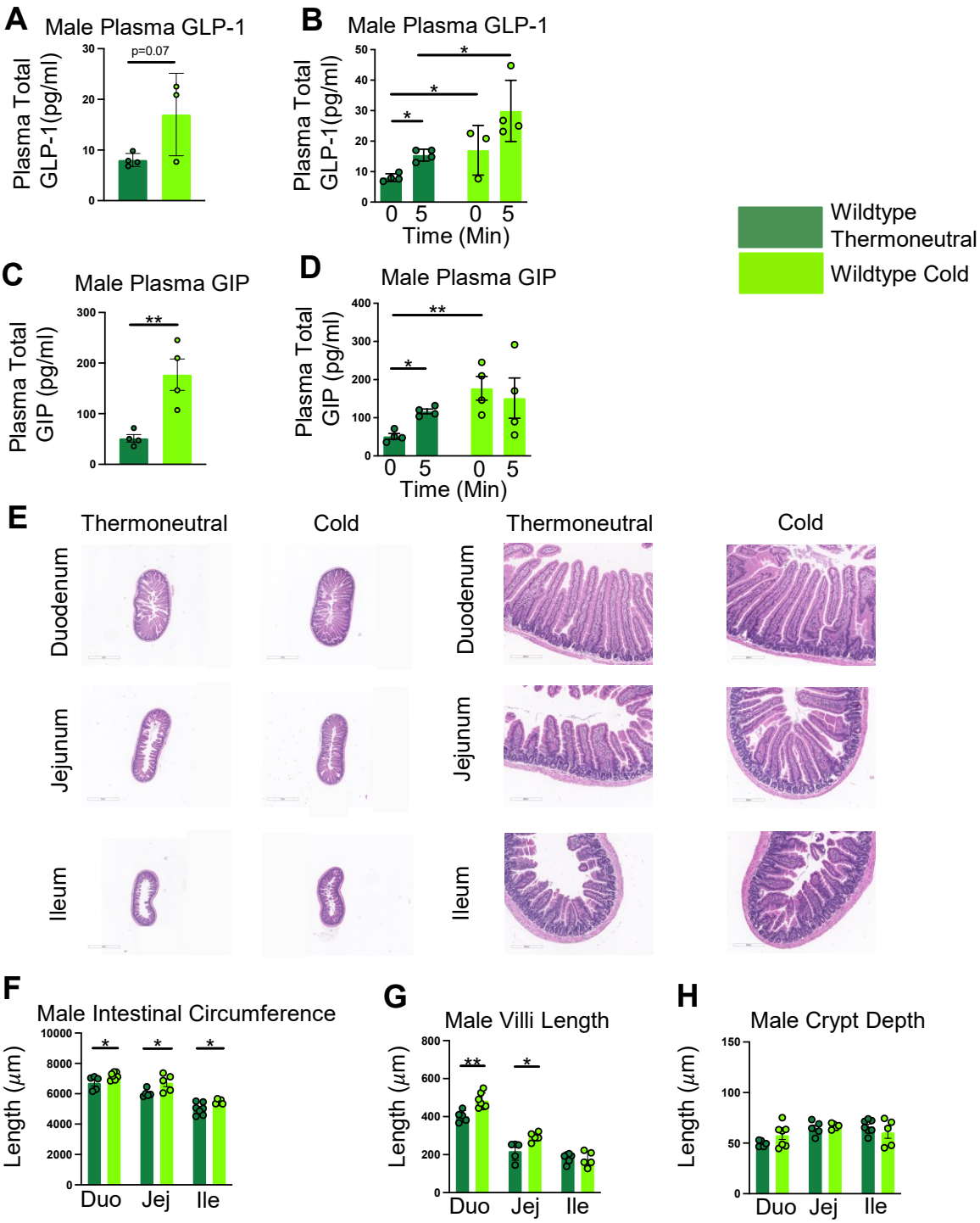


Figure 3.1. Intestinal Circumference and Villi Length Were Increased in 20-Week-Old Cold-Exposed Male Wildtype Mice.

(A-B) Plasma total GLP-1 measured at 0 **(A)**, 5-minutes **(B)** post-olive oil gavage in male mice fasted for 5 hours.

(C-D) Plasma total GIP measured at 0 **(C)**, 5-minutes **(D)** post-olive oil gavage in male mice fasted for 5 hours.

(E-H) Representative H&E staining of duodenum, jejunum, ileum **(E)**, with quantification of intestinal circumference **(F)**, villi length **(G)**, and crypt depth **(H)** after four weeks of cold exposure. Data are presented as mean ± SEM, analyzed by unpaired student's t-test or by two-way ANOVA, *p<0.05, **p<0.01.

3.2. Cold-Exposed Wildtype Mice Demonstrate Increased Food Intake, and Enhanced GLP-1 and GIP Levels

As GLP-1 (and presumably GLP-2) and GIP levels were increased during cold exposure (Fig.3.1a-d), we wanted further to understand the importance of the receptors on intestinal growth. Experiments involving cold exposed mice demonstrate an increased metabolic demand for nutrients and energy stores to compensate for increased energy expenditure (Chevalier et al., 2015). We measured food intake daily in 10-week-old wildtype mice over 3 weeks of chronic cold exposure. Consistent with Chevalier *et al*., cold-exposed male (Fig.3.2a) and female (Fig.3.2b) wildtype mice ate 2-fold, and 3-fold more food per day, respectively, in absolute grams, compared to thermoneutral housed mice. The incretin hormones, GLP-1 and GIP, are important in nutrient uptake and absorption (Diakogiannaki et al., 2012). During cold exposure, plasma total GLP-1 (Fig.3.2c) levels displayed a 1.7-fold increase and GIP (Fig.3.2e) levels displayed a 3.5- fold increase in male wildtype mice housed in chronic cold conditions when compared to thermoneutral housed mice. Female wildtype mice followed a similar trend for GLP-1 levels (Fig.3.2d), along with significantly greater GIP levels in cold exposure (Fig.3.2f). In cold housed female mice, plasma total GIP levels were increased by approximately 5-fold compared to levels in thermoneutral housed mice (Fig. 3.2f).

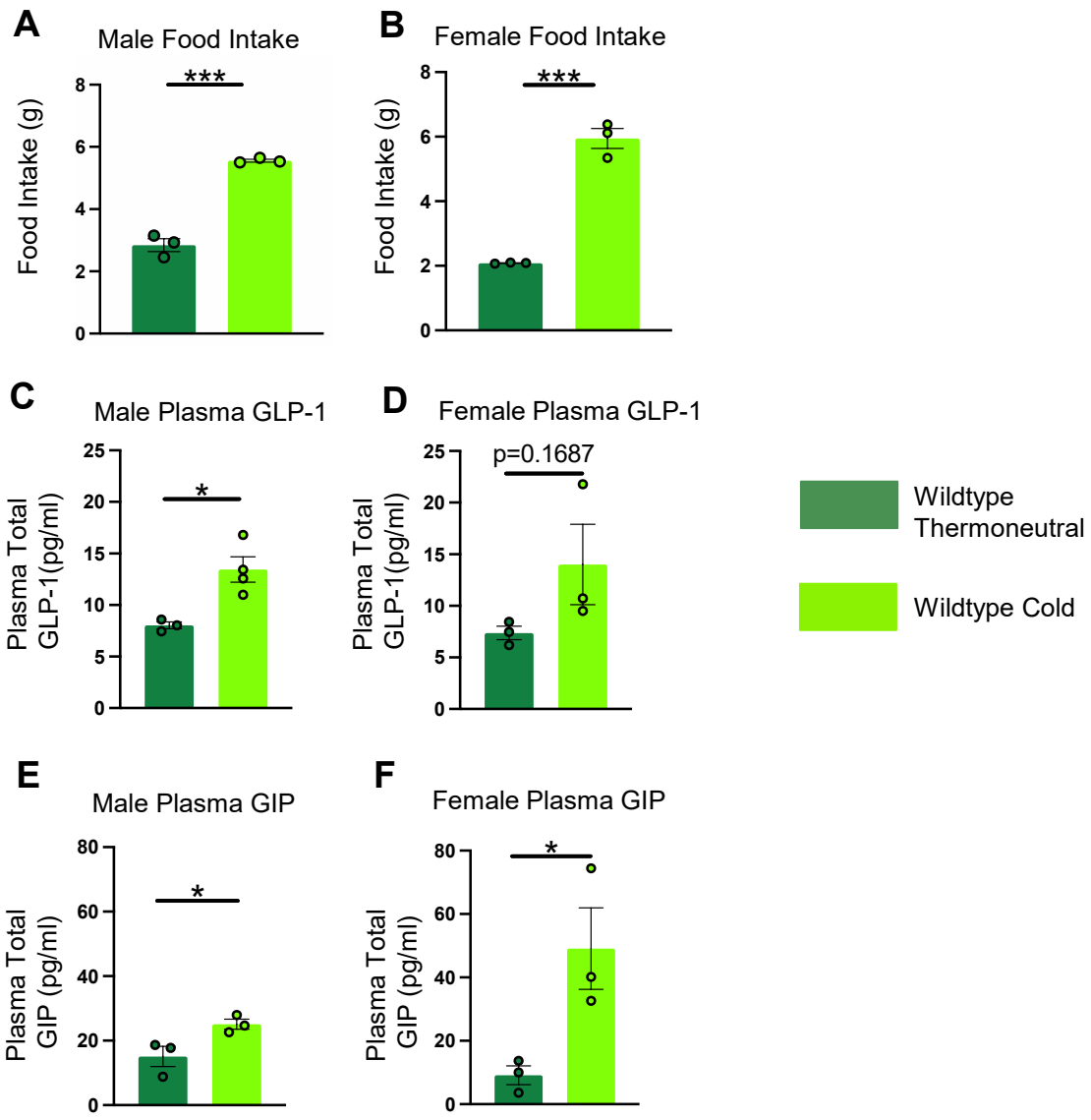


Figure 3.2. Cold-Exposed Wildtype Mice Demonstrate Increased Food Intake, and Enhanced Plasma Concentrations of GLP-1 and GIP.
(A-B) Food was weighed and expressed as an average of grams of chow consumed per day in male **(A)** and female **(B)** wildtype mice.
(C-D) Plasma total GLP-1 measured in male **(C)** and female **(D)** wildtype mice fasted for 5 hours.
(E-F) Plasma total GIP measured in male **(E)** and female **(F)** wildtype mice fasted for 5 hours.
Data are presented as mean ± SEM, analyzed by unpaired student's t-test, *p<0.05, ***p<0.001.

3.3. Cold-Exposed Wildtype Mice Demonstrate Increased Intestinal Circumference and Villi Length

Under cold temperatures, mice experience intestinal remodeling and an increase in villi length, as well as increased gut circumference and weight (Chevalier et al., 2015; Nilaweera & Speakman, 2018). To evaluate this relationship, we measured intestinal circumference, villi length, and crypt depth in 28-35-week-old wild-type mice housed in both cold and thermoneutral temperatures. After chronic housing in the cold, the intestinal circumference and villi length is significantly greater in these mice than their thermoneutral housed counterparts, consistent with previous studies (Chevalier et al., 2015) (Fig.3.3a-d). Male wildtype mice demonstrated significant increases in intestinal circumference in response to cold in the duodenum, jejunum, and ileum (Fig.3.3b). A significant 1.4-fold increase in villi length was observed in the duodenum and jejunum (Fig.3.3c). Following increased intestinal circumference and villi length, we also measured crypt depth; however, this was unchanged in cold exposed male wildtype mice (Fig.3.3d). Furthermore, small, and large bowel weight and length were increased with cold exposure in male (Fig.3.3e) and female (Fig.3.3f) mice. Food intake and intestinal growth were previously reported to increase with cold exposure (Chevalier et al., 2015); thus, our results reproduced these findings in wildtype mice. Our data suggest that wildtype mice respond to cold exposure and may play a role in regulating growth and nutrient absorptive capabilities.

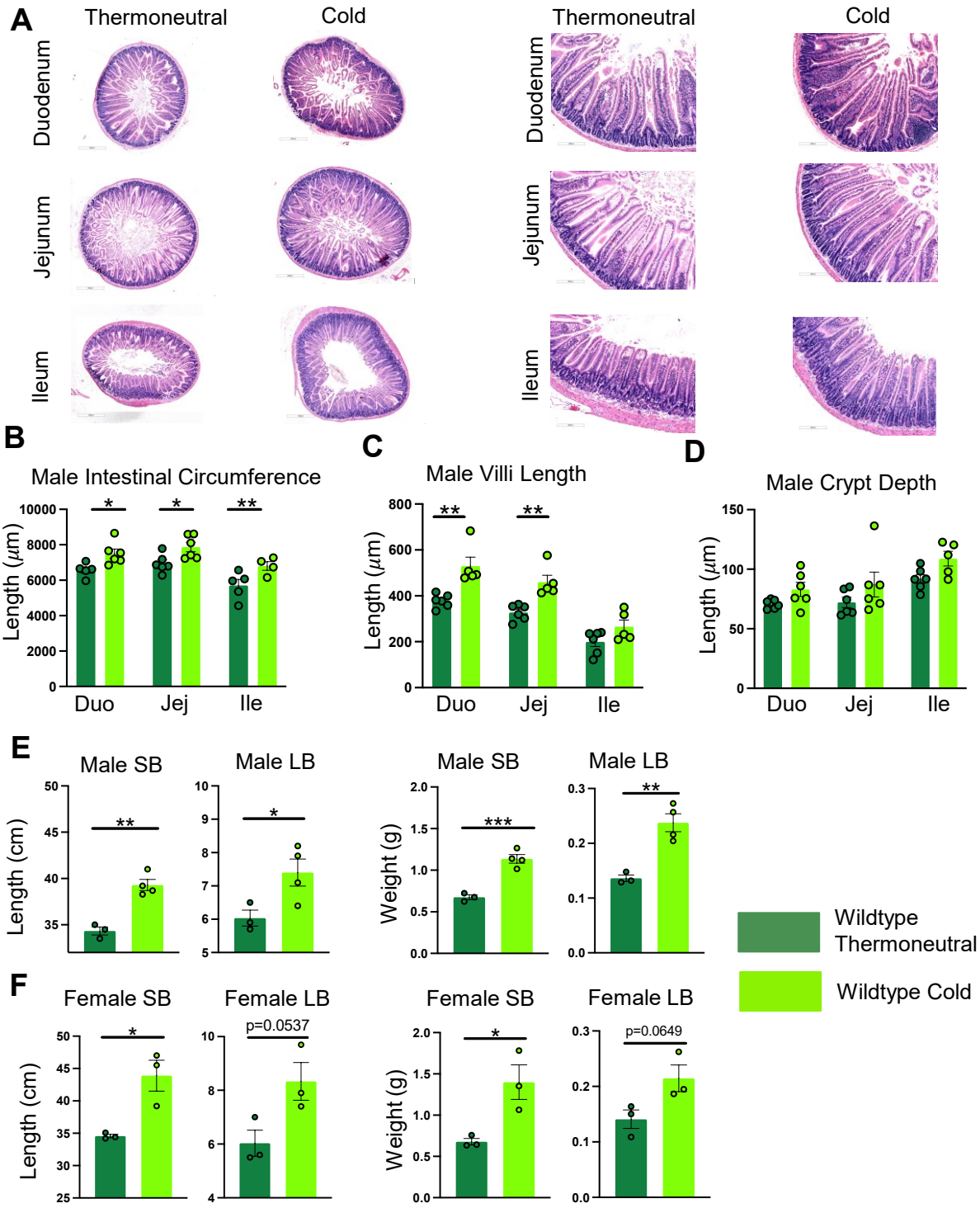


Figure 3.3. Wildtype Mice Housed at 4ºC Demonstrate Increased Intestinal Circumference and Villi Length.

(A-B) Representative hematoxylin and eosin **(**H&E) staining of duodenum, jejunum, ileum in male wildtype mice **(A)**, with quantification of intestinal circumference **(B)**, villi length **(C)**, and crypt depth **(D)** after four weeks of housing at 4ºC.

(E-F) Small bowel (SB), large bowel (LB) weight and length in male **(E)** and female **(F)** wildtype mice. Data are presented as mean ± SEM, analyzed by unpaired student's t-test or by two-way ANOVA, *p<0.05, **p<0.01.

3.4. Cold-Exposed DIRKO Mice Demonstrate Increased Food Intake

To determine whether GLP-1 and GIP receptor signalling is essential towards intestinal growth and other metabolic parameters with cold exposure, we evaluated DIRKO mice under the same conditions. Consistent with wildtype mice, DIRKO mice housed in the cold consumed a 2-fold and 3-fold greater absolute amount of chow diet per day in male (Fig.3.4a) and female (Fig.3.4b) mice, respectively. GLP-1 levels in both male (Fig.3.4c) and female (Fig.3.4d) mice were similar to levels in thermoneutral housed mice in the fasted state. Similarly, total plasma GIP levels do not differ significantly between housing temperature in both male (Fig.3.4e), and female (Fig.3.4f) mice. DIRKO mice lack the GLP-1 receptor; thus, may not increase secretion of GLP-1 and GIP in response to cold exposure.

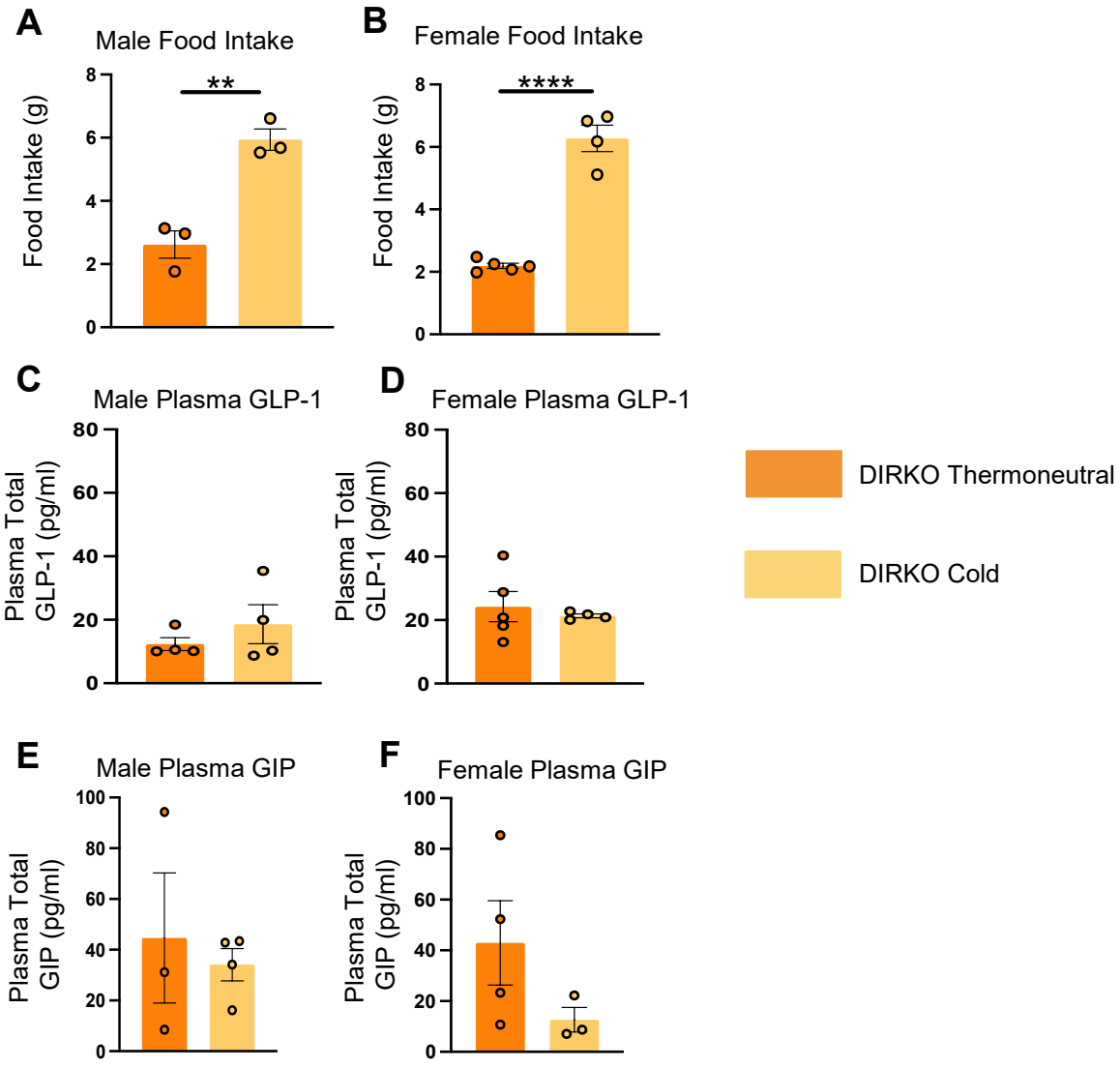


Figure 3.4. Cold-Exposed DIRKO Mice Demonstrate Increased Food Intake.
(A-B) Food was weighed and expressed as an average of grams of chow consumed per day in male **(A)** and female **(B)** DIRKO mice.
(C-D) Plasma total GLP-1 measured at baseline in male **(C)** and female **(D)** DIRKO mice fasted for 5 hours.
(E-F) Plasma total GIP measured at baseline in male **(E)** and female **(F)** DIRKO mice fasted for 5 hours. Data are presented as means ± SEM, analyzed by unpaired student's t-test, **p<0.01, ****p<0.0001.

