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Ecole doctorale
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Glucocorticoids and Intracrine Cortisol Metabolism in human Islets: Impact on Glucose Stimulated Insulin secretion

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Jury members

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Glucocorticoïdes et métabolisme intracrine du cortisol dans les îlots de Langerhans humains : Impact sur la sécrétion d'insuline stimulée par le glucose

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Thèse Soumise à l'Université de Lille en vue de l'obtention du diplôme de Doctorat en Sciences la Vie et de la Santé

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What you do makes a difference,
and you have to decide what kind of difference you want to make
Jane Goodall

SUMMARY

Excessive glucocorticoid (GC) exposure, as seen in patients receiving GC therapy, can lead to β-cell dysfunction and diabetes in up to 40% of the cases. In obesity, increased local cortisol exposure due to altered metabolism contributes to diabetes onset. High doses of GCs like dexamethasone (DEX) are known to inhibit glucose-stimulated insulin secretion (GSIS), but the effects of lower doses and other GCs, such as hydrocortisone (HC) and prednisone (PRED), remain underexplored. The enzyme 5α-reductase type 1 (SRD5A1) is a crucial enzyme for GC degradation, modulating their bioavailability. Inhibition or knockout of *SRD5A1* is associated with impaired insulin sensitivity and increased diabetes risk.

This first part of my thesis investigates the impact of “low therapeutic” doses of PRED (equivalent to 5 to 10 mg administrated orally) and other GCs on glucose stimulated insulin secretion (GSIS). We showed that PRED significantly decreases GSIS, with DEX having a worse effect compared to PRED and HC. BMI, age, or sex do not significantly influence the direct impact of PRED on insulin secretion.

The second part of the work aimed to characterize GC metabolism in human islets. SRD5A1 is the only A-ring reductase expressed in islets, and its expression, along with HSD11B1, is localized within the β-cells of human islets. We demonstrated evidence of intracrine metabolism of cortisol in intact primary human islets cultured under dynamic experimental settings. Expression data reveals significantly diminished expression of both HSD11B1 and *SRD5A1* in T2D donors compared to normoglycemic donors.

The last part aimed to provide proof of concept that decreased cortisol bioavailability via the overexpression of SRD5A1 in human islets mitigates the inhibitory effect of GCs on GSIS. SR5DA1 overexpression attenuated the impact of HC on the first phase of insulin secretion, but not the PRED impact.

To conclude, even at low doses, GCs impair GSIS. The decrease in *SRD5A1* expression in islets may contribute to the development of diabetes in metabolic context. SRD5A1 overexpression protects against the deleterious impact of cortisol on GSIS, providing additional evidence to support the enzyme's role in local cortisol overexposure and the development of diabetes. However, increasing SRD5A1 activity may not be an effective approach to protect against metabolic complications induced by GC therapy. Other aspects of β-cell function, especially cell viability, need to be studied. Moreover, the potential benefits of SRD5A1 in modulating insulin resistance and fatty liver disease should be investigated. These further studies will provide more insight into the potential of SRD5A1 as a therapeutic target.

RESUMÉ

Une exposition excessive aux glucocorticoïdes (GC), comme observée chez les patients recevant une corticothérapie, peut entraîner un dysfonctionnement des cellules β et un diabète chez jusqu'à 40% des patients. Dans l'obésité, une surexposition locale au cortisol secondaire à une altération du métabolisme du cortisol contribue à l'apparition du diabète. Des doses élevées de GC comme la dexaméthasone (DEX) inhibent la sécrétion d'insuline stimulée par le glucose (SISG), mais les effets de doses plus faibles et des autres GC, tels que l'hydrocortisone (HC) et la prednisone (PRED), restent peu étudiés. L'enzyme 5α-réductase de type 1 (SRD5A1) est une enzyme cruciale pour la dégradation des GC, modulant ainsi leur biodisponibilité. L'inhibition de SRD5A1 est associée à une altération de la sensibilité à l'insuline et à un risque accru de diabète.

La première partie de ma thèse étudie l'impact de doses "thérapeutiques faibles" de PRED (équivalentes à 5 à 10 mg par voie orale) et d'autres GC sur la SISG étudiée par périfusion dans des îlots isolés de pancréas humains. Tous les GCs diminuent significativement la SISG, la DEX ayant un impact plus important que la PRED et l'HC. L'IMC, l'âge ou le sexe n'influencent pas significativement l'impact de la PRED sur la sécrétion d'insuline.