3.5. Cold-Exposed DIRKO Mice Demonstrate Increased Food Intake and Increased Intestinal Circumference and Villi Length

Although hormone levels were not significantly different between housing temperatures in DIRKO mice (Fig.3.4), we observed increases in intestinal circumference and villi length (Fig.3.5), similarly to wildtype mice (Fig.3.1) in response to chronic cold exposure. Intestinal circumference significantly increased in the duodenum, jejunum, and the ileum of cold exposed DIRKO mice compared to mice in thermoneutral housing (Fig.3.5b). Villi length in the duodenum, jejunum, and ileum were 1.2, 1.5, 1.4-fold larger in cold exposed male mice, respectively (Fig.3.5c). In parallel with wildtype mice, cold exposed DIRKO mice exhibit only modest differences in crypt depth (Fig.3.5d). Small and large intestinal weight and length are greater in male (Fig.3.5e) and female (Fig.3.5f) mice exposed to chronic 4-6°C, while small bowel length and large bowel weight follow this trend in male mice. Although there is a disruption in incretin receptor action, DIRKO mice remain capable of increasing intestinal area in response to cold.

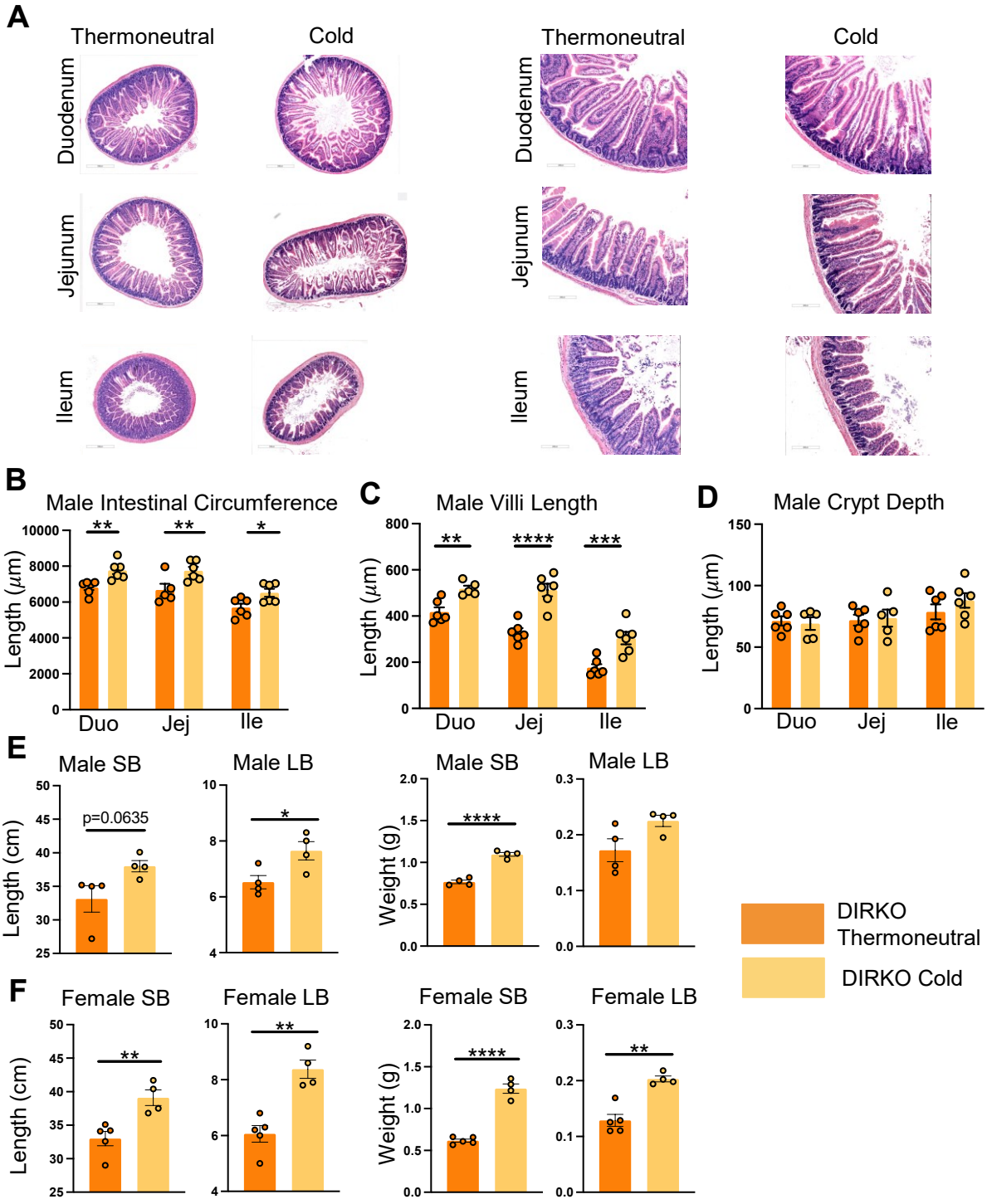


Figure 3.5. Cold-Exposed DIRKO Mice Demonstrate Increased Intestinal Circumference and Villi Length.

(A-B) Representative H&E staining of duodenum, jejunum, ileum in male DIRKO mice **(A)**, with quantification of intestinal circumference **(B)**, villi length **(C)**, and crypt depth **(D** after four weeks of housing at 4ºC.

(E-F) Small bowel (SB), large bowel (LB) weight and length in male **(E)** and female **(F)** DIRKO mice. Data are presented as mean ± SEM, analyzed by unpaired student's t-test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

3.6. GLPDRKO Mice Housed at 4˚C Demonstrate Increased Food Intake, and Increased GLP-1 Levels

As the GLP-2R is mainly responsible for intestinal growth (Bahrami et al., 2010), we further demonstrated the roles of the GLP-1R, GLP-2R by using GLPDRKO mice, which lack the GLP-1R and GLP-2R. Consistent with wildtype and DIRKO mice, food intake was 2-fold and 3-fold increased in male (Fig.3.6a) and female (Fig.3.6b) mice housed in cold temperatures. Plasma GLP-1 levels were 3-fold and 6-fold greater in male (Fig.3.6c) and female (Fig.3.6d) GLPDRKO mice housed at 4-6˚C. In contrast, total plasma GIP levels were similar between housing temperature in both male (Fig.3.6e) and female (Fig.3.6f) mice.

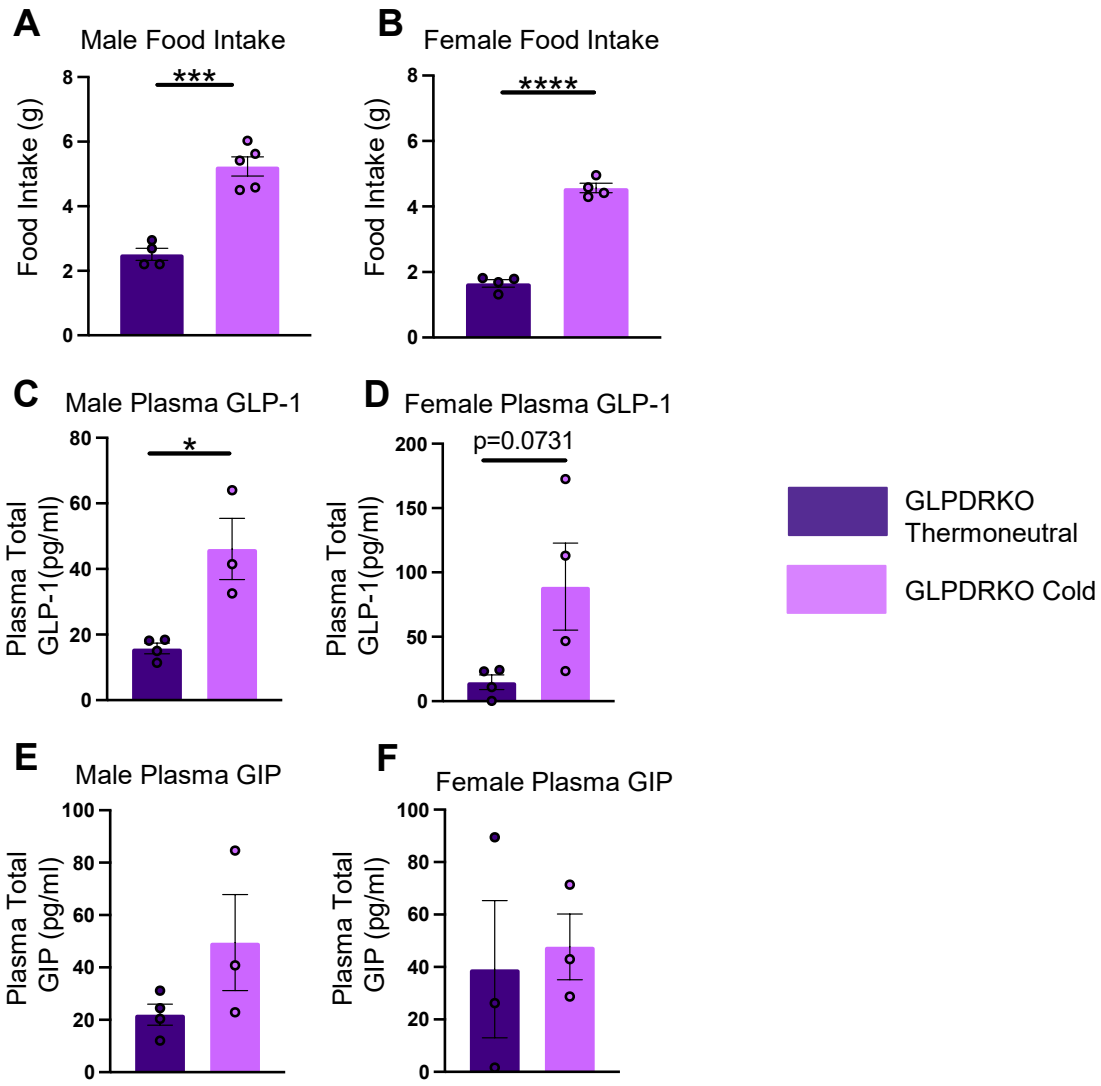


Figure 3.6. GLPDRKO Mice Housed at 4˚C Demonstrate Increased Food Intake, and Increased GLP-1 Levels.
(A-B) Food was weighed and expressed as an average of grams of chow consumed per day in male **(A)** and female **(B)** GLPDRKO mice.
(C-D) Plasma total GLP-1 measured at baseline in male **(C)** and female **(D)** GLPDRKO mice fasted for 5 hours.
(E-F) Plasma total GIP measured at baseline in male **(E)** and female **(F)** GLPDRKO mice fasted for 5 hours. Data are presented as mean ± SEM, analyzed by unpaired student's t-test, *p<0.05, ***p<0.001, ****p<0.0001.

3.7. Absence of the GLP-2R Impairs Intestinal Adaptation to Cold Exposure

In contrast to wildtype and DIRKO mice, GLPDRKO mice housed in the cold did not exhibit any significant differences in intestinal circumference (Fig.3.7b), villi length (Fig.3.7c), and crypt depth (Fig.3.7d), compared to mice housed in thermoneutral conditions. Interestingly, small, and large intestinal weight and lengths were significantly greater in cold exposed male (Fig.3.7e) and female (Fig.3.7f) mice versus thermoneutral housed mice. These results demonstrate that GLPDRKO mice retained modest increases in intestinal weight; however, they fail to increase villi length and intestinal circumference, suggesting the importance of the GLP-2R as a dominant mediator of intestinal remodeling in response to housing in chronic cold.

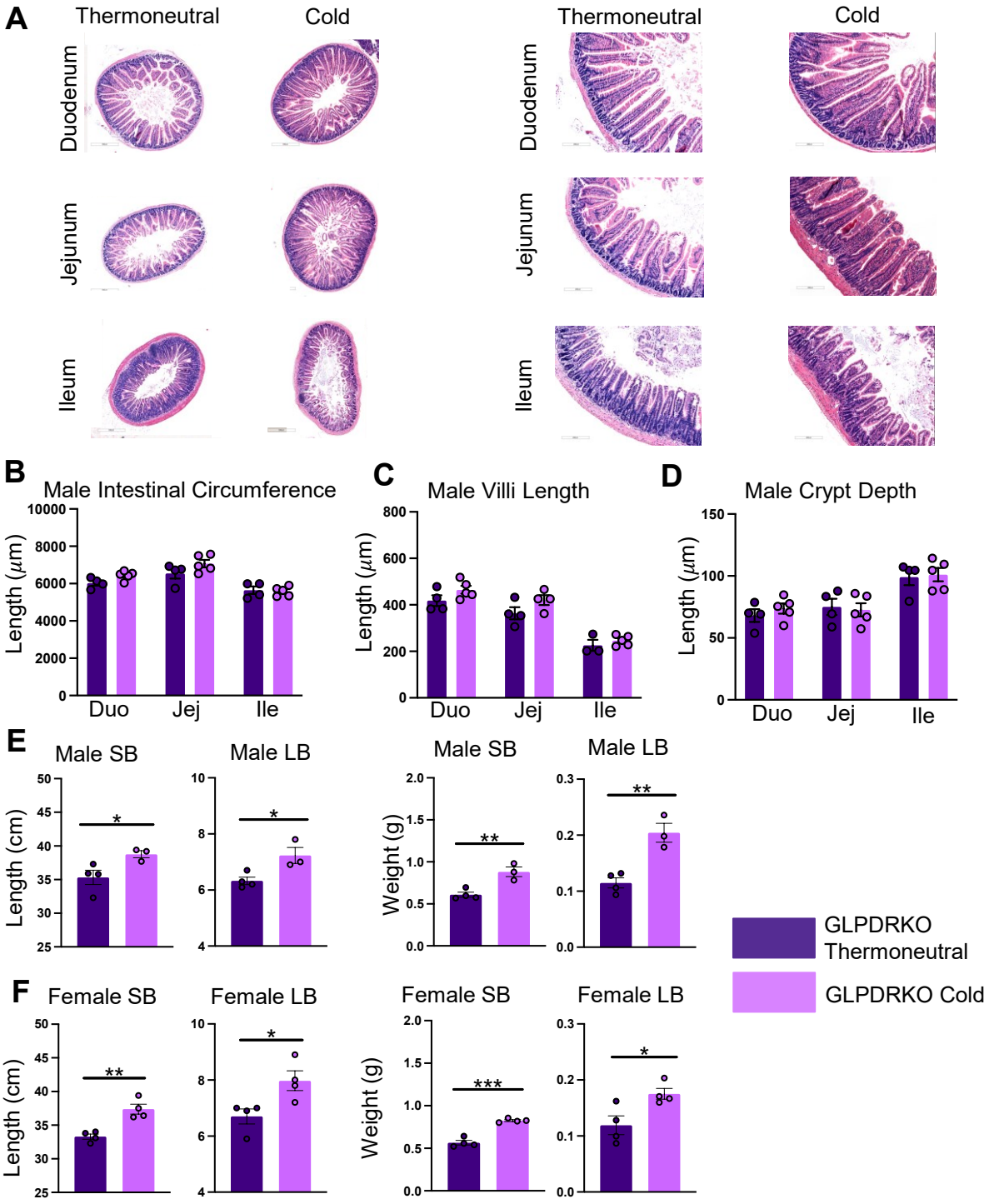


Figure 3.7. Absence of the GLP-2R Impairs Intestinal Adaptation to Cold Exposure.
(A-D) Representative H&E staining of duodenum, jejunum, ileum in male GLPDRKO mice **(A)**, with quantification of intestinal circumference **(B)**, villi length **(C)**, and crypt depth **(D)** after four weeks of housing at 4ºC.
(E-F) Small bowel (SB), large bowel (LB) weight and length in male **(E)** and female **(F)** GLPDRKO mice. Data are presented as mean ± SEM, analyzed by unpaired student's t-test, *p<0.05, **p<0.01, ***p<0.001.

3.8. Regulation of Body Weight During Chronic Cold Exposure

As the preproglucagon peptides have been involved with the control of body weight (Boguszewski, 2015; Burrin et al., 2001), mice were weighed weekly to determine whether GLP-1, GLP-2, and GIP receptor signalling impacts body weight under chronic cold stress. Although food intake was shown to be increased in wildtype, DIRKO and GLPDRKO mice (Fig.3.1, 3.4 and 3.6), body weight did not differ significantly between housing temperatures (Fig.3.8). To compare between genotype, the average body weight of male wildtype and DIRKO mice was about 30g, while the average was between 20-25g in GLPDRKO mice (Fig.3.8a). Similarly, female GLPDRKO weighed less than female wildtype and DIRKO mice (Fig.3.8b). These results suggest that the GLP-2R is linked to the control of body weight.

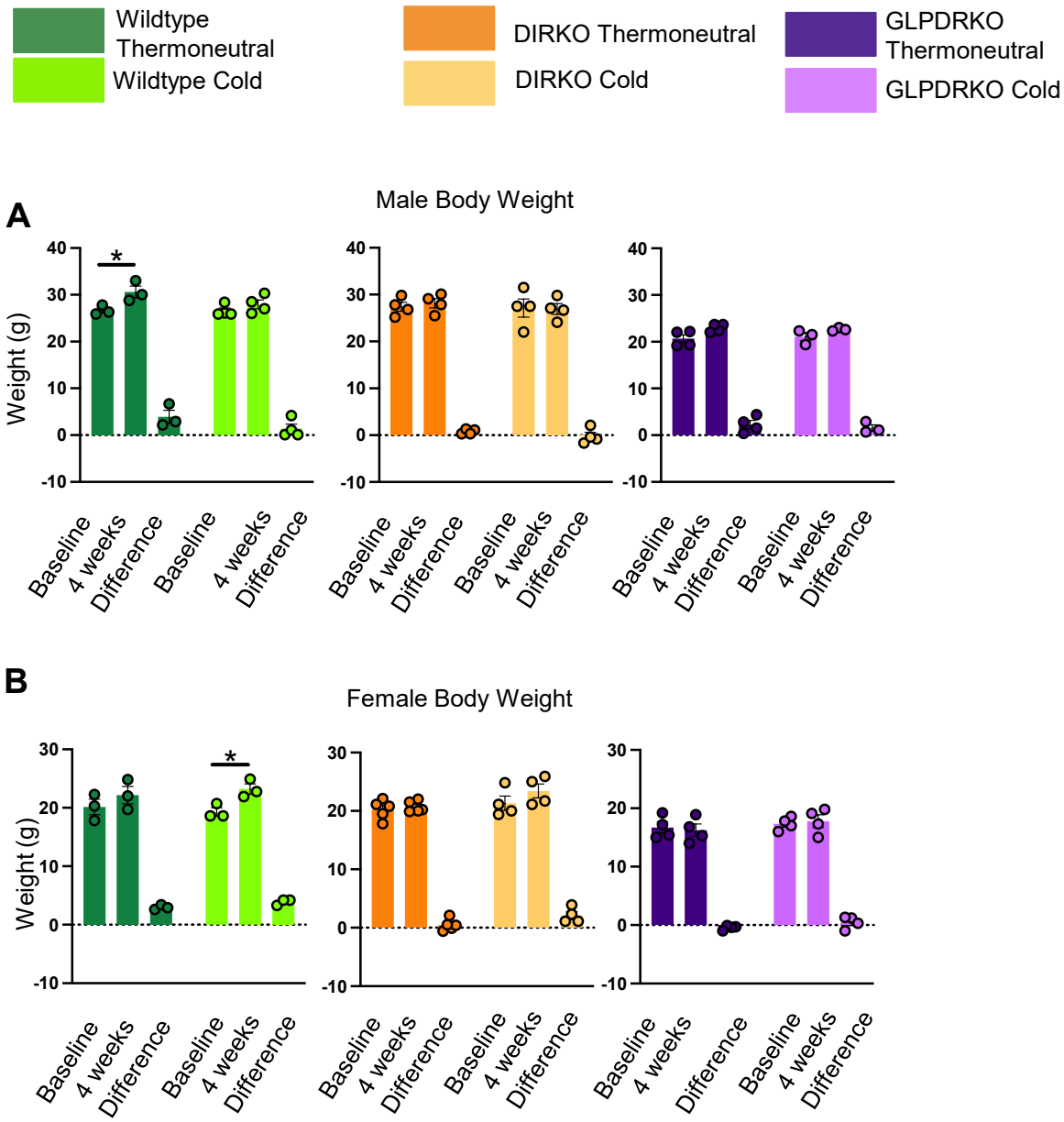


Figure 3.8. Regulation of Body Weight During Chronic Cold Exposure.
(**A-B**) Body weight of male **(A)** and female **(B)** mice measured weekly, and expressed as difference between baseline and after four weeks of cold exposure. Data are presented as mean ± SEM, analyzed by two-way ANOVA, *p<0.05.

3.9. Regulation of Lean Mass During Chronic Cold Exposure

As body weight was similar between housing temperatures and lower in GLPDRKO male mice, we also evaluated lean mass. Quantities of lean mass demonstrated a similar trend (Fig.3.9a). Unlike male mice, lean mass of female wildtype and DIRKO mice was significantly greater with cold exposure after four weeks (Fig.3.9b). These results suggest that the lack of the GLP-2R impacts lean mass, particularly in female mice.

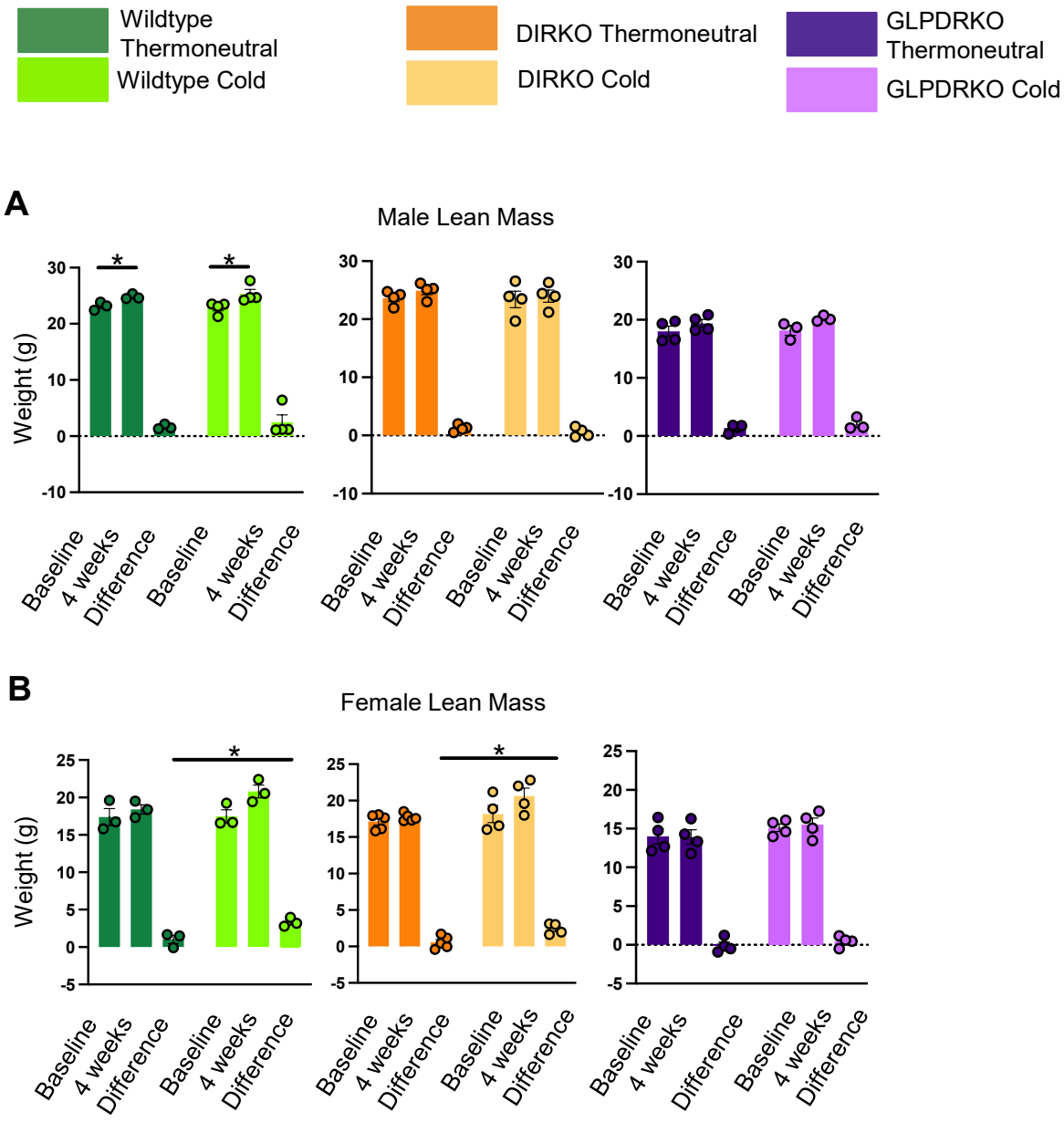


Figure 3.9. Regulation of Lean Mass During Chronic Cold Exposure.
(A-B) Lean mass of male **(A)** and female **(B)** mice determined by EchoMRI, expressed as difference between baseline and after four weeks of cold exposure. Data are presented as mean ± SEM, analyzed by unpaired two-way ANOVA, *p<0.05.

3.10. Postprandial Plasma GLP-1 and GIP Levels are Significantly Greater in Cold-Exposed Male Wildtype Mice

As baseline GLP-1 and GIP levels were shown to be greater in cold-exposed mice (Fig. 3.1,3.2), levels were measured 5 minutes post-olive oil gavage to evaluate post-prandial incretin secretion (Fig.3.10). GLP-1 levels are greater in cold exposed wildtype mice compared to thermoneutral exposed mice (Fig.3.10a,b). Compared to wildtype and DIRKO mice, GLPDRKO mice demonstrate greater GLP-1 levels (Fig.3.10a,b). Total GIP levels are greater with cold exposure only in male wildtype mice (Fig.3.10c), and trend towards increasing in females (Fig.3.10d).

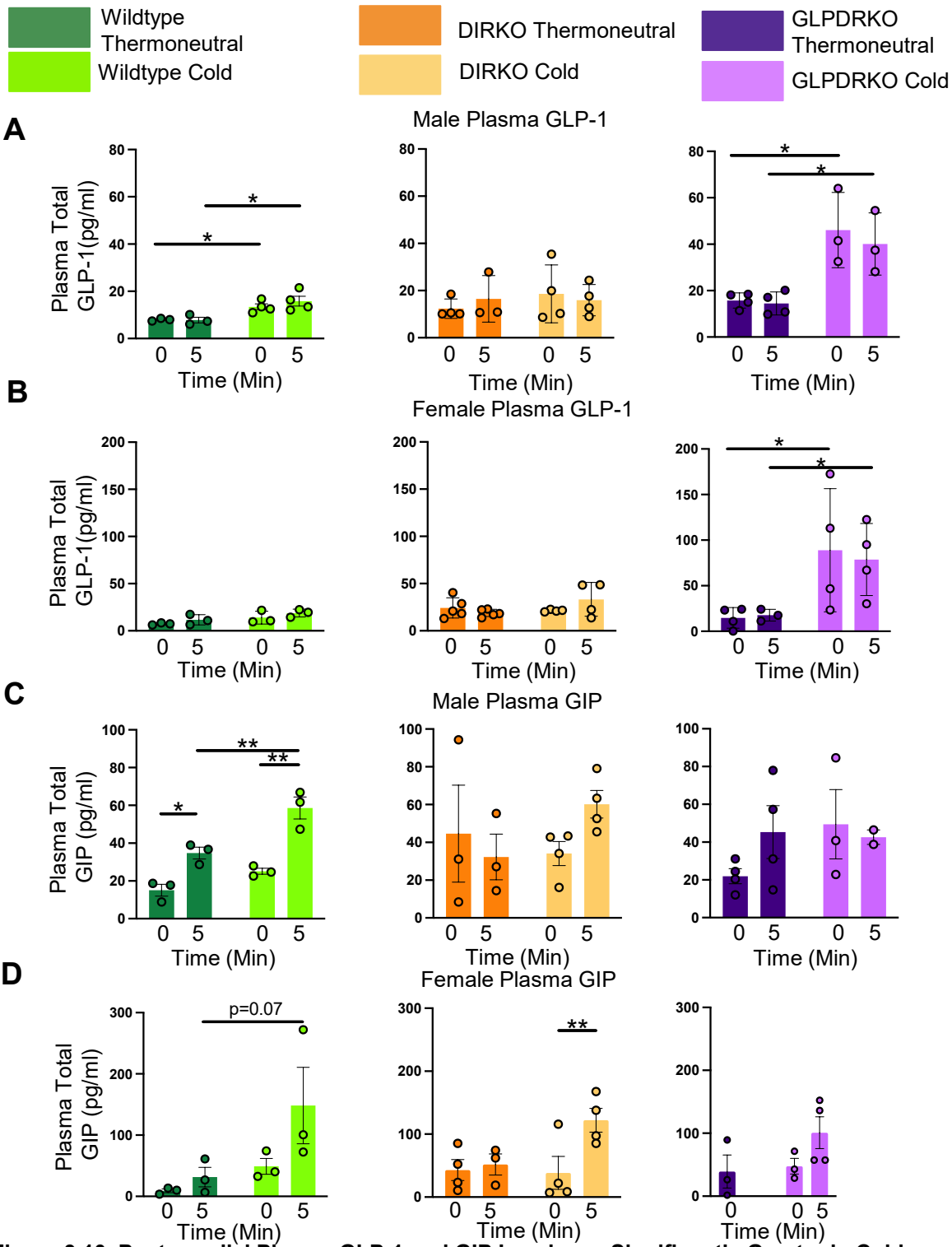


Figure 3.10. Postprandial Plasma GLP-1 and GIP Levels are Significantly Greater in Cold-Exposed Male Wildtype Mice

(A-B) Plasma total GLP-1 measured at 0 and 5 minutes post-olive oil gave in male **(A)** and female **(B)** mice fasted for 5 hours.

(C-D) Plasma total GIP measured at 0 and 5 minutes post-olive oil gave in male **(C)** and female **(D)** mice fasted for 5 hours, thermoneutral GLPDKO 5 minutes is incomplete. Data are presented as mean ± SEM, analyzed by two-way ANOVA, *p<0.05, **p<0.01.

3.11. Cold Exposed Wildtype and DIRKO Mice Demonstrate Greater Small Bowel Weight Compared to GLPDRKO Mice

As the intestine adapts to cold exposure by increasing intestinal circumference and villi length (Fig.3.1 and3.5), small bowel weight was also increased, particularly in wildtype and DIRKO mice (Fig.3.11). These increases are specific to genotype, as small bowel weight is significantly greater in wild-type and DIRKO mice than GLPDRKO mice. Although GLPDRKO retains some ability to grow, the small bowel weight was 1.3-fold lighter than male wildtype and DIRKO mice (Fig.3.11a), while 1.7 and 1.4-fold lighter than female wildtype and DIRKO mice respectively (Fig.3.11b). Together, these data suggest that the activation of the GLP-2R is a dominant mediator of intestinal remodeling, as mice lacking the GLP-2R fail to increase villi length and intestinal circumference in response to cold.

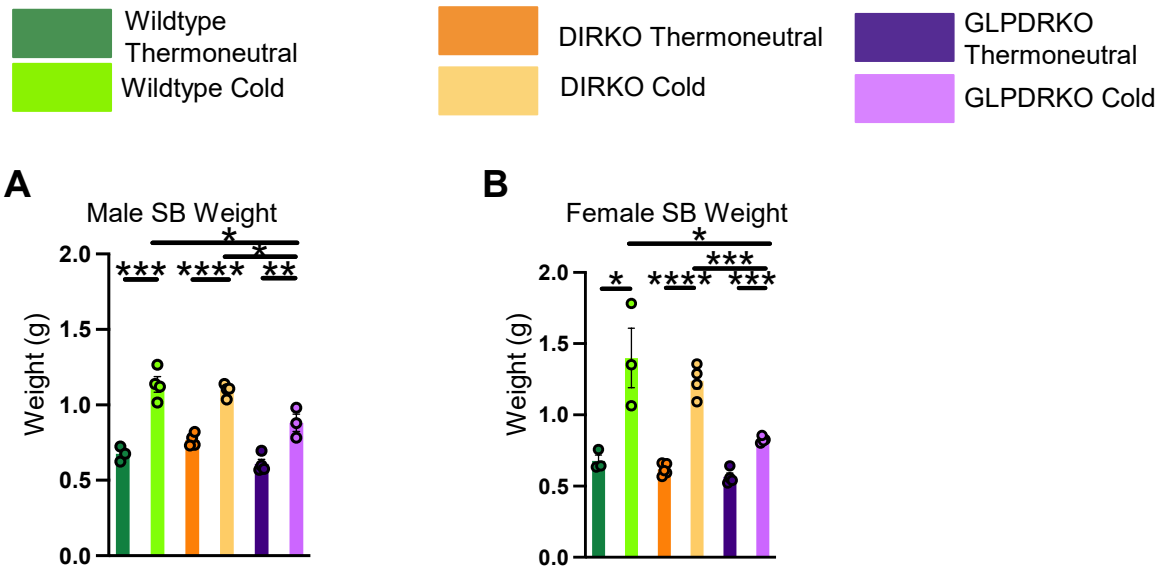


Figure 3.11. Cold-Exposed Wildtype and DIRKO Mice Demonstrate Greater Small Bowel Weight Compared to GLPDRKO Mice.
(A-B) Small bowel (SB) weight in male **(A)** and female **(B)** wildtype mice. Data are presented as mean ± SEM, analyzed by two-way ANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

3.12. Goblet Cell Number Per Villus is Greater in Male GLPDRKO Mice Compared to Male Wildtype and DIRKO Mice

Mucin produced and secreted from goblet cells plays a role in protecting the intestinal epithelium and has been suggested to be a lubricant, enhancing food passage through the intestine (Kong et al., 2018). Cold exposure led to an increase in villi length in wildtype and DIRKO mice, the cell types present on villi may shift in response. Interestingly, goblet cells counted per villi did not differ with cold exposure in wildtype and GLPDRKO mice (Fig.3.12a,c), while goblet cell numbers were greater with cold-exposed DIRKO mice (Fig. 3.12b). Although villi length was not increased in cold exposed GLPDRKO mice, goblet cell number was significantly greater in thermoneutral and cold exposed GLPDRKO mice compared to wildtype and DIRKO mice in both the duodenum and the ileum (Fig.3.12b).

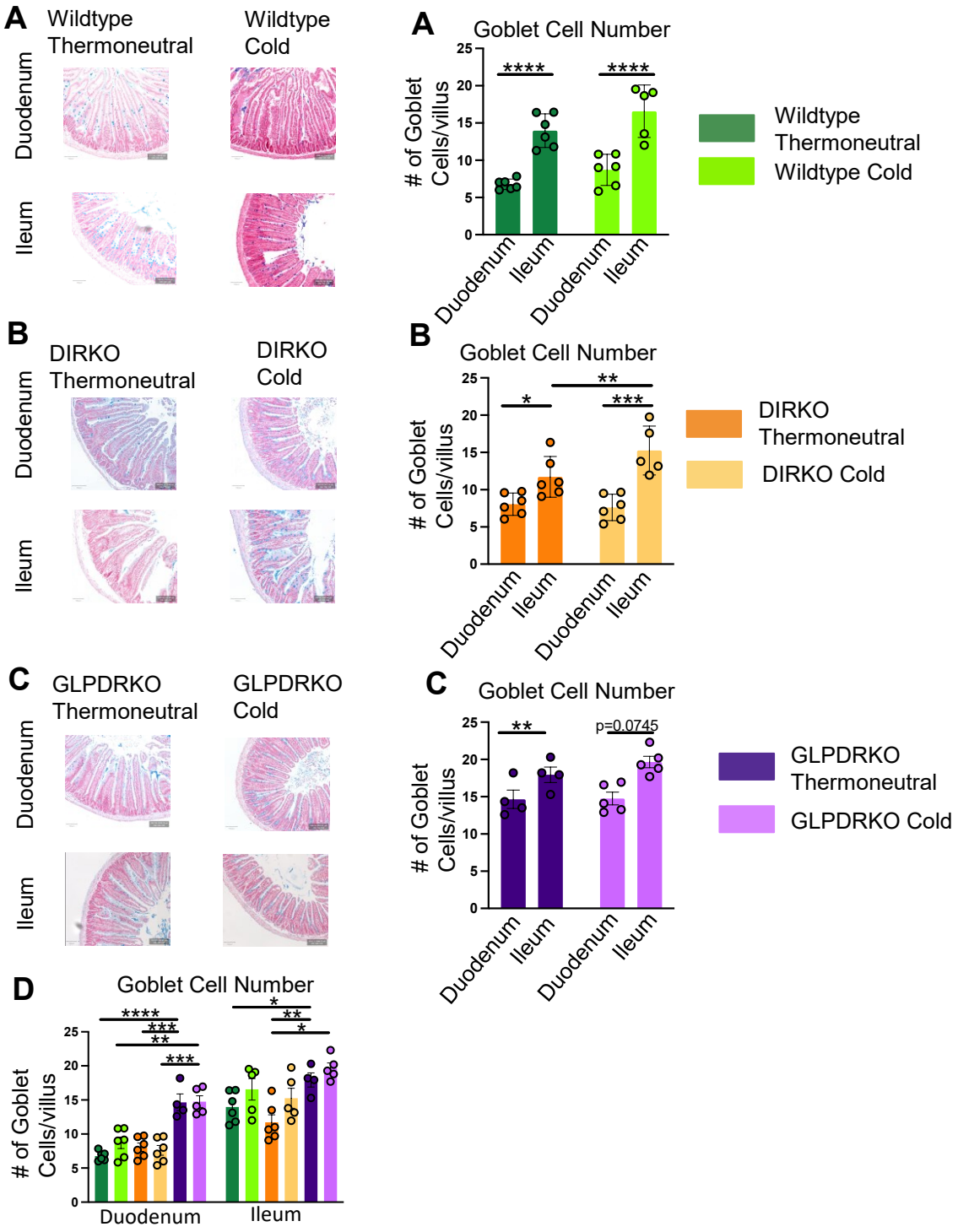


Figure 3.12. Goblet Cell Number Per Villus is Greater in Male GLPDRKO Mice Compared to Male Wildtype and DIRKO Mice.

(A-D) Duodenum and ileum sections were stained with Alcian blue to identify goblet cells in male wildtype **(A)**, DIRKO **(B)**, and GLPDRKO **(C)** mice, where number of goblet cells were averaged per villus.

(D) male wildtype, DIRKO, and GLPDRKO mice, number of goblet cells were averaged per villus. Data are presented as mean ± SEM, analyzed by two-way ANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

3.13. Cold Exposure Improves Glucose Tolerance, While Failing to Improve Insulin Tolerance

Cold acclimation improves glucose tolerance (Chevalier et al., 2015; Dudele et al., 2015), and is also influenced by GLP-1 and GLP-2 (Amato et al., 2016; Scrocchi et al., 1996). Consistent with this, glucose tolerance was improved with cold exposure of female mice of all genotypes (Fig.3.13b). Consistent with the females, male mice (Fig.3.14a) exposed to cold follow a similar trend; however, these differences are not statistically significant. Incretin hormones are both important glucostats necessary to keep post-prandial blood glucose levels stable through glucose-stimulated insulin secretion (Adriaenssens et al., 2019). Plasma insulin levels were measured in the fasting state, where levels do not differ significantly between housing temperature or genotypes in both male (Fig.3.13c) and female (Fig.3.13d) mice. Along with glucose tolerance and plasma insulin levels, insulin sensitivity has been shown to be improved in cold exposed mice (Chevalier et al., 2015). Unexpectedly, cold exposure failed to improve insulin sensitivity in male (Fig.3.13e) and female (Fig.3.13f) mice of all genotypes. These results suggest that GLP-1 and GIP plasma concentrations are greater with cold exposure while also demonstrating improved glucose tolerance, but not insulin sensitivity.

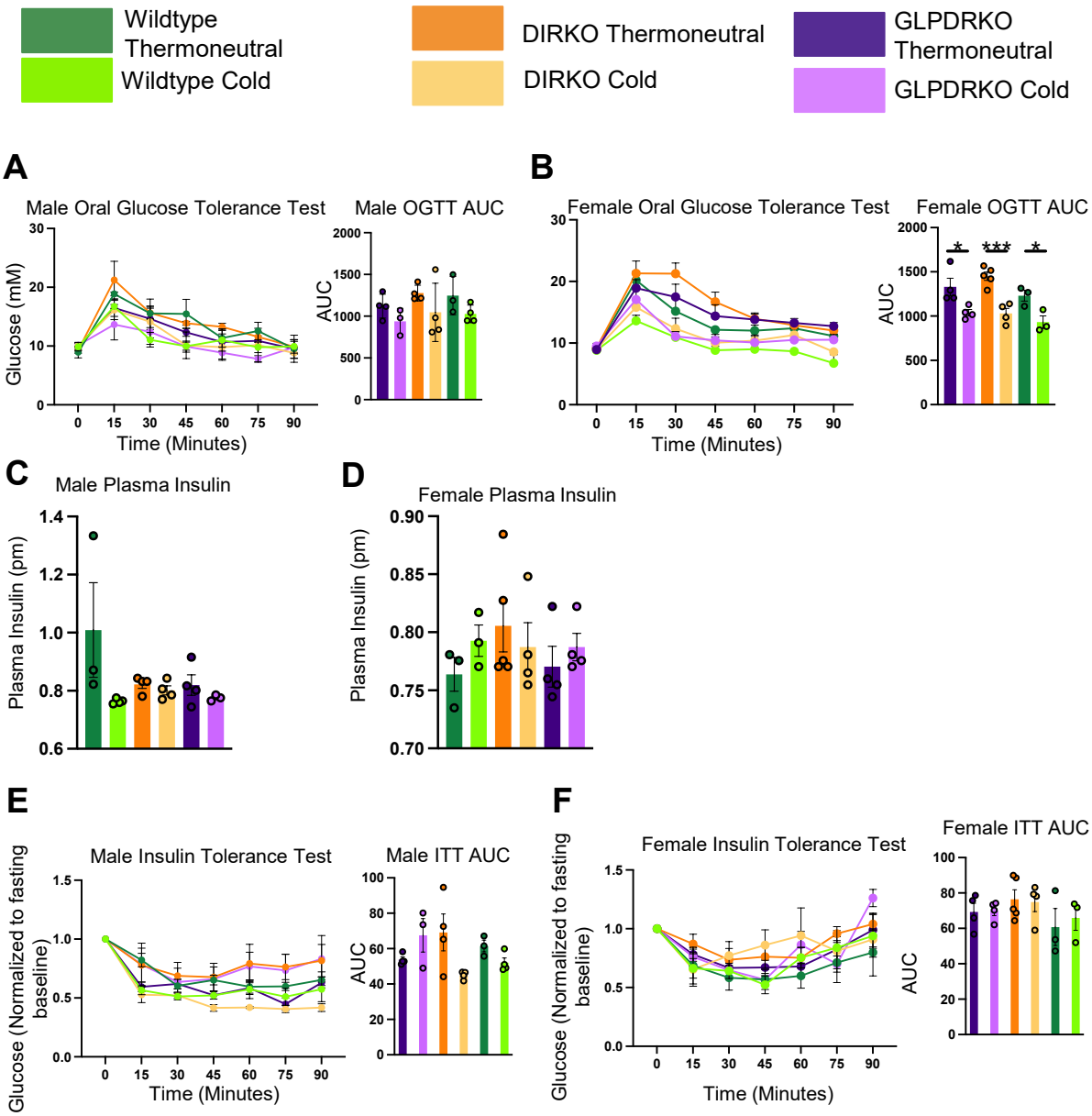


Figure 3.13. Cold Exposure Improves Glucose Tolerance, While Failing to Improve Insulin Tolerance.
(A-B) Oral glucose tolerance test (OGTT). Blood glucose taken 0-90 minutes after administration of oral glucose in male **(A)** and female **(B)** mice.
(C-D) Plasma insulin measured at baseline in male **(C)** and female **(D)** mice fasted for 5 hours.
(E-F) Insulin tolerance test (ITT). Blood glucose taken 0-90 minutes after injection of glucose in male **(E)** and female **(F)** mice. Data are presented as mean ± SEM, analyzed by two-way ANOVA, *p<0.05, ***p<0.001.