La deuxième partie du travail caractérise le métabolisme des GC dans les îlots humains. SRD5A1 est la seule réductase A-ring dans les îlots, et son expression, ainsi que celle de HSD11B1, est localisée dans les cellules β des îlots. Nous avons démontré l'existence d'un métabolisme intracrine du cortisol dans des cultures primaires d'îlots humains. L'expression de *HSD11B1* et *SRD5A1* est significativement

diminuée dans les îlots des donneurs atteints de DT2 par rapport aux donneurs normoglycémiques.

La dernière partie visait à prouver que la diminution de la biodisponibilité du cortisol via la surexpression de SRD5A1 dans les îlots humains atténue l'effet inhibiteur des GC sur la SISG. La surexpression de SR5DA1 a permis d'atténuer l'impact de l'HC sur la première phase de la SISG, mais pas de la PRED.

En conclusion, même à faibles doses, les GC altèrent la SISG. La diminution de l'expression de *SRD5A1* dans les îlots peut contribuer au développement du diabète dans un contexte métabolique. La surexpression de SRD5A1 protège contre l'impact délétère du cortisol sur la SISG. Ces résultats supportent le rôle de SRD5A1 dans la surexposition locale au cortisol et le développement du diabète. Cependant, l'augmentation de l'activité de SRD5A1 ne semble pas efficace pour protéger contre les complications métaboliques induites par la corticothérapie. D'autres aspects de la fonction des cellules β, en particulier la viabilité cellulaire, vont être étudiés. Par ailleurs, le bénéfice potentiel de SRD5A1 dans la modulation de la résistance à l'insuline et de la stéatose hépatique doivent être étudiés. Ces études complémentaires permettront de mieux comprendre le potentiel du gène SRD5A1 dans la modulation de la résistance à l'insuline et de la maladie du foie gras.

TABLE OF CONTENTS

ABBREVIATIONS

GCs – Glucocorticoids
GCID – glucocorticoids induced diabetes
DEX – dexamethasone
PRED – prednisolone
HC-hydrocortisone
COPD – Chronic obstructive pulmonary disease
HPA – Hypothalamic-pituitary-adrenal axis
CRH – corticotropin-releasing hormone
AVP – Arginine vasopressin
PVN – Paraventricular nucleus
ACTH – Adrenocorticotropin hormone
CRHR1 – corticotropin-releasing hormone type-1 receptor
AVP_{1B} – Arginine vasopressin 1B receptor
MC2R – Melanocortin type-2 receptor
11β-HSD1 –11β-hydroxysteroid dehydrogenase type-1
11β-HSD2 –11β-hydroxysteroid dehydrogenase type-2
5α-THF – 5α-tetrahydrocortisol
THF– tetrahydrocortisol
THE – tetrahydrocortisone
SRD5A1 – steroid 5 α-reductase 1
AKR1D1 – aldo-keto reductase family 1 member D1
AKR1C3 – aldo-keto reductase family 1 member C3
AKR1C4 – aldo-keto reductase family 1 member C4
EC50 – 50% maximal effective concentration
NYP – neuropeptide Y family of proteins
GCGR – glucagon receptor
GHSR – growth hormone secretagogue receptor
GLP-1 – glucagon-like peptide-1
GLP -2 – glucagon-like peptide-2
GluR4 – glutamate receptor 4
AA - amino acids
GLUT1 – glucose transporter-1
GLUT2 – glucose transporter-2
GLUT4 – glucose transporter-4
UTR – untranslated regions
NR3C1 – nuclear receptor subfamily 3, group C, member 1
NTD – N-terminal domain
DBD – DNA-binding domain
HR – hinge region
LBD – ligand-binding domain
TNF – tumour necrosis factor
GR – glucocorticoid receptor
GRE – glucocorticoid response elements
TF – transcription factor
GRα – classical cytoplasmic human
GRβ – non-classical GR
mGRα – membrane-bound classical human GR
HSPs – heat shock proteins
GBS – glucocorticoid binding sites
CRC – chromatin remodelling complexes
AP-1 – activator protein -1
NF-κB – nuclear factor kappa-B
IκB – inhibitor of nuclear factor kappa-B
MAPK – mitogen activated protein kinase
cAMP – cyclic adenosine monophosphate
cGMP – cyclic guanosine monophospahate
MR – mineralocorticoid receptor
MRE – mineralocorticoid response element
IC50 – 50% inhibitory concentration
T1D – type-1 diabetes
T2D – type-2 diabetes
CBG – corticosterone binding globulin
P-GP – p glycoprotein
ABCB1 – ATP-binding cassette transporter
CYP3A4/5 - cytochrome P450 family 3, subfamily A4/5
PC1/2 – Prohormone convertases ½
GSK-3β – glycogen synthase kinase 3β
IP3 – inositol 1,4,5-trisphosphate