3.14. Post-Prandial Triglyceride Levels are Similar Between Housing Temperature and Genotype After a Lipid Tolerance Test

TG are significant energy sources, often stored as reserves for later use. GLP-1 action decrease post-prandial TG excursion (Hsieh et al., 2010), while GLP-2 action increases TG secretion (Dash et al., 2014). Plasma TG were measured after a lipid tolerance test; however, were not significantly different between housing temperature or genotype (Fig.3.14a,b). Interestingly, TG levels peak at the two-hour timepoint in males (Fig.3.14a); however, this is not seen in females (Fig.3.14b).

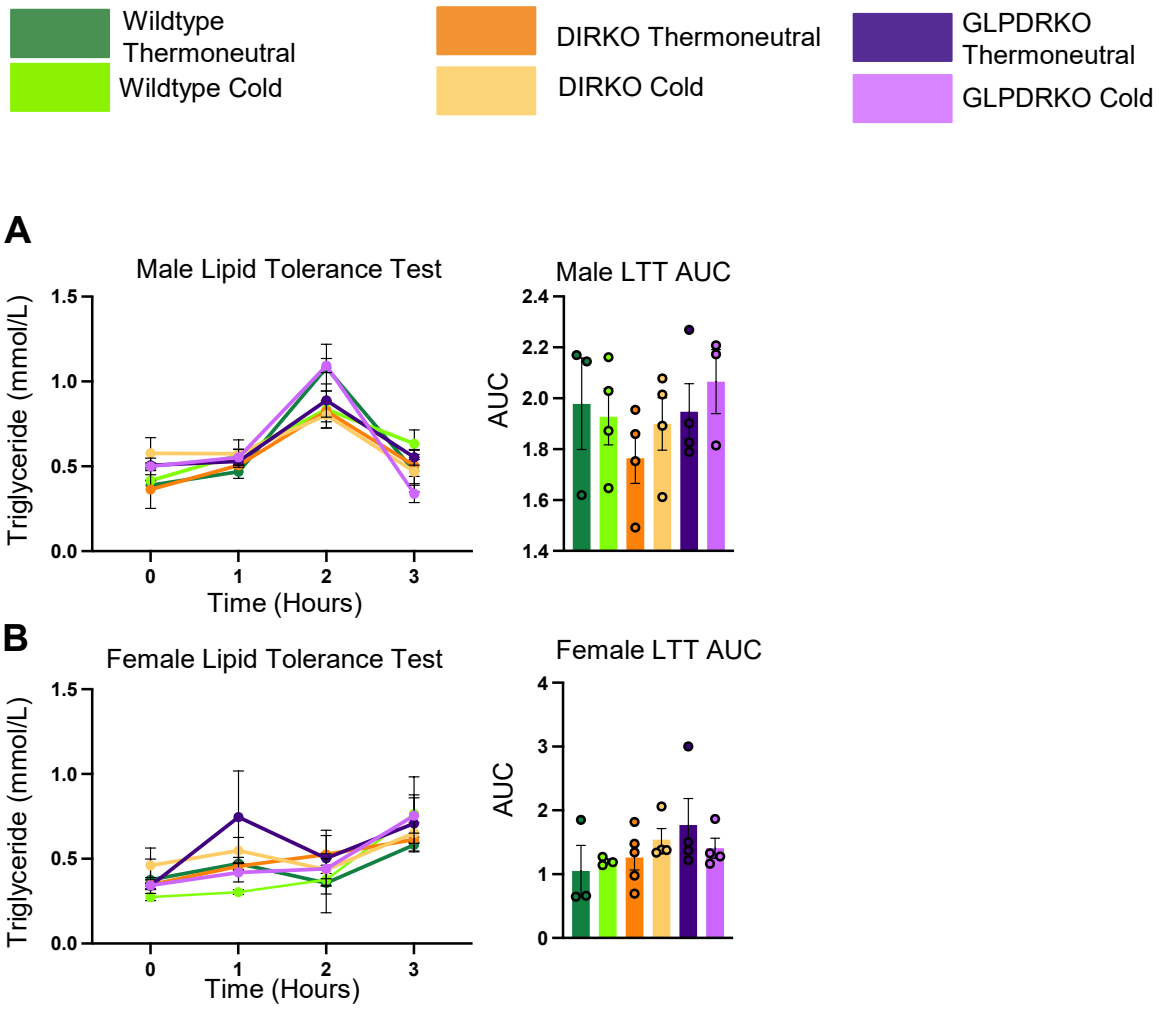


Figure 3.14. Post-Prandial Triglyceride Levels are Similar Between Housing Temperature and Genotype After a Lipid Tolerance Test.
(A-B) Lipid tolerance test (LTT). Plasma triglyceride concentration measured 0, 1, 2, 3 hours post-olive oil gavage in male **(A)** and female **(B)** mice. Data are presented as mean ± SEM, analyzed by two-way ANOVA.

3.15. Cold Exposed Mice Demonstrate Differences in Body Composition and Adipose Tissue Depots

To further investigate changes in body composition and the role of GLP-1, GLP-2, and GIP in adipose tissue regulation, body composition analysis was performed with EchoMRI. Adipose tissue is essential in regulating whole body homeostasis; however, overall fat loss is stimulated with cold exposure (Chevalier et al., 2015). Adipose tissue in thermoneutral exposed mice increased over four weeks, while male wildtype mice exposed to cold exhibited a decrease in adipose tissue (Fig.3.15a). Male GLPDRKO exhibit a similar trend, while male DIRKO mice housed to thermoneutrality drop slightly in adipose tissue weight. In female mice, adipose tissue drops in cold and thermoneutral housed mice of all three models (Fig.3.15b). Various adipose depots are also weighed to determine which forms accrue or are utilized depending on housing temperature (Fig.3.15c,d). BAT is a principle thermogenic organ responsible for increased heat production (Ravussin et al., 2014). Although not significant, BAT weight trends are greater in male (Fig.3.15c) and female (Fig.3.15d) mice housed in the cold and unaffected by genotype. However, BAT weight in male wildtype mice is similar between housing temperatures.

WAT is responsible for energy storage through triglycerides (Ahmadian et al., 2007). ingSAT, epididymal adipose tissue (males only), and pgVAT (females only) are all various depots of WAT. In contrast, the weight of ingSAT, epididymal, and pgVAT are lower in cold exposed mice in all models; however, are not statistically significant (Fig.3.15c,d). Epididymal adipose tissue weight in thermoneutral exposed wildtype mice is significantly greater compared to the weight in thermoneutral exposed DIRKO mice (Fig.3.15c). pgVAT is significantly lower in cold exposed DIRKO mice, compared to those housed in thermoneutrality (Fig.3.15c). Together, this data suggests that metabolic demand is increased with cold exposure, such that adipose tissue is catabolized.

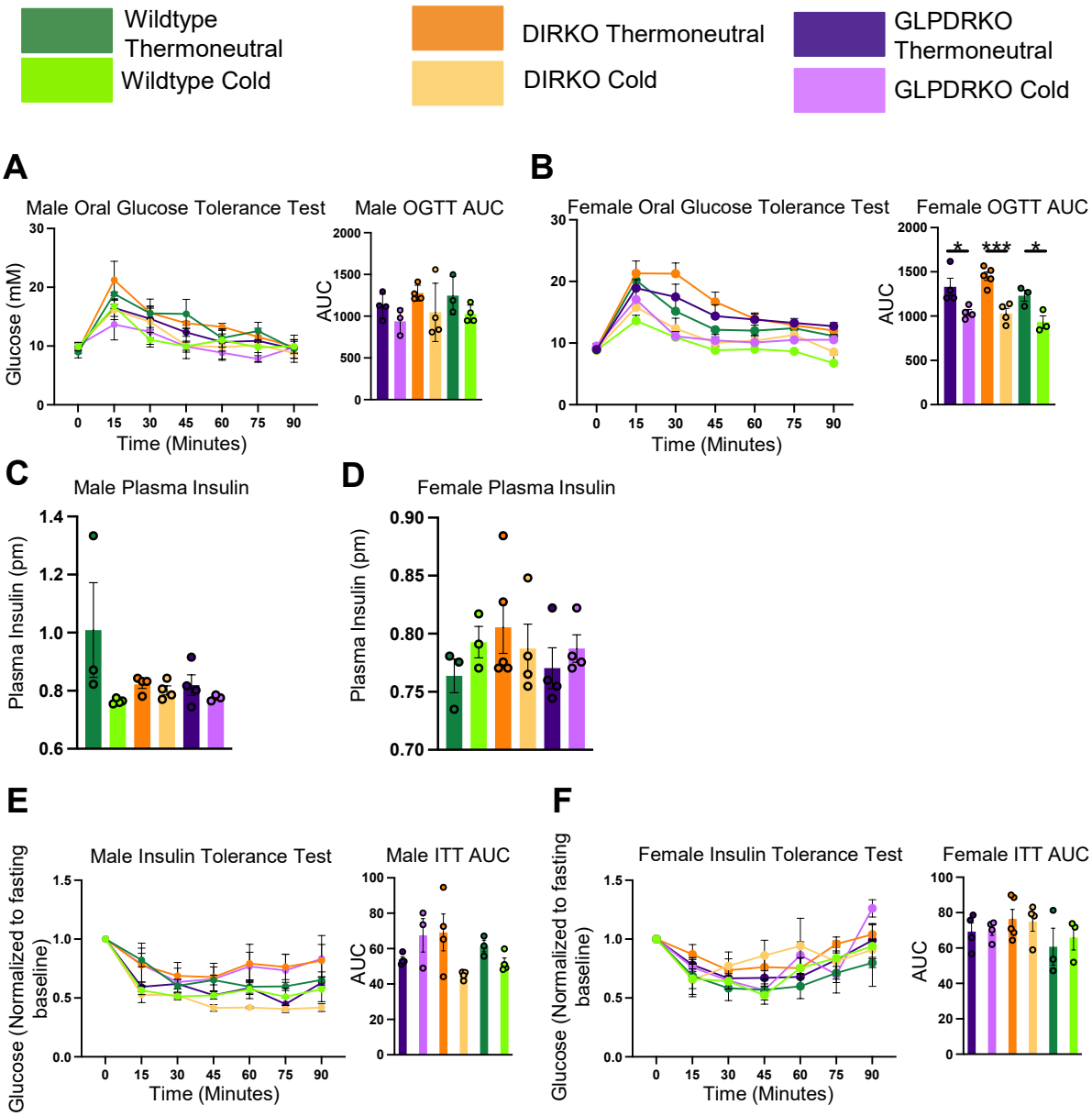


Figure 3.13. Cold Exposure Improves Glucose Tolerance, While Failing to Improve Insulin Tolerance.
(A-B) Oral glucose tolerance test (OGTT). Blood glucose taken 0-90 minutes after administration of oral glucose in male **(A)** and female **(B)** mice.
(C-D) Plasma insulin measured at baseline in male **(C)** and female **(D)** mice fasted for 5 hours.
(E-F) Insulin tolerance test (ITT). Blood glucose taken 0-90 minutes after injection of glucose in male **(E)** and female **(F)** mice. Data are presented as mean ± SEM, analyzed by two-way ANOVA, *p<0.05, ***p<0.001.

3.16. Plasma Leptin Concentrations in Cold Exposed Mice

Leptin is an essential hormone which is released by adipocytes and regulates food intake by activating a neural circuit involving anorexigenic or orexigenic neuropeptides (Kelesidis et al., 2010). Leptin signals the central nervous system to decrease food intake. Cold exposure and fat mass have been positively correlated while circulating leptin concentration was reduced in cold exposed mice (Ravussin et al., 2014). Similarly, our data demonstrate that leptin concentration is lower in male (Fig.3.16a) and female (Fig.3.16b) cold exposed mice; however, not statistically significant in male GLPDRKO and female wildtype female mice. This trend is not seen in male DIRKO mice.

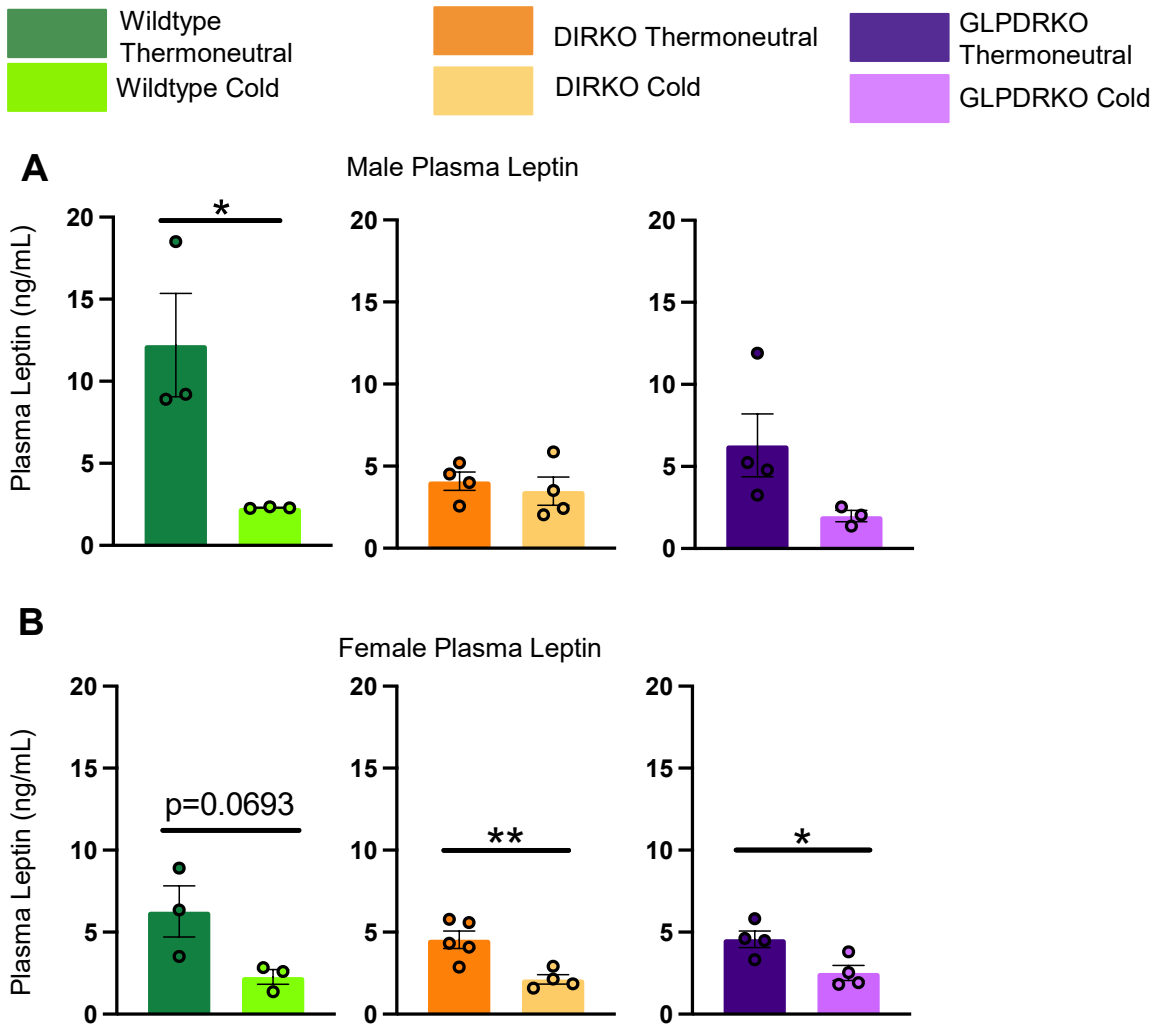


Figure 3.16. Plasma Leptin Concentrations in Cold Exposed Mice
(A-B) Plasma leptin measured from cardiac puncture in male **(A)** and female **(B)** wildtype, DIRKO, GLPDRKO mice fasted for 5 hours. Data are presented as mean ± SEM, analyzed by unpaired student's t-test, *p<0.05, **p<0.01.

4. Discussion, Future Directions, and Limitations

4.1 Discussion

All animals must possess a compensatory mechanism for adaptation when exposed to unfavourable conditions. When the external housing environment for mice is consistently at 4-6˚C, the mice stimulate energy expenditure to produce heat and regulate body temperature making accessing increased energy supply through accelerated nutrient absorption and food intake imperative to survival (Ravussin et al., 2014). A mouse's core temperature is remarkably close to that of a human; typically, 1°C below that of a human. Studies involving cold exposure to elucidate metabolic rate typically expose mice at 5±1°C (Chevalier et al., 2015; Ravussin et al., 2014; Xu et al., 2019; Zhao, 2012). Mice housed in temperatures below 5°C may go into torpor; a state of physical inactivity, reduced body temperature and metabolic expenditure (Gavrilova et al., 1999). In contrast to mice in a hibernation state, cold exposure has been seen to increase energy expenditure, food intake, and intestinal growth (Chevalier et al., 2015; Price et al., 2013). Mice prefer an ambient temperature within the thermoneutral zone, which is between 26-34°C. (Kaikaew et al., 2017). This is when their metabolic rate is the lowest; termed, basal metabolic rate. When housing temperatures are within the TNZ, mice do not experience cold-induced thermogenesis (Neff, 2020). Animal facilities generally set room temperature between 20-24°C, which imposes a moderate cold-stress (Kaikaew et al., 2017). Between 20-23°C, mice expend massive quantities of energy to maintain body temperature; one third of food intake is used towards thermogenesis (Neff, 2020).

Although chronic cold exposure time varies between studies (10 days to 4 weeks) (Chevalier et al., 2015; Price et al., 2013; Ravussin et al., 2014), we housed mice in cold or thermoneutral housing for 4-5 weeks. Following this protocol allows the mice two weeks to adapt to a metabolic stimulus such as cold or high-fat diet (HFD), where parameters stabilize (Obernier & Baldwin, 2006; Wang & Liao, 2012). This allowed an appropriate amount of time to evaluate physiological increases in the incretin and gut-derived hormones, and other metabolic testing.

We first reproduced the increased food intake, villi length and intestinal circumference observed in mice exposed to housing in cold temperatures (Chevalier et al., 2015). This pilot study housed wildtype C57BL/6J male mice at 4-6°C for four weeks (Fig.3.1). Although intestinal growth has previously been observed under cold exposure, the incretin hormone receptor action has not yet been thoroughly explored in the context of cold stress. Together with greater villi length and intestinal circumference (Fig.3.1e-g), the mice housed at 4°C demonstrated greater plasma GLP-1 (Fig.3.1a,b) and GIP (Fig.3.1c,d) concentrations at baseline and 5-minutes post-olive oil gavage, compared to wildtype mice housed at 27°C.

Incretin and Preproglucagon Peptide Secretion is Elevated During Chronic Cold Exposure

In the genotype studies, we set out to determine the importance of the signaling of the preproglucagon peptides; GLP-1 and GLP-2, along with GIP in mediating nutrient absorption and adaptation within the gut in response to housing at 4-6˚C. As sex may influence various metabolic effects, we included the analysis of both male and female mice. Cold exposure increases food intake to compensate for the increased energy expenditure (Langeveld et al., 2016; Ravussin et al., 2014). Our data demonstrate that food intake rises with cold exposure, although body weight does not change (Fig.3.8), commonly seen with cold exposure (Ravussin et al., 2014). Across all genotypes, food intake was increased in response to cold exposure (Fig. 3.2, 3.4 and 3.6) as all mice must compensate for an increased energy demand regardless of the hormone receptors present.

Male C57BL/6J mice have a baseline GLP-1 concentration of approximately 3.5 pM (Zhang et al., 2011). This is in line with our wildtype mice which had ~8 pM at baseline plasma (Fig.3.1a,b, 3.2c,d). Surprisingly, GLP-1 levels in both male and female DIRKO mice are similar between cold and thermoneutral housing temperatures (Fig.3.4c,d) but elevated compared to wildtype mice at ~15-20 pM. Therefore, increases in secretion may be observed in compensation for the lack of intact GLP-1R and GIPR signaling in DIRKO mice. GLP-1 levels in cold exposed GLPDRKO mice (Fig.3.6c,d) are far greater than wildtype and DIRKO mice.

GLPDRKO mice lacked morphometric changes within the intestine in response to housing at 4-6˚C and therefore may upregulate GLP-1 and GLP-2 secretion, attempting to maximize intestinal absorption and production of energy through thermogenesis.

GIP levels in wildtype mice were increased approximately 2-fold (Fig. 3.1c,d) and 3.5-fold (Fig.3.2e) with chronic cold exposure, consistent with previous studies where plasma GIP is increased between days 18-24 of cold exposure in rats (Irwin et al., 2011). Strikingly, total GIP levels in both male and female DIRKO (Fig. 3.4e,f) and GLPDRKO (Fig.3.6e,f) mice did not differ between housing temperatures. Therefore, it is unlikely that GIP signaling is involved in gut remodelling in response to cold.