SGLT1 – sodium-dependent glucose transporter 1

SGLT2 – sodium-dependent glucose transporter 2

HOMA-β – homeostatic model assessment -β-cell

HOMA-IR – homeostatic model assessment -estimated insulin resistance

HOMA-IR - homeostatic model assessment -estimated insulin resistance

INS – human insulin gene

IVGTT – intravenous glucose tolerance test

Ins1, *Ins2* – mouse insulin genes 1 and 2

OGTT – oral glucose tolerance test

ER – endoplasmic reticulum

RANKL – receptor activator of nuclear factor kappa-B ligand

SAT – subcutaneous adipose tissues

OPG – osteoprotegerin

VAT – visceral adipose tissues

BMP – bone morphogenetic protein

BMI – body mass index

NO – nitric oxide

HbA1c – glycated hemoglobin A1c

AMPK – AMP-activated protein kinase

ATP – adenosine triphosphate

Compound RU486 – mifepristone

ADP – adenosine diphosphate

Compound BVT.2733 – 3-chloro-2-methyl-N-(4-(2-(4-methyl-1-piperazinyl)-2-oxoethyl)-1,3-thiazol-2-yl)benzenesulfonamide

K_{ATP} – potassium - ATP gated channels

Compound INCB13739 – N/A

IP3 – inositol 1,4,5-trisphosphate

Compound MK-0916 – 3-((1s,3s)-1-(4-chlorophenyl)-3-fluorocyclobutyl)-4,5-dicyclopropyl-4H-1,2,4-triazole

DAG – diacylglycerides

GSK-3β – glycogen synthase kinase 3β

GLIZ– glucocorticoid induced leucine zipper

Compound MK-0736 – 4H-1,2,4 Triazole, 3-(4 (3(ethylsulfonyl)propyl)bicyclo(2.2.2)o t-1-yl)-4-methyl-5-(2 (trifluoromethyl)phenyl)