GLP-2R Signalling Contributes to Intestinal Remodelling in Response to Cold Exposure

The absorptive capacity of nutrients in the small intestine has been seen to increase with cold exposure through intestinal remodelling (Nilaweera & Speakman, 2018). The intestine has a remarkable ability to adapt depending on energy needs (Vaanholt et al., 2009). The small intestine adapts to this increased energy demand by increasing the surface area to maximize potential nutrient uptake (Chevalier et al., 2015). In agreement with previous data (Chevalier et al., 2015), we demonstrate that wildtype mice exhibit increased intestinal circumference and villi length (Fig.3.1e-h, 3.3a-c). Villi within the small intestine are crucial for nutrient absorption, and areas responsible for most nutrient uptake typically have longer villi (Jolma et al., 1980). The ileum absorbs far fewer nutrients than the duodenum and the jejunum (Collins & Bhimji, 2018), and expectedly, our data shows villi length within the ileum are not significantly different between housing temperatures (Fig.3.1g, 3.3c). Energy expenditure and nutrient metabolism greatly depend on incretin and other gut-derived hormone action through their respective receptors. We used C57BL/6J mice which are commonly used in many studies pertaining to intestinal growth (Fig. 3.1, 3.3) (Baldassano et al., 2013; Chevalier et al., 2015). To understand the importance of incretin and gut-derived hormone action, we used DIRKO mice (Fig.3.5) as they lack the incretin receptors and GLPDRKO (Fig.3.7),

which lack the GLP-1 and GLP-2 receptors. By incorporating both DIRKO and GLPDRKO mice, we can understand the role of the GLP-1R, GIPR, and GLP-2R signaling individually and whether the intact receptors may compensate for the genetically eliminated receptors.

We reveal that villi length and intestinal circumference are increased with cold exposed DIRKO mice (Fig.3.5a-c), even though there is a disruption in incretin hormone response, consistent with data identifying GLP-2's role in intestinal growth (Bahrami et al., 2010). In contrast to both wildtype and DIKRO mice, in cold-exposed GLPDRKO mice, there is no observed increase in intestinal circumference or villi length in cold exposure (Fig.3.7a-c). This suggests that increases in villi length and intestinal circumference rely on intact GLP-2R signaling, consistent with the role of GLP-2R signaling in intestinal growth as suggested in previous studies (Rowland et al., 2011). Moreover, it has also been previously reported that blocking endogenous GLP-2 reduced intestinal growth (Hartmann et al., 2000). Given the clear effect in GLPDRKO mice on intestinal circumference and villi length, crypt cell depth was also measured. Previous work has shown that the administration of GLP-2 increases crypt cell proliferation (Drucker, 2019). Surprisingly, our results do not demonstrate this relationship in crypt cell depth (Fig.3.1h, 3.3d, 3.5d and 3.7d). This may be due to differences in endogenous signaling vs. signaling induced and sustained with exogenous or pharmacological concentrations of GLP-2, which may have a greater impact on growth. Lastly, intestinal length and weight was measured. Our results reveal small bowel weight and length increases in cold-exposed mice (Fig.3.3e,f, 3.5e,f and 3.7ef); however, we did not see significant differences throughout the large intestine between genotypes. Small intestinal length was significantly greater with cold exposure, but not between genotypes, while small intestinal weight is greater in wildtype and DIRKO mice compared to small intestinal weight in GLPDRKO. This weight difference may be attributed to the increases in villi length and intestinal circumference, which are absent in GLPDRKO. Our data, along with other studies has shown that intestinal remodeling is occurring when mice are exposed to cold. Furthermore, we show that in cold exposure, GLP-2 is

playing a role in intestinal remodeling and therefore essential for maintenance of homeostasis.

Another cell group analyzed were goblet cells and their abundance in the duodenum and ileum (Fig.3.12). The average number of goblet cells per villi did not significantly differ between genotypes. It was observed, however, that the absolute number of goblet cells per villi were significantly greater in GLPDRKO mice (Fig.3.12c) compared to both male wildtype (Fig.3.12a) and DIRKO (Fig.3.12b) mice. No published studies link goblet cell number to the incretin hormones; however, as shown previously (Chevalier et al., 2015), goblet cell number did not increase with cold exposure.

Metabolic Changes with Chronic Cold Exposure

In general, C57BL/6J male mice weigh more than C57BL/6J female mice (*Body Weight Information for C57BL/6J*, Retrieved August 9, 2021). Rectal temperatures between male and female rats do not differ significantly when exposed to cold (4°C) for 2 hours while also demonstrating no significant differences in body weight (Elmarzouki et al., 2014). With cold exposure, female mice eat more food and export more milk for lactation, where the limits to heat dissipation are removed (Król & Speakman, 2003; Zhao, 2012). Swiss female mice exposed to cold (5°C) exhibited increased food intake compared to those exposed to warm temperatures (23°C), and decreased body weight (Zhao, 2012). Similarly, male C57BL/6J mice exposed to cold (4°C) for 4 hours a day demonstrated increased food intake, but no reduction in body weight or adiposity (Ravussin et al., 2014). The diet may additionally contribute to changes in phenotype (Pellizzon, 2016). Standard laboratory chow diet is commonly used in mouse studies, composed mainly of complex carbohydrates, with fats from vegetable sources (Warden & Fisler, 2008). Chow diet typically comprises 20% fiber (soluble and insoluble) while purified diets contain about 5% insoluble fiber (Pellizzon, 2016). Fiber composition is important particularly to the gut, as bacteria can ferment soluble fibre, which releases SCFA; ultimately, supplying colonocytes with energy and improved insulin action (Pellizzon,

2016). To minimize variability and influence positive health of the gut, all mice were fed with standard chow diet by Harlan Teklad 2019, containing an energy density of 3.3kcal/g (22% calories from fat). Chow diet was provided to mice ad libitum, unless otherwise noted.

Lean mass measures muscle tissue mass equivalent to all body parts containing water. After four weeks, lean mass in all mice was only significantly greater in wildtype and DIRKO female mice housed at 4°C (Fig.3.9). There were no significant differences between genotypes, suggesting that the incretin hormones and GLP-2 may not be prominent in these processes. Cold exposure places the body in a catabolic state despite increased food intake. Between wildtype and *Glp1r*$^{-/-}$ male and female mice, food intake and body weight did not differ (Scrocchi, Brown, MacLusky, et al., 1996), suggesting the disruption of receptor signaling is not associated with alterations in body weight or feeding behaviour. Between chow-fed wildtype and DIRKO mice, total energy intake (kCal/day) was similar (Hansotia et al., 2007). Although not shown, 24-hour food intake is greater in DIRKO mice than wild-type mice.

In the current study, body weight and lean mass tend to be greater in male mice than in female mice (Fig. 3.8 and 3.9). Although overall body weight and lean mass remain consistent over the exposure period, GLPDRKO mice weigh less (Fig. 3.8,3.9). It is unknown whether GLP-2 plays a role in the homeostatic control of body weight (Guan, 2014); however, body weight of *POMC-Glp2r*$^{-/-}$ mice trended towards being slightly lower than the body weight of *POMC-Glp2r* wildtype mice (Guan, 2014). These data suggest that even though there was a disruption in receptor signaling, all mice increased food intake to compensate for the increased caloric need during cold exposure. Incretin hormones play an essential role in orchestrating the body's post-prandial response to nutrients entering the gut (Diakogiannaki et al., 2012). GLP-1 and GIP are essential for glucose-stimulated insulin secretion (MacDonald et al., 2002). These data demonstrate that incretin hormones GLP-1 and GIP are greater after an olive oil gavage in male and female cold exposed wildtype mice, suggesting they are important in metabolic processes when energy demand is increased. Along with insulin secretion, GLP-1 has been

linked to favouring thermogenesis in brown adipose tissue (González-García et al., 2019), influencing food intake (Katsurada & Yada, 2016), and intestinal growth (Drucker & Yusta, 2014). It is known that there are several homeostatic mechanisms regulating metabolism that have defined sex differences. Although studies focusing on adipose tissue are typically performed in male mice, a study compared intracellular glucose metabolism in BAT, which revealed similar cold-induced changes (6°C) between male and female mice (Wang et al., 2020). Sex differences in our study are summarized in Table 4.1 and 4.2.

Glucose metabolism is a central process in the body which allows for efficient energy production (Nakrani et al., 2021), and a variety of factors may influence glucose and insulin tolerance. Observing glucose tolerance and insulin sensitivity in cold exposed mice may depict which energy processes are important in different genotypes and how incretin hormones may play a role. An oral glucose tolerance test determines how mice respond to orally administered glucose. Glucose tolerance is shown to improve with cold exposure, where orally administered glucose is rapidly taken up in cold exposed mice (Chevalier et al., 2015). Glucose tolerance was similar in young male and female mice (24 week old). Aged female mice (72 week old) displayed similar glucose tolerance, while aged male mice display significant deterioration (Reynolds et al., 2019). Although few studies compare insulin tolerance between male and female C57BL/6J mice, a study demonstrated improved insulin tolerance in female mice (Macotela et al., 2009). Interestingly C57BL/6J male and female mice demonstrated increased glucose tolerance compared to male and female DIRKO mice (Hansotia et al., 2004). DIRKO mice also fail to demonstrate improved glycemic response after treatment with administration of GIP, exendin-4, and valine pyrrolidide (Val-Pyr)- a DPP4 inhibitor, further emphasizing the importance of the GLP-1 and GIP receptors in the post-prandial glucose response. *Glp-1r*$^{-/-}$ male and female mice demonstrate an increased blood glucose response following an OGTT compared to wildtype mice (Scrocchi, Brown, MacLusky, et al., 1996).This pattern is consistent with our data (Fig.3.13a,b); however, there are no significant differences between genotype. However, although not significant, trends suggest that male and female DIRKO mice

are more glucose intolerant than wildtype and GLPDRKO, which has previously been observed in compared to C57BL/6J mice (Hansotia et al., 2004). During the OGTT, plasma insulin levels are also measured. We did not observe any differences in plasma insulin levels between housing temperatures or between genotype (Fig.3.13 c and d). In previous studies, DIRKO mice had lower plasma insulin levels 15 minutes post glucose gavage (Hansotia et al., 2004).

Chevalier *et al.* demonstrate that insulin sensitivity is increased with cold exposure. We, however, did not observe any significant differences between housing temperature or genotype (Fig.3.13 e and f). Insulin sensitivity trends to improve in male wildtype and DIRKO mice but not in GLPDRKO mice; however, these results are not statistically significant. It has been previously demonstrated that insulin tolerance is improved with cold exposure, as activation of brown adipose tissue improves whole body insulin tolerance (Poher et al., 2015).

Contrasting results regarding cold exposure and triglycerides have been reported. TG levels have been demonstrated to increase with cold exposure (Saari et al., 2020), while other studies have reported decreased levels (Nie et al., 2015). Studies have also reported that TG levels do not differ significantly between 4-6˚C and room temperature mice (Chevalier et al., 2015; Ravussin et al., 2014). Our data did not demonstrate any significant differences between housing temperatures (Fig.3.14a,b). Interestingly, the female mice did not demonstrate a peak (Fig.3.14b), suggesting that TG production never overwhelmed the TG clearance. In contrast, the male mice demonstrate a peak at two hours post-gavage, as entry of TG into circulation is greater than TG clearance. Plasma TG levels are typically lower in female mice compared to males, where delipidation of CM is caused by increased activity of LPL (Shearer et al, 2000). The gut-derived peptides modulate intestinal triglyceride secretion (Cho & Kieffer, 2010; Mulvihill, 2018). GLP-1R signaling decreases TG levels, while GIPR and GLP-2R signaling increase TG levels. Unexpectedly, we did not observe this in our wildtype mice compared to other genotypes. Although many of our results did not show statistical significance, post-prandial metabolism is a complicated system with many routes of regulation that compensate for the lack of incretin and preproglucgon signaling.

Impact of Cold Exposure on Adipose Tissue

Remodelling of adipose tissue occurs in response to a moderate decrease in environmental temperature, such that body weight and total fat mass have been observed to decrease (Stelt et al., 2017). Consistent with body weight, total adipose tissue seems to diminish in male mice exposed to cold (Fig.3.15a), and female mice exhibit decreases in total adipose tissue weight in both thermoneutral and cold housing (Fig.3.15b). It is expected that total adipose tissue weight decreases with cold exposure, as observed in fat pad mass (Chevalier et al., 2015). Between genotypes, adipose tissue mass in male GLPDRKO does not decrease compared to wildtype and DIRKO mice. This may be attributed to GIP action increasing lipid accumulation and compensating for the lack of GLP-1R and GLP-2R signaling. IngSAT, pgVAT, and epididymal adipose tissue are all forms of WAT, which have greater weights in thermoneutral mice as compared to cold exposed mice. These specific depots of adipose tissue have been observed to decrease with cold exposure (Chevalier et al., 2015). In line with previously published data (Chevalier et al., 2015), our results demonstrate that epididymal adipose tissue is decreased with cold exposure, while ingSAT and pgVAT trend towards decreased mass (Fig.3.15c,d).

Leptin is a hormone strongly correlated to the amount of white adipose tissue present in the body, such that circulating leptin concentration is directly related to adipose tissue size. Leptin regulates food intake, by signaling the central nervous system to decrease food intake. This is consistent with our data, as food intake is greater in cold exposed mice, while white adipose tissue and leptin concentration is greater in thermoneutral exposed mice. Another form of adipose tissue is interscapular brown adipose tissue, which is responsible for non-shivering thermogenesis. Cold-transplanted microbiota in mice led to a trend of decreased BAT mass, while intermittent cold exposure revealed similar results (Ravussin et al., 2014). Our data trends towards greater amounts of brown adipose tissue in cold exposed mice (Fig.3.16). Consistent with more significant amounts of WAT in wildtype mice (Fig.3.15c,d), leptin levels are notably higher in male wildtype mice compared to DIRKO and GLPDRKO mice housed at 27°C (Fig.3.16a). These data

demonstrate the importance of WAT and BAT during cold exposure; however, the intestinally-derived peptides role in regulating these adipose depots remain unclear.

In summary, our data demonstrates that the secretion of incretin hormones and GLP-2 are increased during chronic cold exposure. Intestinal remodeling occurs to increase intestinal circumference and villi length in wildtype and DIRKO mice. GLPDRKO mice lack this ability and have the lowest body weight and fat pad mass suggesting that growth requires activation of the GLP-2 receptor.

Table 4.1. Summary table of results from male wildtype, DIRKO, GLPDRKO mice are exposed to cold (4ºC) compared to thermoneutral (27ºC) for 5 weeks. Direction of arrow represents effect of cold compared to thermoneutral housing temperature.

	Wildtype	DIRKO	GLPDRKO
Villi/Circumference			
Food Intake			
GLP-1 Levels			
GIP Levels			
Body Weight			
Glucose Tolerance			
Insulin Tolerance			
Triglyceride Levels			
Adipose Tissue Mass			
BAT Mass			
ingSAT/pgVAT Mass			
Leptin Levels			

Table 4.2. Summary table of results from female wildtype, DIRKO, GLPDRKO mice are exposed to cold (4ºC) compared to thermoneutral (27ºC) for 5 weeks. Direction of arrow represents effect of cold compared to thermoneutral housing temperature.

	Wildtype	DIRKO	GLPDRKO
Villi/Circumference			
Food Intake			
GLP-1 Levels			
GIP Levels			
Body Weight			
Glucose Tolerance			
Insulin Tolerance			
Triglyceride Levels			
Adipose Tissue Mass			
BAT Mass			
ingSAT/pgVAT Mass			
Leptin Levels			

4.2 Future Directions

Although all mice exposed to cold consume a more significant amount of food to compensate for increased metabolic need, GLPDRKO mice failed to maximize potential intestinal growth. Our future studies will confirm these findings of intestinal histology in female mice, to further determine whether intestinal remodeling occurs in wildtype and DIRKO mice, but not in GLPDRKO mice. Non-shivering thermogenesis heavily relies on UCP1 in BAT (Fedorenko et al., 2012), and *Ucp1* mRNA expression increases with cold exposure (Chevalier et al., 2015). Thus, staining BAT and subcutaneous WAT for UCP1 will reveal whether GLP-2 receptor signaling plays a direct or indirect role in thermogenesis.

The intestinal microbiome's importance is becoming increasingly recognized and has been reported to influence host digestion and other physiologic functions (Shreiner et al., 2015). Although there are many species identified, many are unculturable; thus, their role has not been highly explored. Androgens have been demonstrated to influence the microbiome. Specifically, female pathogen-free non-obese diabetic (NOD) mice were up to four times higher more likely to develop type-1 diabetes (T1D) (Yurkovetskiy et al., 2013). Furthermore, microbiota modulate energy regulation and nutrient metabolism. *Lactobassilus rhamnosus* PL60 was seen to decrease fat content, as it produces conjugated linoleic acid; known to decrease fat. Body weight also decreased, without decreasing energy intake (Lee et al., 2006). Moreover, the gastrointestinal microbiome plays a critical role in energy harvest, storage, regulation and nutrient metabolism (Krajmalnik-Brown et al., 2012; Xiao & Kang, 2020). Ingestible nutrients that reach the colon make up approximately 10-30% of ingested energy, where the microbiome provides enzymes required for metabolism. This further emphasizes the importance of the colonic microbiome indigestion (Bergman, 1990; Krajmalnik-Brown et al., 2012). *Bacteroides thetaiotaomicron* was identified to produce 226 glycoside hydrolases and 15 polysaccharide lyases, improving the host's energy balance (Krajmalnik-Brown et al., 2012; Sonnenburg et al., 2005). Although there are many studies pertaining to the microbiome's influence on intestinal eubiosis and dysbiosis, very few have

linked them with intestinal growth (Chevalier et al., 2015). Increased intestinal weight and length were demonstrated in cold exposed microbiota depleted mice, and these increases were more pronounced with antibiotic treatment (150% and 35% greater, respectively). Compared to room temperature-transplanted mice, cold microbiota transplanted mice revealed greater intestinal weight and length, suggesting that the cold microbiota are significantly involved in this phenotypic change (Chevalier et al., 2015). The composition of the intestinal microbiome is influenced by various physiological changes (Chevalier et al., 2015) and may also contribute to generating signaling molecules important for the control of intestinal morphology. Therefore, evaluating the intestinal microbiome populations in our models to determine how abundance and population diversity may relate to intestinal remodelling during exposure to cold is of interest.

4.3 Limitations

Although incretin therapies are becoming increasingly adopted for the treatment of diabetes (Neumiller, 2015), many published studies use receptor agonists and other exogenous treatment forms. While these methods may be practical and discern the pharmacological properties of these therapies, the exact mechanism of endogenous incretins and other gut-derived peptides remains unclear. Our studies only evaluate endogenous signaling circuits and the effects may be indirect through additional mediators which have not yet been revealed as discussed above.

Moreover, experiments and timelines were affected by the COVID-19 pandemic. Due to ACVS and UOHI restrictions, our ability to perform in-person experiments was hindered. Female and male mouse studies were severely delayed due to COVID-19 lab restrictions and reductions in the breeding colony.

5. References

Abdulla, H., Phillips, B., Smith, K., Wilkinson, D., Atherton, P., & Idris, I. (2014). Physiological Mechanisms of Action of Incretin and Insulin in Regulating Skeletal Muscle Metabolism. *Current Diabetes Reviews*, *10*(5), 327–335. https://doi.org/10.2174/15733998106661410171537 49

Adriaenssens, A. E., Biggs, E. K., Darwish, T., Tadross, J., Sukthankar, T., Girish, M., … Reimann, F. (2019). Glucose-Dependent Insulinotropic Polypeptide Receptor-Expressing Cells in the Hypothalamus Regulate Food Intake. *Cell Metabolism*, *30*(5), 987-996.e6. https://doi.org/10.1016/j.cmet.2019.07.013

Ahmadian, M., Duncan, R. E., Jaworski, K., Sarkadi-Nagy, E., & Sul, H. S. (2007, April). Triacylglycerol metabolism in adipose tissue. *Future Lipidology*, Vol. 2, pp. 229–237. https://doi.org/10.2217/17460875.2.2.229

Ali, A., Tan, H. Y., & Kaiko, G. E. (2020, December 7). Role of the Intestinal Epithelium and Its Interaction With the Microbiota in Food Allergy. *Frontiers in Immunology*, Vol. 11, p. 3222. https://doi.org/10.3389/fimmu.2020.604054

Ali, S., Lamont, B. J., Charron, M. J., & Drucker, D. J. (2011). Dual elimination of the glucagon and GLP-1 receptors in mice reveals plasticity in the incretin axis. *The Journal of Clinical Investigation*, *121*(5), 1917. https://doi.org/10.1172/JCI43615

Allaire, J. M., Crowley, S. M., Law, H. T., Chang, S. Y., Ko, H. J., & Vallance, B. A. (2018, September 1). The Intestinal Epithelium: Central Coordinator of Mucosal Immunity. *Trends in Immunology*, Vol. 39, pp. 677–696. https://doi.org/10.1016/j.it.2018.04.002

Amato, A., Baldassano, S., & Mulè, F. (2016, May 1). GLP2: An underestimated signal for improving glycaemic control and insulin sensitivity. *Journal of Endocrinology*, Vol. 229, pp. R57–R66. https://doi.org/10.1530/JOE-16-0035

Andersen, D. B., Grunddal, K. V, Pedersen, J., Kuhre, R. E., Lund, M. L., Holst, J. J., & Ørskov, C. (2021). Using a Reporter Mouse to Map Known and Novel Sites of GLP-1 Receptor Expression in Peripheral Tissues of Male Mice. *Endocrinology*, *162*(3), 1–19. https://doi.org/10.1210/endocr/bqaa246

Anini, Y., & Brubaker, P. (2003). Role of leptin in the regulation of glucagon-like peptide-1 secretion. *Diabetes*, *52*(2), 252–259.

https://doi.org/10.2337/DIABETES.52.2.252

Asmar, M., Asmar, A., Simonsen, L., Gasbjerg, L. S., Sparre-Ulrich, A. H., Rosenkilde, M. M., … Bülow, J. (2017). The Gluco-and Liporegulatory and Vasodilatory Effects of Glucose-Dependent Insulinotropic Polypeptide (GIP) Are Abolished by an Antagonist of the Human GIP Receptor. *Diabetes*, *66*. https://doi.org/10.2337/db17-0480

Ayala, J. E., Bracy, D. P., James, F. D., Burmeister, M. A., Wasserman, D. H., & Drucker, D. J. (2010). Glucagon-Like Peptide-1 Receptor Knockout Mice Are Protected from High-Fat Diet-Induced Insulin Resistance. *Endocrinology*, *151*(10), 4678. https://doi.org/10.1210/EN.2010-0289

Bahrami, J., Longuet, C., Baggio, L. L., Li, K., & Drucker, D. J. (2010). Glucagon-like peptide-2 receptor modulates islet adaptation to metabolic stress in the ob/ob mouse. *Gastroenterology*, *139*(3), 857–868. https://doi.org/10.1053/j.gastro.2010.05.006

Bahrami, J., Yusta, B., & Drucker, D. J. (2010). ErbB Activity Links the Glucagon-Like Peptide-2 Receptor to Refeeding-Induced Adaptation in the Murine Small Bowel. *Gastroenterology*, *138*(7), 2447–2456. https://doi.org/10.1053/j.gastro.2010.03.006

Baldassano, S, Rappa, F., Amato, A., Cappello, F., & Mulè, F. (2015). GLP-2 as Beneficial Factor in the Glucose Homeostasis in Mice Fed a High Fat Diet. *Journal of Cellular Physiology*, *230*(12), 3029–3036. https://doi.org/10.1002/JCP.25039

Baldassano, Sara, Amato, A., Cappello, F., Rappa, F., & Mulè, F. (2013). Glucagon-like peptide-2 and mouse intestinal adaptation to a high-fat diet. *Journal of Endocrinology*, *217*(1), 11–20. https://doi.org/10.1530/JOE-12-0500

Baldassano, Sara, Gasbjerg, L. S., Kizilkaya, H. S., Rosenkilde, M. M., Holst, J. J., & Hartmann, B. (2019). Increased Body Weight and Fat Mass After Subchronic GIP Receptor Antagonist, but Not GLP-2 Receptor Antagonist, Administration in Rats. *Frontiers in Endocrinology*, *10*, 492. https://doi.org/10.3389/FENDO.2019.00492

Barrera, J. G., Sandoval, D. A., D'Alessio, D. A., & Seeley, R. J. (2011, September).

GLP-1 and energy balance: An integrated model of short-term and long-term control. *Nature Reviews Endocrinology*, Vol. 7, pp. 507–516. https://doi.org/10.1038/nrendo.2011.77

Beaudry, J. L., Kaur, K. D., Varin, E. M., Baggio, L. L., Cao, X., Mulvihill, E. E., … Drucker, D. J. (2019). Physiological roles of the GIP receptor in murine brown adipose tissue. *Molecular Metabolism*, *28*, 14. https://doi.org/10.1016/J.MOLMET.2019.08.006

Beiroa, D., Imbernon, M., Gallego, R., Senra, A., Herranz, D., Villarroya, F., … Nogueiras, R. (2014). GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. *Diabetes*, *63*(10), 3346–3358. https://doi.org/10.2337/db14-0302

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). *Triacylglycerols Are Highly Concentrated Energy Stores*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK22369/

Bergman, E. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews*, *70*(2), 567–590. https://doi.org/10.1152/PHYSREV.1990.70.2.567

Birchenough, G. M. H., Johansson, M. E. V., Gustafsson, J. K., Bergström, J. H., & Hansson, G. C. (2015, July 25). New developments in goblet cell mucus secretion and function. *Mucosal Immunology*, Vol. 8, pp. 712–719. https://doi.org/10.1038/mi.2015.32

Blanpain, C., Mohrin, M., Sotiropoulou, P., & Passegué, E. (2011). DNA-damage response in tissue-specific and cancer stem cells. *Cell Stem Cell*, *8*(1), 16–29. https://doi.org/10.1016/J.STEM.2010.12.012

Boer, G. A., Keenan, S. N., Miotto, P. M., Holst, J. J., & Watt, M. J. (2021). GIP receptor deletion in mice confers resistance to high-fat diet-induced obesity via alterations in energy expenditure and adipose tissue lipid metabolism. *Https://Doi.Org/10.1152/Ajpendo.00646.2020*, *320*(4), E835–E845. https://doi.org/10.1152/AJPENDO.00646.2020

Boguszewski, C. (2015). The role of the gastrointestinal tract in the control of energy balance - Boguszewski - Translational Gastrointestinal Cancer. Retrieved May

10, 2021, from https://tgc.amegroups.com/article/view/4304/5761

Brestoff, J. R., & Artis, D. (2015). Immune regulation of metabolic homeostasis in health and disease. *Cell*, *161*(1), 146–160. https://doi.org/10.1016/J.CELL.2015.02.022

Brøns, C., Jensen, C., Storgaard, H., Hiscock, N., White, A., Appel, J., … Vaag, A. (2009). Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men. *The Journal of Physiology*, *587*(Pt 10), 2387–2397. https://doi.org/10.1113/JPHYSIOL.2009.169078

Burrin, D. G., Petersen, Y., Stoll, B., & Sangild, P. (2001b, April 1). Glucagon-like peptide 2: A nutrient-responsive gut growth factor. *Journal of Nutrition*, Vol. 131, pp. 709–712. https://doi.org/10.1093/jn/131.3.709

Camilleri, M., Lyle, B. J., Madsen, K. L., Sonnenburg, J., Verbeke, K., & Wu, G. D. (2019). Role for diet in normal gut barrier function: developing guidance within the framework of food-labeling regulations. *Https://Doi.Org/10.1152/Ajpgi.00063.2019*, *317*(1), G17–G39. https://doi.org/10.1152/AJPGI.00063.2019

Campbell, J. E. (2021). Targeting the GIPR for obesity: To agonize or antagonize? Potential mechanisms. *Molecular Metabolism*, *46*. https://doi.org/10.1016/J.MOLMET.2020.101139

Chehade, J., Alcalde, R., Naem, E., Mooradian, A., Wong, N., & Haas, M. (2013). Induction of apolipoprotein A-I gene expression by glucagon-like peptide-1 and exendin-4 in hepatocytes but not intestinal cells. *Metabolism: Clinical and Experimental*, *62*(2), 265–274. https://doi.org/10.1016/J.METABOL.2012.07.005

Chen, A., Singh, A., Xiao, F., Dringenberg, U., Wang, J., Engelhardt, R., … Seidler, U. (2010). Gene ablation for PEPT1 in mice abolishes the effects of dipeptides on small intestinal fluid absorption, short-circuit current, and intracellular pH. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *299*(1). https://doi.org/10.1152/AJPGI.00055.2010

Chen, Y., Michalak, M., & Agellon, L. B. (2018). Focus: Nutrition and Food Science: Importance of Nutrients and Nutrient Metabolism on Human Health. *The Yale*

Journal of Biology and Medicine, *91*(2), 95. Retrieved from /pmc/articles/PMC6020734/

Chevalier, C., Stojanović, O., Colin, D. J., Suarez-Zamorano, N., Tarallo, V., Veyrat-Durebex, C., … Trajkovski, M. (2015b). Gut Microbiota Orchestrates Energy Homeostasis during Cold Copy. *Cell*, *163*(6), 1360–1374. https://doi.org/10.1016/j.cell.2015.11.004

Cho, Y. M., & Kieffer, T. J. (2010). K-cells and Glucose-Dependent Insulinotropic Polypeptide in Health and Disease. *Vitamins and Hormones*, *84*(C), 111–150. https://doi.org/10.1016/B978-0-12-381517-0.00004-7

Christian, M. (2017). The browning of white fat | Society for Endocrinology. Retrieved July 8, 2021, from https://www.endocrinology.org/endocrinologist/126-winter17/features/the-browning-of-white-fat/

Clarke, Z. (2007). Leptin. *XPharm: The Comprehensive Pharmacology Reference*, 1–2. https://doi.org/10.1016/B978-008055232-3.62020-3

Cohen, P., & Spiegelman, B. M. (2016). Cell biology of fat storage. *Molecular Biology of the Cell*, *27*(16), 2523. https://doi.org/10.1091/MBC.E15-10-0749

Collins, J. T., & Bhimji, S. S. (2018). Anatomy, Abdomen and Pelvis, Small Intestine. In *StatPearls*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/29083773

Connor, E., Evock-Clover, C., Wall, E., Baldwin, R., Santin-Duran, M., Elsasser =, T., & Bravo, D. (2016). Glucagon-like peptide 2 and its beneficial effects on gut function and health in production animals. *Domestic Animal Endocrinology*, *56 Suppl*, S56–S65. https://doi.org/10.1016/J.DOMANIEND.2015.11.008

Contreras, C., Nogueiras, R., Diéguez, C., Medina-Gómez, G., & López, M. (2016). Hypothalamus and thermogenesis: Heating the BAT, browning the WAT. *Molecular and Cellular Endocrinology*, *438*, 107–115. https://doi.org/10.1016/j.mce.2016.08.002

Cuevas-Ramos, D., Mehta, R., & Aguilar-Salinas, C. A. (2019). Fibroblast Growth Factor 21 and Browning of White Adipose Tissue. *Frontiers in Physiology*, *0*(FEB), 37. https://doi.org/10.3389/FPHYS.2019.00037

D'Agostino, D., Cordle, R. A., Kullman, J., Erlanson-Albertsson, C., Muglia, L. J., &

Lowe, M. E. (2002). Decreased Postnatal Survival and Altered Body Weight Regulation in Procolipase-deficient Mice. *The Journal of Biological Chemistry*, *277*(9), 7170. https://doi.org/10.1074/JBC.M108328200

Dailey, M. J. (2014, December 1). Nutrient-induced intestinal adaption and its effect in obesity. *Physiology and Behavior*, Vol. 136, pp. 74–78. https://doi.org/10.1016/j.physbeh.2014.03.026

Dailey, M. J., & Moran, T. H. (2013). Glucagon-like peptide 1 and appetite. *Trends in Endocrinology and Metabolism: TEM*, *24*(2), 85. https://doi.org/10.1016/J.TEM.2012.11.008

Dao, D.-P. D., & Le, P. H. (2021). Histology, Goblet Cells. *StatPearls*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK553208/

Dash, S., Xiao, C., Morgantini, C., Connelly, P. W., Patterson, B. W., & Lewis, G. F. (2014). Glucagon-like peptide-2 regulates release of chylomicrons from the intestine. *Gastroenterology*, *147*(6), 1275-1284.e4. https://doi.org/10.1053/j.gastro.2014.08.037

Davidson, N. O., & Shelness, G. S. (2000). Apolipoprotein B: mRNA Editing, lipoprotein assembly, and presecretory degradation. *Annual Review of Nutrition*, Vol. 20, pp. 169–193. https://doi.org/10.1146/annurev.nutr.20.1.169

De Iuliis, G., & Pulerà, D. (2011). The Shark. *The Dissection of Vertebrates*, 27–77. https://doi.org/10.1016/B978-0-12-375060-0.00003-6

de Lartigue, G., & Raybould, H. E. (2012). The Gastrointestinal Tract and Control of Food Intake. *Physiology of the Gastrointestinal Tract*, *2*, 1963–1978. https://doi.org/10.1016/B978-0-12-382026-6.00073-7

de Sousa e Melo, F., & de Sauvage, F. J. (2019, January 3). Cellular Plasticity in Intestinal Homeostasis and Disease. *Cell Stem Cell*, Vol. 24, pp. 54–64. https://doi.org/10.1016/j.stem.2018.11.019

Diakogiannaki, E., Gribble, F. M., & Reimann, F. (2012). Nutrient detection by incretin hormone secreting cells. *Physiology and Behavior*, *106*(3), 387–393. https://doi.org/10.1016/j.physbeh.2011.12.001

Dixon, J. B. (2010). Mechanisms of chylomicron uptake into lacteals. *Annals of the New York Academy of Sciences*, *1207*(Suppl 1), E52.

https://doi.org/10.1111/J.1749-6632.2010.05716.X

Donohoe, C., & Reynolds, J. (2010). Short bowel syndrome. *The Surgeon : Journal of the Royal Colleges of Surgeons of Edinburgh and Ireland*, *8*(5), 270–279. https://doi.org/10.1016/J.SURGE.2010.06.004

Drucker, D. J. (2019). The Discovery of GLP-2 and development of teduglutide for short bowel syndrome. *ACS Pharmacology and Translational Science*, *2*(2), 134–142. https://doi.org/10.1021/acsptsci.9b00016

Drucker, D. J., Ehrlich, P., Asa, S. L., & Brubaker, P. L. (1996). Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(15), 7911–7916. https://doi.org/10.1073/pnas.93.15.7911

Drucker, D., Shi, Q., Crivici, A., Sumner-Smith, M., Tavares, W., Hill, M., … Brubaker, P. (1997). Regulation of the biological activity of glucagon-like peptide 2 in vivo by dipeptidyl peptidase IV. *Nature Biotechnology*, *15*(7), 673–677. https://doi.org/10.1038/NBT0797-673

Drucker, D., & Yusta, B. (2014). Physiology and pharmacology of the enteroendocrine hormone glucagon-like peptide-2. *Annual Review of Physiology*, *76*, 561–583. https://doi.org/10.1146/ANNUREV-PHYSIOL-021113-170317

Dudele, A., Rasmussen, G. M., Mayntz, D., Malte, H., Lund, S., & Wang, T. (2015). Effects of ambient temperature on glucose tolerance and insulin sensitivity test outcomes in normal and obese c57 male mice. *Physiological Reports*, *3*(5). https://doi.org/10.14814/phy2.12396

Egecioglu, E., Anesten, F., Schéle, E., & Palsdottir, V. (2018). Interleukin-6 is important for regulation of core body temperature during long-term cold exposure in mice. *Biomedical Reports*, *9*(3), 206. https://doi.org/10.3892/BR.2018.1118

Ejarque, M., Sabadell-Basallote, J., Beiroa, D., Calvo, E., Keiran, N., Nuñez-Roa, C., … Fernández-Veledo, S. (2020). Adipose tissue is a key organ for the beneficial effects of GLP-2 metabolic function. *British Journal of Pharmacology*, bph.15278. https://doi.org/10.1111/bph.15278

Elmarzouki, H., Aboussaleh, Y., Bitiktas, S., Suer, C., Artis, A. S., Dolu, N., & Ahami, A. (2014). Effects of cold exposure on behavioral and electrophysiological parameters related with hippocampal function in rats. *Frontiers in Cellular Neuroscience*, *0*(SEP), 253. https://doi.org/10.3389/FNCEL.2014.00253

Engevik, A. C., & Goldenring, J. R. (2018). Trafficking Ion Transporters to the Apical Membrane of Polarized Intestinal Enterocytes. *Cold Spring Harbor Perspectives in Biology*, *10*(1). https://doi.org/10.1101/CSHPERSPECT.A027979

Fedorenko, A., Lishko, P. V., & Kirichok, Y. (2012). Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. *Cell*, *151*(2), 400–413. https://doi.org/10.1016/j.cell.2012.09.010

Feingold, K. R. (2021). Introduction to Lipids and Lipoproteins. *Endotext*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK305896/

Fesler, Z., Mitova, E., & Brubaker, P. (2020). GLP-2, EGF, and the Intestinal Epithelial IGF-1 Receptor Interactions in the Regulation of Crypt Cell Proliferation. *Endocrinology*, *161*(4). https://doi.org/10.1210/ENDOCR/BQAA040

Foufelle, F., & Ferré, P. (2013). Mechanism of Storage and Synthesis of Fatty Acids and Triglycerides in White Adipocytes. *Physiology and Physiopathology of Adipose Tissue*, 101–121. https://doi.org/10.1007/978-2-8178-0343-2_8

Fu, W., Farache, J., Clardy, S. M., Hattori, K., Mander, P., Lee, K., … Mathis, D. (2014). Epigenetic modulation of type-1 diabetes via a dual effect on pancreatic macrophages and β cells. *ELife*, *3*(November), 1–20. https://doi.org/10.7554/eLife.04631

Fu, Z., Gilbert, E. R., & Liu, D. (2013). Regulation of Insulin Synthesis and Secretion and Pancreatic Beta-Cell Dysfunction in Diabetes. *Current Diabetes Reviews*, *9*(1), 25. Retrieved from /pmc/articles/PMC3934755/

Fuchs, S., Yusta, B., Baggio, L. L., Varin, E. M., Matthews, D., & Drucker, D. J. (2020). Loss of Glp2r signaling activates hepatic stellate cells and exacerbates diet-induced steatohepatitis in mice. *JCI Insight*, *5*(8). https://doi.org/10.1172/jci.insight.136907

Galgani, J., & Ravussin, E. (2008). Energy metabolism, fuel selection and body weight regulation. *International Journal of Obesity (2005)*, *32*(Suppl 7), S109. https://doi.org/10.1038/IJO.2008.246

Gavrilova, O., Leon, L. R., Marcus-Samuels, B., Mason, M. M., Castle, A. L., Refetoff, S., … Reitman, M. L. (1999). Torpor in mice is induced by both leptin-dependent and -independent mechanisms. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(25), 14623. https://doi.org/10.1073/PNAS.96.25.14623

Giammanco, A., Cefalù, A. B., Noto, D., & Averna, M. R. (2015). The pathophysiology of intestinal lipoprotein production. *Frontiers in Physiology*, *0*(MAR), 61. https://doi.org/10.3389/FPHYS.2015.00061

González-García, I., Milbank, E., Diéguez, C., López, M., & Contreras, C. (2019). Glucagon, GLP-1 and thermogenesis. *International Journal of Molecular Sciences*, *20*(14). https://doi.org/10.3390/ijms20143445

Graaf, C. de, Donnelly, D., Wootten, D., Lau, J., Sexton, P. M., Miller, L. J., … Wang, M.-W. (2016). Glucagon-Like Peptide-1 and Its Class B G Protein–Coupled Receptors: A Long March to Therapeutic Successes. *Pharmacological Reviews*, *68*(4), 954. https://doi.org/10.1124/PR.115.011395

Gribble, F. M., Williams, L., Simpson, A. K., & Reimann, F. (2003). A Novel Glucose-Sensing Mechanism Contributing to Glucagon-Like Peptide-1 Secretion From the GLUTag Cell Line. *Diabetes*, *52*(5), 1147–1154. https://doi.org/10.2337/DIABETES.52.5.1147

Guan, X. (2014). The CNS glucagon-like peptide-2 receptor in the control of energy balance and glucose homeostasis. *Https://Doi.Org/10.1152/Ajpregu.00096.2014*, *307*(6), R585–R596. https://doi.org/10.1152/AJPREGU.00096.2014

Guillen, B., & Atherton, N. S. (2020). Short Bowel Syndrome. *Blackwell's Five-Minute Veterinary Consult Clinical Companion: Small Animal Gastrointestinal Diseases*, 526–530. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK536935/

Hansotia, T, Maida, A., Flock, G., Y, Y., K, T., Y, S., & DJ, D. (2007).

Extrapancreatic incretin receptors modulate glucose homeostasis, body weight, and energy expenditure. *The Journal of Clinical Investigation*, *117*(1), 143–152. https://doi.org/10.1172/JCI25483

Hansotia, Tanya, Baggio, L. L., Delmeire, D., Hinke, S. A., Yamada, Y., Tsukiyama, K., … Drucker, D. J. (2004). Double Incretin Receptor Knockout (DIRKO) Mice Reveal an Essential Role for the Enteroinsular Axis in Transducing the Glucoregulatory Actions of DPP-IV Inhibitors. *Diabetes*, *53*(5), 1326–1335. https://doi.org/10.2337/diabetes.53.5.1326

Hansson, G., & Johansson, M. (2010). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Gut Microbes*, *1*(1), 51–54. https://doi.org/10.4161/GMIC.1.1.10470

Hartmann, B., Harr, M. B., Jeppesen, P. B., Wojdemann, M., Deacon, C. F., Mortensen, P. B., & Holst, J. J. (2000). In Vivo and in Vitro Degradation of Glucagon-Like Peptide-2 in Humans1. *The Journal of Clinical Endocrinology & Metabolism*, *85*(8), 2884–2888. https://doi.org/10.1210/jcem.85.8.6717

Hein, G., Baker, C., Hsieh, J., Farr, S., & Adeli, K. (2013). GLP-1 and GLP-2 as yin and yang of intestinal lipoprotein production: evidence for predominance of GLP-2-stimulated postprandial lipemia in normal and insulin-resistant states. *Diabetes*, *62*(2), 373–381. https://doi.org/10.2337/DB12-0202

Herz, C., & Kiefer, F. (2019). Adipose tissue browning in mice and humans. *The Journal of Endocrinology*, *241*(3), R97–R109. https://doi.org/10.1530/JOE-18-0598

Higgins, P. B., Shade, R. E., Rodríguez-Sánchez, I. P., Garcia-Forey, M., Tejero, M. E., Voruganti, V. S., … Folli, F. (2016). CNS Control of Metabolism: Central GIP signaling stimulates peripheral GIP release and promotes insulin and pancreatic polypeptide secretion in nonhuman primates. *American Journal of Physiology - Endocrinology and Metabolism*, *311*(4), E661. https://doi.org/10.1152/AJPENDO.00166.2016

Hildebrand, H., Borgström, B., Békássy, A., Erlanson-Albertsson, C., & Helin, I. (1982). Isolated co-lipase deficiency in two brothers. *Gut*, *23*(3), 243. https://doi.org/10.1136/GUT.23.3.243

Hill, M. E., Asa, S. L., & Drucker, D. J. (1999). Essential Requirement for Pax6 in Control of Enteroendocrine Proglucagon Gene Transcription. *Molecular Endocrinology*, *13*(9), 1474–1486. https://doi.org/10.1210/MEND.13.9.0340

Holst, J. J., & Gromada, J. (2004, August). Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *American Journal of Physiology - Endocrinology and Metabolism*, Vol. 287. https://doi.org/10.1152/ajpendo.00545.2003

Holst, J. J., & Ørskov, C. (2004a). The Incretin Approach for Diabetes Treatment. *Diabetes*, *53*(suppl 3), S197–S204. https://doi.org/10.2337/DIABETES.53.SUPPL_3.S197

Hooper, L. V. (2015). Epithelial Cell Contributions to Intestinal Immunity. *Advances in Immunology*, *126*, 129–172. https://doi.org/10.1016/BS.AI.2014.11.003

Hruby, V. J. (1997). Glucagon:: Molecular Biology and Structure-Activity. *Principles of Medical Biology*, *10*(C), 387–401. https://doi.org/10.1016/S1569-2582(97)80161-4

Hsieh, J, Longuet, C., Baker, C., Qin, B., Federico, L., Drucker, D., & Adeli, K. (2010). The glucagon-like peptide 1 receptor is essential for postprandial lipoprotein synthesis and secretion in hamsters and mice. *Diabetologia*, *53*(3), 552–561. https://doi.org/10.1007/S00125-009-1611-5

Hsieh, Joanne, Longuet, C., Maida, A., Bahrami, J., Xu, E., Baker, C. L., … Adeli, K. (2009). Glucagon-Like Peptide-2 Increases Intestinal Lipid Absorption and Chylomicron Production via CD36. *Gastroenterology*, *137*(3), 997-1005.e4. https://doi.org/10.1053/J.GASTRO.2009.05.051

Hussain, M. M., Rava, P., Walsh, M., Rana, M., & Iqbal, J. (2012). Multiple functions of microsomal triglyceride transfer protein. *Nutrition & Metabolism 2012 9:1*, *9*(1), 1–16. https://doi.org/10.1186/1743-7075-9-14

Hussain, M., Shi, J., & Dreizen, P. (2003). Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. *Journal of Lipid Research*, *44*(1), 22–32. https://doi.org/10.1194/JLR.R200014-JLR200

Hylander, B. L., & Repasky, E. A. (2016, April 1). Thermoneutrality, Mice, and Cancer: A Heated Opinion. *Trends in Cancer*, Vol. 2, pp. 166–175.

https://doi.org/10.1016/j.trecan.2016.03.005

Iqbal, J., & Hussain, M. M. (2009, June). Intestinal lipid absorption. *American Journal of Physiology - Endocrinology and Metabolism*, Vol. 296, p. E1183. https://doi.org/10.1152/ajpendo.90899.2008

Irwin, N., Francis, J. M. E., & Flatt, P. R. (2011). Alterations of glucose-dependent insulinotropic polypeptide (GIP) during cold acclimation. *Regulatory Peptides*, *167*(1), 91–96. https://doi.org/10.1016/j.regpep.2010.12.001

Janssen, P., Rotondo, A., Mulé, F., & Tack, J. (2013). Review article: a comparison of glucagon-like peptides 1 and 2. *Alimentary Pharmacology & Therapeutics*, *37*(1), 18–36. https://doi.org/10.1111/APT.12092

Jasleen, J., Shimoda, N., Shen, E., A, T., EE, W., DO, J., … SW, A. (2000). Signaling mechanisms of glucagon-like peptide 2-induced intestinal epithelial cell proliferation. *The Journal of Surgical Research*, *90*(1), 13–18. https://doi.org/10.1006/JSRE.2000.5818

Jensen, J., Rustad, P. I., Kolnes, A. J., & Lai, Y.-C. (2011). The Role of Skeletal Muscle Glycogen Breakdown for Regulation of Insulin Sensitivity by Exercise. *Frontiers in Physiology*, *0*, 112. https://doi.org/10.3389/FPHYS.2011.00112

Jeppesen, P. B., Sanguinetti, E. L., Buchman, A., Howard, L., Scolapio, J. S., Ziegler, T. R., … Mortensen, P. B. (2005). Teduglutide (ALX-0600), a dipeptidyl peptidase IV resistant qlucagon-like peptide 2 analogue, improves intestinal function in short bowel syndrome patients. *Gut*, *54*(9), 1224–1231. https://doi.org/10.1136/gut.2004.061440

Jolma, V. M., Kendall, K., & Koldovský, O. (1980). Differences in the development of jejunum and ileum as observed in fetal rat intestinal isografts. Possible implications related to the villus size gradient. *American Journal of Anatomy*, *158*(2), 211–215. https://doi.org/10.1002/aja.1001580209

Jorsal, T., Rhee, N. A., Pedersen, J., Wahlgren, C. D., Mortensen, B., Jepsen, S. L., … Knop, F. K. (2018). Enteroendocrine K and L cells in healthy and type 2 diabetic individuals. *Diabetologia*, *61*(2), 284–294. https://doi.org/10.1007/s00125-017-4450-9

Kaikaew, K., Steenbergen, J., Themmen, A. P. N., Visser, J. A., & Grefhorst, A.

(2017). Sex difference in thermal preference of adult mice does not depend on presence of the gonads. *Biology of Sex Differences 2017 8:1*, *8*(1), 1–10. https://doi.org/10.1186/S13293-017-0145-7

Kalra, A., Yetiskul, E., Wehrle, C. J., & Tuma, F. (2021). Physiology, Liver. *StatPearls*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK535438/

Kamagate, A., Qu, S., Perdomo, G., Su, D., Kim, D. H., Slusher, S., … Dong, H. H. (2008). FoxO1 mediates insulin-dependent regulation of hepatic VLDL production in mice. *The Journal of Clinical Investigation*, *118*(6), 2347. https://doi.org/10.1172/JCI32914

Katsurada, K., & Yada, T. (2016). Neural effects of gut- and brain-derived glucagon-like peptide-1 and its receptor agonist. *Journal of Diabetes Investigation*, *7*, 64–69. https://doi.org/10.1111/JDI.12464

Kelesidis, T., Kelesidis, I., Chou, S., & Mantzoros, C. S. (2010, January 19). Narrative review: The role of leptin in human physiology: Emerging clinical applications. *Annals of Internal Medicine*, Vol. 152, pp. 93–100. https://doi.org/10.7326/0003-4819-152-2-201001190-00008

Kersten, S. (2001). Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Reports*, *2*(4), 282. https://doi.org/10.1093/EMBO-REPORTS/KVE071

Khan, R., Tomas, A., & Rutter, G. (2020). Effects on pancreatic Beta and other Islet cells of the glucose-dependent insulinotropic polypeptide. *Peptides*, *125*. https://doi.org/10.1016/J.PEPTIDES.2019.170201

Kiela, P. R., & Ghishan, F. K. (2016, April 1). Physiology of intestinal absorption and secretion. *Best Practice and Research: Clinical Gastroenterology*, Vol. 30, pp. 145–159. https://doi.org/10.1016/j.bpg.2016.02.007

Killion, E. A., Wang, J., Yie, J., D-H Shi, S., Bates, D., Min, X., … Lloyd, D. J. (2018). Anti-obesity effects of GIPR antagonists alone and in combination with GLP-1R agonists in preclinical models. *Sci. Transl. Med*, *10*, 3392. Retrieved from http://stm.sciencemag.org/

Kim, W., & Kim, G. (2020). An intestinal model with a finger-like villus structure fabricated using a bioprinting process and collagen/SIS-based cell-laden bioink.

Theranostics, *10*(6), 2495–2508. https://doi.org/10.7150/thno.41225

Klok, M., Jakobsdottir, S., & Drent, M. (2007). The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. *Obesity Reviews : An Official Journal of the International Association for the Study of Obesity*, *8*(1), 21–34. https://doi.org/10.1111/J.1467-789X.2006.00270.X

Knoop, K. A., & Newberry, R. D. (2018). Goblet cells: multifaceted players in immunity at mucosal surfaces. *Mucosal Immunology 2018 11:6*, *11*(6), 1551–1557. https://doi.org/10.1038/s41385-018-0039-y

Koehler, J. A., Baggio, L. L., Yusta, B., Longuet, C., Rowland, K. J., Cao, X., … Drucker, D. J. (2015). GLP-1R agonists promote normal and neoplastic intestinal growth through mechanisms requiring Fgf7. *Cell Metabolism*, *21*(3), 379–391. https://doi.org/10.1016/j.cmet.2015.02.005

Kong, S., Zhang, Y. H., & Zhang, W. (2018). Regulation of intestinal epithelial cells properties and functions by amino acids. *BioMed Research International*, *2018*. https://doi.org/10.1155/2018/2819154

Konturek. (2011). Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options - PubMed. Retrieved May 9, 2021, from https://pubmed.ncbi.nlm.nih.gov/22314561/

Krajmalnik-Brown, R., Ilhan, Z.-E., Kang, D.-W., & DiBaise, J. K. (2012). Effects of Gut Microbes on Nutrient Absorption and Energy Regulation. *Nutrition in Clinical Practice : Official Publication of the American Society for Parenteral and Enteral Nutrition*, *27*(2), 201. https://doi.org/10.1177/0884533611436116

Król, E., & Speakman, J. R. (2003). Limits to sustained energy intake VI. Energetics of lactation in laboratory mice at thermoneutrality. *Journal of Experimental Biology*, *206*(23), 4255–4266. https://doi.org/10.1242/JEB.00674

Langeveld, M., Tan, C. Y., Soeters, M. R., Virtue, S., Ambler, G. K., Watson, L. P. E., … Vidal-Puig, A. (2016). Mild cold effects on hunger, food intake, satiety and skin temperature in humans. *Endocrine Connections*, *5*(2), 65–73. https://doi.org/10.1530/EC-16-0004

Le Gall, M., Thenet, S., Aguanno, D., Jarry, A.-C., Genser, L., Ribeiro-Parenti, L., … Le Beyec, J. (2019). Intestinal plasticity in response to nutrition and

gastrointestinal surgery. *Nutrition Reviews*, *77*(3), 129–143. https://doi.org/10.1093/NUTRIT/NUY064

Lee, A. A., & Owyang, C. (2019). Sugars, sweet taste receptors, and brain responses. *Molecular Nutrition Carbohydrates*, 265–283. https://doi.org/10.1016/B978-0-12-849886-6.00020-3

Lee, H., Park, J., Seok, S., Baek, M., DJ, K., KE, L., … JH, P. (2006). Human originated bacteria, Lactobacillus rhamnosus PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. *Biochimica et Biophysica Acta*, *1761*(7), 736–744. https://doi.org/10.1016/J.BBALIP.2006.05.007

Li, B., Li, L., Li, M., Lam, S. M., Wang, G., Wu, Y., … Speakman, J. R. (2019). Microbiota Depletion Impairs Thermogenesis of Brown Adipose Tissue and Browning of White Adipose Tissue. *Cell Reports*, *26*(10), 2720-2737.e5. https://doi.org/10.1016/J.CELREP.2019.02.015

Liao, T., Hamosh, P., & Hamosh, M. (1984). Fat digestion by lingual lipase: mechanism of lipolysis in the stomach and upper small intestine. *Pediatric Research*, *18*(5), 402–409. https://doi.org/10.1203/00006450-198405000-00002

Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D., & Darnell, J. (2000). *G Protein –Coupled Receptors and Their Effectors*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK21718/

Lueschow, S. R., & McElroy, S. J. (2020, April 3). The Paneth Cell: The Curator and Defender of the Immature Small Intestine. *Frontiers in Immunology*, Vol. 11, p. 587. https://doi.org/10.3389/fimmu.2020.00587

Ma, T. Y., Nighot, P., & Al-Sadi, R. (2018). Tight Junctions and the Intestinal Barrier. In *Physiology of the Gastrointestinal Tract: Sixth Edition* (Vol. 1–2, pp. 587–639). https://doi.org/10.1016/B978-0-12-809954-4.00025-6

MacDonald, P. E., El-kholy, W., Riedel, M. J., Salapatek, A. M. F., Light, P. E., & Wheeler, M. B. (2002). The Multiple Actions of GLP-1 on the Process of Glucose-Stimulated Insulin Secretion. *Diabetes*, *51*(suppl 3), S434–S442. https://doi.org/10.2337/DIABETES.51.2007.S434

Macotela, Y., Boucher, J., Tran, T. T., & Ronald Kahn, C. (2009). *The sexual*

dimorphism of insulin resistance: Gender and depot differences in insulin sensitivity. Retrieved from http://diabetes.diabetesjournals.orghttp//diabetes.diabetesjournals.org.

Mansbach, C. M., & Siddiqi, S. A. (2010). The Biogenesis of Chylomicrons. *Annual Review of Physiology*, *72*, 315. https://doi.org/10.1146/ANNUREV-PHYSIOL-021909-135801

Mao, J., Hu, X., Xiao, Y., Yang, C., Ding, Y., Hou, N., … Zhang, X. (2013). Overnutrition Stimulates Intestinal Epithelium Proliferation Through β-Catenin Signaling in Obese Mice. *Diabetes*, *62*(11), 3736–3746. https://doi.org/10.2337/DB13-0035

Mapelli, C., Natarajan, S., Meyer, J., Bastos, M., Bernatowicz, M., Lee, V., … WR, E. (2009). Eleven amino acid glucagon-like peptide-1 receptor agonists with antidiabetic activity. *Journal of Medicinal Chemistry*, *52*(23), 7788–7799. https://doi.org/10.1021/JM900752A

Markovic, M. A., & Brubaker, P. L. (2019a). The roles of glucagon-like peptide-2 and the intestinal epithelial insulin-like growth factor-1 receptor in regulating microvillus length. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-49510-5

Martin, A. M., Sun, E. W., & Keating, D. J. (2020, January 1). Mechanisms controlling hormone secretion in human gut and its relevance to metabolism. *Journal of Endocrinology*, Vol. 244, pp. R1–R15. https://doi.org/10.1530/JOE-19-0399

Matikainen, N., Björnson, E., Söderlund, S., Borén, C., Eliasson, B., Pietiläinen, K. H., … Taskinen, M.-R. (2016). Minor Contribution of Endogenous GLP-1 and GLP-2 to Postprandial Lipemia in Obese Men. *PLOS ONE*, *11*(1), e0145890. https://doi.org/10.1371/JOURNAL.PONE.0145890

McCauley, H. A., Matthis, A. L., Enriquez, J. R., Nichol, J. T., Sanchez, J. G., Stone, W. J., … Wells, J. M. (2020). Enteroendocrine cells couple nutrient sensing to nutrient absorption by regulating ion transport. *Nature Communications 2020 11:1*, *11*(1), 1–10. https://doi.org/10.1038/s41467-020-18536-z

McConnell, R. E., Higginbotham, J. N., Shifrin, D. A., Jr., Tabb, D. L., Coffey, R. J.,

& Tyska, M. J. (2009). The enterocyte microvillus is a vesicle-generating organelle. *The Journal of Cell Biology*, *185*(7), 1285. https://doi.org/10.1083/JCB.200902147

Meeran, K., O'Shea, D., Edwards, C., MD, T., MM, H., I, G., … SR, B. (1999). Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. *Endocrinology*, *140*(1), 244–250. https://doi.org/10.1210/ENDO.140.1.6421

Meloni, A. R., DeYoung, M. B., Lowe, C., & Parkes, D. G. (2013). GLP-1 receptor activated insulin secretion from pancreatic β-cells: mechanism and glucose dependence. *Diabetes, Obesity & Metabolism*, *15*(1), 15. https://doi.org/10.1111/J.1463-1326.2012.01663.X

Miyawaki, K., Yamada, Y., Ban, N., Ihara, Y., Tsukiyama, K., Zhou, H., … Seino, Y. (2002). Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nature Medicine 2002 8:7*, *8*(7), 738–742. https://doi.org/10.1038/nm727

Miyawaki, K., Yamada, Y., Yano, H., Niwa, H., Ban, N., Ihara, Y., … Seino, Y. (1999). Glucose intolerance caused by a defect in the entero-insular axis: A study in gastric inhibitory polypeptide receptor knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(26), 14843. https://doi.org/10.1073/PNAS.96.26.14843

Moller, N., O'Brien, P., & Nair, K. S. (1998). Disruption of the Relationship between Fat Content and Leptin Levels with Aging in Humans. *The Journal of Clinical Endocrinology & Metabolism*, *83*(3), 931–934. https://doi.org/10.1210/JCEM.83.3.4620

Moriya, R., Shirakura, T., Ito, J., Mashiko, S., & Seo, T. (2009). Activation of sodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating incretin secretion in mice. *American Journal of Physiology. Endocrinology and Metabolism*, *297*(6). https://doi.org/10.1152/AJPENDO.00412.2009

Müller, T. D., Finan, B., Bloom, S. R., D'Alessio, D., Drucker, D. J., Flatt, P. R., … Tschöp, M. H. (2019). Glucagon-like peptide 1 (GLP-1). *Molecular Metabolism*, *30*, 72. https://doi.org/10.1016/J.MOLMET.2019.09.010

Mulvihill, E. E. (2018, April 1). Regulation of intestinal lipid and lipoprotein

metabolism by the proglucagon-derived peptides glucagon like peptide 1 and glucagon like peptide 2. *Current Opinion in Lipidology*, Vol. 29, pp. 95–103. https://doi.org/10.1097/MOL.0000000000000495

Mulvihill, E. E., & Drucker, D. J. (2014). Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. *Endocrine Reviews*, Vol. 35, pp. 992–1019. https://doi.org/10.1210/er.2014-1035

Nakajima, K., Nagamine, T., Fujita, M. Q., Ai, M., Tanaka, A., & Schaefer, E. (2014). Apolipoprotein B-48: A unique marker of chylomicron metabolism. *Advances in Clinical Chemistry*, *64*, 117–177. https://doi.org/10.1016/B978-0-12-800263-6.00003-3

Nakrani, M. N., Wineland, R. H., & Anjum, F. (2021). Physiology, Glucose Metabolism. *StatPearls*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK560599/

Nauck, M., & Meier, J. (2016). The incretin effect in healthy individuals and those with type 2 diabetes: physiology, pathophysiology, and response to therapeutic interventions. *The Lancet. Diabetes & Endocrinology*, *4*(6), 525–536. https://doi.org/10.1016/S2213-8587(15)00482-9

Navale, A. M., & Paranjape, A. N. (2016). Glucose transporters: physiological and pathological roles. *Biophysical Reviews*, *8*(1), 5. https://doi.org/10.1007/S12551-015-0186-2

Neff, E. P. (2020). A point on thermoneutrality for mice. *Lab Animal 2020 49:6*, *49*(6), 169–169. https://doi.org/10.1038/s41684-020-0560-y

Neumiller, J. (2015). Incretin-based therapies. *The Medical Clinics of North America*, *99*(1), 107–129. https://doi.org/10.1016/J.MCNA.2014.08.013

Nie, Y., Yan, Z., Yan, W., Xia, Q., & Zhang, Y. (2015). Cold exposure stimulates lipid metabolism, induces inflammatory response in the adipose tissue of mice and promotes the osteogenic differentiation of BMMSCs via the p38 MAPK pathway in vitro. *International Journal of Clinical and Experimental Pathology*, *8*(9), 10875. Retrieved from /pmc/articles/PMC4637617/

Nilaweera, K. N., & Speakman, J. R. (2018). Regulation of intestinal growth in response to variations in energy supply and demand. *Obesity Reviews*, *19*, 61–

72. https://doi.org/10.1111/OBR.12780

Noah, T. K., Donahue, B., & Shroyer, N. F. (2011). Intestinal development and differentiation. *Experimental Cell Research*, Vol. 317, pp. 2702–2710. https://doi.org/10.1016/j.yexcr.2011.09.006

Obernier, J. A., & Baldwin, R. L. (2006). Establishing an Appropriate Period of Acclimatization Following Transportation of Laboratory Animals. *ILAR Journal*, *47*(4), 364–369. https://doi.org/10.1093/ILAR.47.4.364

Park, J. H., Kotani, T., Konno, T., Setiawan, J., Kitamura, Y., Imada, S., … Matozaki, T. (2016). Promotion of intestinal epithelial cell turnover by commensal bacteria: Role of short-chain fatty acids. *PLoS ONE*, *11*(5). https://doi.org/10.1371/journal.pone.0156334

Patel, V., Joharapurkar, A., Shah, G., & Jain, M. (2014). Effect of GLP-1 based therapies on diabetic dyslipidemia. *Current Diabetes Reviews*, *10*(4), 238–250. https://doi.org/10.2174/1573399810666140707092506

Pelleymounter, M., Cullen, M., Healy, D., Hecht, R., Winters, D., & McCaleb, M. (1998). Efficacy of exogenous recombinant murine leptin in lean and obese 10- to 12-mo-old female CD-1 mice. *The American Journal of Physiology*, *275*(4). https://doi.org/10.1152/AJPREGU.1998.275.4.R950

Pellizzon, M. (2016). Choice of laboratory animal diet influences intestinal health. *Lab Animal*, *45*(6), 238–239. https://doi.org/10.1038/LABAN.1014

Poher, A. L., Altirriba, J., Veyrat-Durebex, C., & Rohner-Jeanrenaud, F. (2015). Brown adipose tissue activity as a target for the treatment of obesity/insulin resistance. *Frontiers in Physiology*, Vol. 6. https://doi.org/10.3389/fphys.2015.00004

Price, E. R., Ruff, L. J., Guerra, A., & Karasov, W. H. (2013). Cold exposure increases intestinal paracellular permeability to nutrients in the mouse. *Journal of Experimental Biology*, *216*(21), 4065–4070. https://doi.org/10.1242/jeb.088203

Ravussin, Y., Xiao, C., Gavrilova, O., & Reitman, M. L. (2014a). Effect of intermittent cold exposure on brown fat activation, obesity, and energy Homeostasis in mice. *PLoS ONE*, *9*(1), 85876. https://doi.org/10.1371/journal.pone.0085876

Reynolds, T. H., Dalton, A., Calzini, L., Tuluca, A., Hoyte, D., & Ives, S. J. (2019). The impact of age and sex on body composition and glucose sensitivity in C57BL/6J mice. *Physiological Reports*, *7*(3). https://doi.org/10.14814/PHY2.13995

Richards, P., Parker, H. E., Adriaenssens, A. E., Hodgson, J. M., Cork, S. C., Trapp, S., … Reimann, F. (2014). Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. *Diabetes*, *63*(4), 1224–1233. https://doi.org/10.2337/db13-1440

Ritzel, U., Fromme, A., Ottleben, M., Leonhardt, U., & Ramadori, G. (1997). Release of glucagon-like peptide-1 (GLP-1) by carbohydrates in the perfused rat ileum. *Acta Diabetologica*, *34*(1), 18–21. https://doi.org/10.1007/S005920050059

Rix, I., Nexøe-Larsen, C., Bergmann, N. C., Lund, A., & Knop, F. K. (2019). Glucagon Physiology. *Canadian Journal of Diabetes*, *34*(3), 187–188. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK279127/

Ronveaux, C. C., Tomé, D., & Raybould, H. E. (2015). Glucagon-Like Peptide 1 Interacts with Ghrelin and Leptin to Regulate Glucose Metabolism and Food Intake through Vagal Afferent Neuron Signaling. *The Journal of Nutrition*, *145*(4), 672. https://doi.org/10.3945/JN.114.206029

Rowland, K. J., Trivedi, S., Lee, D., Wan, K., Kulkarni, R. N., Holzenberger, M., & Brubaker, P. L. (2011). Loss of Glucagon-Like Peptide-2-Induced Proliferation Following Intestinal Epithelial Insulin-Like Growth Factor-1-Receptor Deletion. *YGAST*, *141*, 2166-2175.e7. https://doi.org/10.1053/j.gastro.2011.09.014

Saari, T. J., Raiko, J., U-Din, M., Niemi, T., Taittonen, M., Laine, J., … Virtanen, K. A. (2020). Basal and cold-induced fatty acid uptake of human brown adipose tissue is impaired in obesity. *Scientific Reports 2020 10:1*, *10*(1), 1–11. https://doi.org/10.1038/s41598-020-71197-2

Sandoval, D. A., & D'Alessio, D. A. (2015). Physiology of proglucagon peptides: Role ofglucagon and GLP-1 in health and disease. *Physiological Reviews*, *95*(2), 513–548. https://doi.org/10.1152/physrev.00013.2014

Schwartz, E., Koska, J., Mullin, M., Syoufi, I., Schwenke, D., & Reaven, P. (2010).

Exenatide suppresses postprandial elevations in lipids and lipoproteins in individuals with impaired glucose tolerance and recent onset type 2 diabetes mellitus. *Atherosclerosis*, *212*(1), 217–222. https://doi.org/10.1016/J.ATHEROSCLEROSIS.2010.05.028

Scrocchi, L. A., Brown, T. J., Maclusky, N., Brubaker, P. L., Auerbach, A. B., Joyner, A. L., & Drucker, D. J. (1996). Glucose intolerance but normal satiety in mice with a null mutation in the glucagon–like peptide 1 receptor gene. *Nature Medicine 1996 2:11*, *2*(11), 1254–1258. https://doi.org/10.1038/nm1196-1254

Seino, Y., Fukushima, M., & Yabe, D. (2010, February). GIP and GLP-1, the two incretin hormones: Similarities and differences. *Journal of Diabetes Investigation*, Vol. 1, pp. 8–23. https://doi.org/10.1111/j.2040-1124.2010.00022.x

Senn, J. R., Maushart, C. I., Gashi, G., Michel, R., Lalive d'Epinay, M., Vogt, R., … Betz, M. J. (2018). Outdoor Temperature Influences Cold Induced Thermogenesis in Humans. *Frontiers in Physiology*, *0*(AUG), 1184. https://doi.org/10.3389/FPHYS.2018.01184

Shearer, G. C., Joles, J. A., Jones, H., Walzem, R. L., & Kaysen, G. A. (2000). Estrogen effects on triglyceride metabolism in analbuminemic rats. *Kidney International*, *57*(6), 2268–2274. https://doi.org/10.1046/J.1523-1755.2000.00087.X

Sherwood, N. M., & Adams, B. A. (2004). Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP)/Glucagon Superfamily. *Encyclopedia of Endocrine Diseases*, 629–635. https://doi.org/10.1016/B0-12-475570-4/01417-7

Shi, X., Zhou, F., Li, X., Chang, B., Li, D., Wang, Y., … Guan, X. (2013). Central GLP-2 enhances hepatic insulin sensitivity via activating PI3K signaling in POMC neurons. *Cell Metabolism*, *18*(1), 86–98. https://doi.org/10.1016/j.cmet.2013.06.014

Shin, E. D., Drucker, D. J., & Brubaker, P. L. (2005). *Glucagon-like peptide 2: an update*.

Shin, E. D., Estall, J. L., Izzo, A., Drucker, D. J., & Brubaker, P. L. (2005). Mucosal adaptation to enteral nutrients is dependent on the physiologic actions of

glucagon-like peptide-2 in mice. *Gastroenterology*, *128*(5), 1340–1353. https://doi.org/10.1053/j.gastro.2005.02.033

Shreiner, A. B., Kao, J. Y., & Young, V. B. (2015). The gut microbiome in health and in disease. *Current Opinion in Gastroenterology*, *31*(1), 69. https://doi.org/10.1097/MOG.0000000000000139

Shroyer, N. F., & Kocoshis, S. A. (2011). Anatomy and Physiology of the Small and Large Intestines. In *Pediatric Gastrointestinal and Liver Disease* (pp. 324–336). https://doi.org/10.1016/B978-1-4377-0774-8.10031-4

Sigalet, D. L., Bawazir, O., Martin, G. R., Wallace, L. E., Zaharko, G., Miller, A., & Zubaidi, A. (2006). Glucagon-like Peptide-2 Induces a Specific Pattern of Adaptation in Remnant Jejunum. *Digestive Diseases and Sciences 2006 51:9*, *51*(9), 1557–1566. https://doi.org/10.1007/S10620-006-9077-5

Singh, A. K. (2014, November 1). Dipeptidyl peptidase-4 inhibitors: Novel mechanism of actions. *Indian Journal of Endocrinology and Metabolism*, Vol. 18, pp. 753–759. https://doi.org/10.4103/2230-8210.141319

Sonnenburg, J., Xu, J., Leip, D., CH, Chen, C., BP, W., J, W., … JI, G. (2005). Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science (New York, N.Y.)*, *307*(5717), 1955–1959. https://doi.org/10.1126/SCIENCE.1109051

Sørensen, L. B., Flint, A., Raben, A., Hartmann, B., Holst, J. J., & Astrup, A. (2003). No effect of physiological concentrations of glucagon-like peptide-2 on appetite and energy intake in normal weight subjects. *International Journal of Obesity 2003 27:4*, *27*(4), 450–456. https://doi.org/10.1038/sj.ijo.0802247

Spreckley, E., & Murphy, K. G. (2015, July 20). The L-Cell in Nutritional Sensing and the Regulation of Appetite. *Frontiers in Nutrition*, Vol. 2, p. 1. https://doi.org/10.3389/fnut.2015.00023

Stelt, I. van der, Hoevenaars, F., Široká, J., Ronde, L. de, Friedecký, D., Keijer, J., & Schothorst, E. van. (2017). Metabolic Response of Visceral White Adipose Tissue of Obese Mice Exposed for 5 Days to Human Room Temperature Compared to Mouse Thermoneutrality. *Frontiers in Physiology*, *8*(MAR). https://doi.org/10.3389/FPHYS.2017.00179

Suzuki, K., Harada, N., Yamane, S., Nakamura, Y., Sasaki, K., Nasteska, D., … Inagaki, N. (2013). Transcriptional regulatory factor X6 (Rfx6) increases gastric inhibitory polypeptide (GIP) expression in enteroendocrine K-cells and is involved in GIP hypersecretion in high fat diet-induced obesity. *The Journal of Biological Chemistry*, *288*(3), 1929–1938. https://doi.org/10.1074/JBC.M112.423137

Tabuchi, C., & Sul, H. S. (2021). Signaling Pathways Regulating Thermogenesis. *Frontiers in Endocrinology*, *0*, 243. https://doi.org/10.3389/FENDO.2021.595020

Timper, K., Grisouard, J., Sauter, N., Herzog-Radimerski, T., Dembinski, K., Peterli, R., … Christ-Crain, M. (2013). Glucose-dependent insulinotropic polypeptide induces cytokine expression, lipolysis, and insulin resistance in human adipocytes. *American Journal of Physiology. Endocrinology and Metabolism*, *304*(1). https://doi.org/10.1152/AJPENDO.00100.2012

Trefts, E., Gannon, M., & Wasserman, D. H. (2017). The liver. *Current Biology : CB*, *27*(21), R1147. https://doi.org/10.1016/J.CUB.2017.09.019

Tseng, C. C., Jarboe, L. A., Landau, S. B., Williams, E. K., & Wolfe, M. M. (1993). Glucose-dependent insulinotropic peptide: structure of the precursor and tissue-specific expression in rat. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(5), 1992. https://doi.org/10.1073/PNAS.90.5.1992

Tso, P., & Fujimoto, K. (1991). The absorption and transport of lipids by the small intestine. *Brain Research Bulletin*, *27*(3–4), 477–482. https://doi.org/10.1016/0361-9230(91)90145-A

Ugleholdt, R., Poulsen, M. L. H., Holst, P. J., Irminger, J. C., Orskov, C., Pedersen, J., … Holst, J. J. (2006). Prohormone convertase 1/3 is essential for processing of the glucose-dependent insulinotropic polypeptide precursor. *Journal of Biological Chemistry*, *281*(16), 11050–11057. https://doi.org/10.1074/jbc.M601203200

Usdin, T. B., Mezey, E., Button, D. C., Brownstein, M. J., & Bonner, T. I. (1993). Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive

intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. *Endocrinology*, *133*(6), 2861–2870. https://doi.org/10.1210/ENDO.133.6.8243312

Ussar, S., Haering, M. F., Fujisaka, S., Lutter, D., Lee, K. Y., Li, N., … Ronald Kahn, C. (2017). Regulation of glucose uptake and enteroendocrine function by the intestinal epithelial insulin receptor. *Diabetes*, *66*(4), 886–896. https://doi.org/10.2337/db15-1349

Vaanholt, L. M., Daan, S., Schubert, K. A., & Visser, G. H. (2009). Metabolism and aging: Effects of cold exposure on metabolic rate, body composition, and longevity in mice. *Physiological and Biochemical Zoology*, *82*(4), 314–324. https://doi.org/10.1086/589727

van der Flier, L. G., & Clevers, H. (2009). Stem Cells, Self-Renewal, and Differentiation in the Intestinal Epithelium. *Annual Review of Physiology*, *71*(1), 241–260. https://doi.org/10.1146/annurev.physiol.010908.163145

van Tilbeurgh, H., Bezzine, S., Cambillau, C., Verger, R., & Carrière, F. (1999). Colipase: structure and interaction with pancreatic lipase. *Biochimica et Biophysica Acta*, *1441*(2–3), 173–184. https://doi.org/10.1016/S1388-1981(99)00149-3

Wahbeh, G. T., & Christie, D. L. (2011). Basic Aspects of Digestion and Absorption. *Pediatric Gastrointestinal and Liver Disease*, 10-19.e2. https://doi.org/10.1016/B978-1-4377-0774-8.10002-8

Walsh, N. A., Yusta, B., DaCambra, M. P., Anini, Y., Drucker, D. J., & Brubaker, P. L. (2003). Glucagon-Like Peptide-2 Receptor Activation in the Rat Intestinal Mucosa. *Endocrinology*, *144*(10), 4385–4392. https://doi.org/10.1210/en.2003-0309

Wang, C.-Y., & Liao, J. K. (2012). A Mouse Model of Diet-Induced Obesity and Insulin Resistance. *Methods in Molecular Biology (Clifton, N.J.)*, *821*, 421. https://doi.org/10.1007/978-1-61779-430-8_27

Wang, Z., Ning, T., Song, A., Rutter, J., Wang, Q. A., & Jiang, L. (2020). Chronic cold exposure enhances glucose oxidation in brown adipose tissue. *EMBO Reports*, *21*(11). https://doi.org/10.15252/EMBR.202050085

Warden, C. H., & Fisler, J. S. (2008). Comparisons of diets used in animal models of high fat feeding. *Cell Metabolism*, *7*(4), 277. https://doi.org/10.1016/J.CMET.2008.03.014

Wilcox, G. (2005). Insulin and Insulin. *The Clinical Biochemist. Reviews*, *26*(May), 19–39. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16278749

Wright, E. M., Loo, D. D. F., & Hirayama, B. A. (2011). Biology of Human Sodium Glucose Transporters. *Https://Doi.Org/10.1152/Physrev.00055.2009*, *91*(2), 733–794. https://doi.org/10.1152/PHYSREV.00055.2009

Wu, T., Rayner, C. K., Jones, K., & Horowitz, M. (2010). Chapter 3 - Dietary Effects on Incretin Hormone Secretion. *Vitamins and Hormones: Incretins and Insulin Secretion*, *84*, 81–110. https://doi.org/10.1016/S0083-6729(10)84015-4

Xiao, H., & Kang, S. (2020). The Role of the Gut Microbiome in Energy Balance With a Focus on the Gut-Adipose Tissue Axis. *Frontiers in Genetics*, *0*, 297. https://doi.org/10.3389/FGENE.2020.00297

Xu, Z., You, W., Zhou, Y., Chen, W., Wang, Y., & Shan, T. (2019). Cold-induced lipid dynamics and transcriptional programs in white adipose tissue. *BMC Biology*, *17*(1), 1–21. https://doi.org/10.1186/s12915-019-0693-x

Yabe, D., & Seino, Y. (2011). Two incretin hormones GLP-1 and GIP: Comparison of their actions in insulin secretion and β cell preservation. *Progress in Biophysics and Molecular Biology*, *107*(2), 248–256. https://doi.org/10.1016/J.PBIOMOLBIO.2011.07.010

Yamane, S., & Harada, N. (2019). Gastric inhibitory polypeptide/glucose-dependent insulinotropic polypeptide signaling in adipose tissue. *Journal of Diabetes Investigation*, *10*(1), 3. https://doi.org/10.1111/JDI.12942

Yang, D., Zhou, C., Liu, Q., & Wang, M. (2015). Landmark studies on the glucagon subfamily of GPCRs: from small molecule modulators to a crystal structure. *Acta Pharmacologica Sinica*, *36*(9), 1033. https://doi.org/10.1038/APS.2015.78

Yurkovetskiy, L., Burrows, M., Khan, A. A., Graham, L., Volchkov, P., Becker, L., … Chervonsky, A. V. (2013). Gender bias in autoimmunity is influenced by microbiota. *Immunity*, *39*(2), 400–412. https://doi.org/10.1016/J.IMMUNI.2013.08.013

Yusta, B., Matthews, D., Koehler, J., Pujadas, G., Kaur, K., & Drucker, D. (2019). Localization of Glucagon-Like Peptide-2 Receptor Expression in the Mouse. *Endocrinology*, *160*(8), 1950–1963. https://doi.org/10.1210/EN.2019-00398

Zhang, Q., Delessa, C. T., Augustin, R., Bakhti, M., Colldén, G., Drucker, D. J., … Müller, T. D. (2021). The glucose-dependent insulinotropic polypeptide (GIP) regulates body weight and food intake via CNS-GIPR signaling. *Cell Metabolism*, *33*(4), 833-844.e5. https://doi.org/10.1016/J.CMET.2021.01.015

Zhao, Z.-J. (2012). Effect of cold exposure on energy budget and thermogenesis during lactation in Swiss mice raising large litters. *Biology Open*, *1*(4), 397–404. https://doi.org/10.1242/BIO.2012661

6. Curriculum Vitae

Antonio Hanson

Education

Masters of Biochemistry 2019-present
University of Ottawa, ON

Honours Bachelor of Science with Specialization in Biomedical Science 2015-2019
University of Ottawa, ON

Major Projects:

- Presenter at University of Ottawa Faculty of Medicine Research Day (Online Format) (2021)
- Presenter at University of Ottawa Cardiovascular Research Day (Online Format)
- Presenter at University of Ottawa Faculty of Medicine Department of Biochemistry Research Day (Online Format) (2021)
- Presenter at Insulin 100 (Online Format) (2021)
- Presenter at 2020 Canadian Vascular & Lipid Summit (Canadian Lipoprotein Conference) (Online Format) (2020)
- University of Ottawa Heart Institute Work in Progress (Online Format) (2020)
- Presenter at University of Ottawa Faculty of Medicine Day (Online Format) (2020)
- Selected for poster presentation at the University of Ottawa Heart Institute Research Day (Cancelled) (2020)
- Presenter at University of Ottawa Faculty of Medicine Day (2020)
- Presenter at University of Ottawa Faculty of Medicine Department of Biochemistry Research Day (2020)
- Selected for poster presentation at the University of Ottawa Heart Institute Research Day (2020)
- Presenter at Montreal Diabetes Research Centre Conference. Montreal, QC (2020)
- Presenter at Canadian Lipoprotein Conference. Banff, AB (2019)
- Presenter at University of Ottawa Heart Institute Summer Student Research Day (2019)
- Presenter at Ottawa Cardiovascular Research Day (2019)
- Fourth year honour's project with Dr. Erin Mulvihill's lab and poster presentation (2019)
  "Determining the Role of the Preproglucagon Peptides in Gut Adaptation and Nutrient Absorption"
- Seminar critique and presentation of scientific article

Relevant Courses:

- Biochemistry lab: learned different lab techniques such as calculating serial dilution, percent yield, gel filtration chromatography, colorimetric assays, ion-exchange chromatography, SDS polyacrylamide gel electrophoresis, enzyme kinetics, thin layer chromatography, isolating DNA
- Microbiology: discussed cellular and acellular microorganisms, classification and characteristics of different microorganisms, viruses, immunology, staining methods, bacterial anatomy, anatomy of eukaryotic cells, cell culturing, bacterial growth, vaccines, epidemiology
- Molecular biology: explored DNA replication, DNA recombination, protein synthesis, RNA structure and function, gene expression in prokaryotes and eukaryotes
- Organic chemistry lab: performed thin layer chromatography, column chromatography, gas chromatography, solvent crystallization, extractions, distillations, sublimation
- Spectroscopy: studied elemental analysis, mass spectrometry, infrared spectroscopy, ultraviolet and visible spectroscopy, nuclear magnetic resonance spectroscopy, proton and carbon NMR, x-ray crystallography, cryo-electron microscopy, heteronuclear single quantum coherence
- Seminar: read/analyzed scientific journals and papers related to infectious diseases, presented research journals, created a research proposal

Research Experience

Research Publications:

- Morrow, N.M.; Trzaskalski N.A.; **Hanson, A.A.**; Fadzeyeva, E.; Telford, D.E.; Chhoker, S.S.; Sutherland, B.G.; Edwards, J.Y.; Huff, M.W.; Mulvihill, E.E. (2021) Nobiletin Prevents High-Fat Diet-Induced Dysregulation of Intestinal Lipid Metabolism and Attenuates Postprandial Lipemia. *Arteriosclerosis, Thrombosis, and Vascular Biology* https://www.ahajournals.org/doi/abs/10.1161/ATVBAHA.121.316896

- Morrow, N.M.; **Hanson, A.A.**; Mulvihill, E.E. (2021) Distinct Identity of GLP-1R, GLP-2R, and GIPR Expressing Cells and Signaling Circuits Within the Gastrointestinal Tract. *Frontiers in Cell and Developmental Biology* https://www.frontiersin.org/articles/10.3389/fcell.2021.703966/full

- Varin, E.M.; **Hanson, A.A**.; Beaudry, J.L.; Nguyen, M.-A.; Cao, X.; Baggio, L.L.; Mulvihill, E.E; Drucker, D.J. (2020) Hematopoietic vs. enterocyte-derived dipeptidyl peptidase-4 differentially regulates triglyceride excursion in mice. *JCI Insight*. https://insight.jci.org/articles/view/140418

Awards:

- University of Ottawa Faculty of Medicine Department of Biochemistry Research Day Metabolism Presentation Award Winner

- Voted best presentation/research project by Faculty of Medicine

Research Assistant/Honours Student (Dr. Erin Mulvihill Lab) 2017-present
Faculty of Medicine, University of Ottawa, ON

- Observing physical changes of the gut of mice under cold and warm conditions
- Observing changes in gene expression of mice under cold and warm conditions
- Determining role of incretin hormones in a various mouse genotypes
- Performed DPP4 activity assay, PCR, BSA assay, ELISA, histological analysis, RNA isolation, cryosection, western blot, work with mice, Microsoft Excel
- Fourth year honours research presentation
- Variety of oral/poster presentations

Research Assistant (Dr. Subash Sad Lab) 2017-2018
Faculty of Medicine, University of Ottawa, ON

- Observed transcription factor FoxO3A as a key role in regulating inflammatory response in mice
- Effect of pseudomonas aeruginosa in mice, and relationship with cystic fibrosis
- Performed western blot, protein assay, cell culture work, colorimetric assay, animal work

Research Assistant (Dr. Howard Rundle Lab) 2016-2017
Faculty of Science, University of Ottawa, ON

- Observed sexual selection of drosophila under specific conditions
- Preparing the meals and environment for the flies, recording results of the assays performed

Past Work Experiences

University of Ottawa Adventures in Engineering and Science
2017

- Taught children about mathematics, biology, chemistry, physics
- School presentations

Vezina Opticians/Eye Care Clinic 2016-2017

- Optometrist assistant
- Performed eye exams
- Administration

Vector Marketing Canada 2016-2017

- Sales and presentations for top quality cutlery

Community Involvement

Ottawa Greek Fest Zorba Show Head Teacher/Organizer 2016-present

- Head teacher and performer for the Zorba Show
- Booking live bands
- Ordering props
- In charge of financials related to the show

Project Pulse Ambassador 2015-present

- Ticket sales and organization of the event
- Presentations to high school students

SSA and Capital Catwalk model 2016-present

- Modelled clothing for Science Student association
- Catwalk model for Narcisse Palaise Haute Couture

Greek Dance Performer 2010-present

- Zorba Show head instructor and organizer
- Zorba Show performer
- Wedding performer

References

Available upon request