TLR – toll-like receptor

NOX4 – NADPH oxidase 4

DUSP1 – dual-specificity protein phosphatase 1

TXNIP – thioredoxin-interacting protein

GLIZ. – glucocorticoid-induced leucine zipper

GSTP1 – glutathione S-transferase P1

MAPK – mitogen activated protein kinase

PKA/B- protein kinase A/B

MKP1 – mitogen activated protein kinase phosphatase-1

ChREBP – carbohydrate response element binding protein

JNK – JUN N terminal kinase

SREBP – sterol regulating element binding protein

ERK – extracellular signal-regulated kinase

PGC-1α – peroxisome proliferator-activated receptor-ycoactivator

TNFα – tumor necrosis factor-α

CRTC2 – CREB regulated transcription coactivator 2

IL – interleukins

TCA – Tricarboxylic acid cycle

CCL2 – chemokine ligand-2

ATP – adenosine triphosphate

CXCL – chemokine (C-X-C motif) ligand-1

PEPCK – phosphoenolpyruvate carboxykinase

TSLP – thymic stroma lymphopoietin

G6P – glucose-6-phospaphate

FASN – fatty acid synthase

ACC1/2 – acetyl-CoA carboxylases 1 and 2

PDX1 – pancreatic and duodenal homebox 1

GSK33β – glycogen synthase kinase 3 beta

ROS – reactive oxygen species

TXNIP – thioredoxin interacting protein

PIP2/3 – Phosphatidylinositol -2/3 – Kinase

PDK-1 – PIP3 dependent protein kinase

ERK – extracellular signal regulated kinase

GSTP1 – glutathione S-transferase pi 1

Bcl-2 – B-cells lymphoma 2

NAD(P)H – nicotinamide adenine dinucleotide phosphate hydrogenase

DAG – diacylglycerol

$InsP_3$ – inositol 1,4,5, triphosphate

MCP-1 – monocyte chemoattractant protein-1

RANKL – receptor activator of nuclear factor kappa beta

OPG – osteoprotegerin

COX-2 – cyclooxygenase 2

AKT – protein kinase B

IRS-1/2 – insulin receptor substrate -/21

NOX4 – NADPH oxidase 4

TXNIP – thioredoxin-interacting protein

GSTP1 – glutathione S-transferase P1

PKA/B – protein kinase A/B

PLC – phospholipase C

SOX9 – sex determining region-Y box transcription factor

Ngn3 – neurogenin -3

PAC – p1 derived artificial chromosome

PI3 – phophoinositide-3 kinase

CHAPTER 1
INTRODUCTION

INTRODUCTION

1.1 Generalities on glucocorticoids

1.1.1 Glucocorticoids chemistry and physiology

Glucocorticoids (GCs) are ubiquitous steroid hormones that regulate several physiological processes, such as glucose, lipids and protein metabolism, and immune response (**Table 1**) (Arlt & Stewart, 2005). Cortisol plays a significant role in lipid and glucose metabolism through its action on liver, adipose tissue, muscles and pancreas. GCs encompass both the endogenously produced cortisol in humans and corticosterone in rodents, along with their synthetic derivatives. These synthetic GCs have been designed to a have a more potent anti-inflammatory, and immunomodulatory function, and are also prescribed in clinical therapy to treat a variety of diseases, including asthma, allergies, COPD, rheumatoid arthritis, multiple sclerosis, lupus, etc (Barnes, 1998; Alex Rafacho et al., 2014). Hydrocortisone is the synthetic preparation of cortisol given to patients presenting with adrenal deficiency.

Table 1: Physiological function of cortisol and consequences of cortisol excess

Physiological function of cortisol	Consequence of cortisol excess
Glucose metabolism	
- Promote gluconeogenesis and hepatic glycogen formation - Decrease peripheral glucose uptake - Permissive effects on catecholamines and glucagon	- Glucose intolerance - Diabetes
Lipid metabolism	
- Stimulation of adipocyte lipolysis, leading to an increase in circulating free fatty acids	- ↑Total cholesterol - ↑Triglycerides - ↑HDL
- Stimulation of adipocytes differentiation and adipogenesis, mainly at the visceral level	- Facial and muscle fat distribution - Buffalo neck - Filling the supraclavicular recesses

Physiological function of cortisol	Consequence of cortisol excess
Immunomodulating function (anti-inflammatory)	
- Direct action on lymphocytes, inhibiting immunoglobulin and cytokines production, and increasing apoptosis - Inhibition of monocytes and macrophage differentiation - Inhibition of macrophage activity	- ↓Lymphocyte - ↓PNN - ↓PNE - Susceptibility to infection
Salt and water homeostasis	
- Increased sensitivity to angiotensin II - Increased angiotensinogen production - Increased glomerular filtration rate, epithelial sodium transport and free water clearance - Mineralocorticoid action at high doses (by exceeding the enzymatic activity of 11β-HSD2)	- Hypertension - Hypokalemia - Lower limb edema
Skin, muscle	
- Inhibition of epidermal cell division - Inhibition of collagen production	- Skin fragility bruising, stretch marks - Amyotrophy, proximal myopathy
Bones, phospho-calcium metabolism	
- Inhibition of osteoblast function in bones - Increased intestinal calcium absorption - Increased renal calcium excretion	- Osteoporosis - Growth retardation (renal lithiasis, nephrocalcinosis)
Central nervous system	
- Role in brain and hippocampus	- Asthenia - Impaired concentration and changes in appetite - Memory impairment - Depression, psychosis - Insomnia, irritability
- Suppression of thyrotropic and gonadotropic axis	- Hypothyroidism - Cycle disorder, amenorrhea - Decreased libido
Coagulation	
- Increased synthesis of many coagulations factor - Decreased fibrinolysis capacity - Hyperhomocysteinemia	- Thrombosis
Others	
- Glucocorticoid receptor expression in the digestive tract	- Gastric ulcer
- Increased production of aqueous humor in the eye	- Glaucoma - Cataracts
- Peripheral conversion of cortisone to testosterone	- Hirsutism - Acne

a) Glucocorticoids biosynthesis

The natural GC, cortisol, is synthesized in the zona fasciculata of the adrenal cortex from precursor cholesterol. Its biosynthesis is under the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. This signalling cascade begins with the release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from the parvocellular neurons in the hypothalamic paraventricular nucleus (PVN) into the pituitary portal circulation (Gillies et al., 1982). In the pituitary, CRH and AVP binds their respective receptors, CRH type 1 receptor and AVP_{1B} receptor, to stimulate the release of adrenocorticotropic hormone (ACTH) into circulation. AVP is mainly involved in ACTH release in cases of acute stressors while CRH is the main regulator of the pituitary-adrenal axis and is involved in the regulation under basal conditions but also stress. The principal target for the circulating ACTH is the adrenal cortex where it binds to melanocortin type 2 receptor (MC2R), and stimulate the synthesis and secretion of cortisol from the zona fasciculata (Angelousi et al., 2000; Deussing & Chen, 2018; Dickmeis, 2009; Zelena et al., 2009). Cortisol secretion follows a circadian rhythm, resulting in a morning diurnal peaks (6-8 a.m.) and a decrease secretion until its midnight nadir (11 p.m.) (Dickmeis, 2009). Temporarily increased cortisol secretion is observed in response to physiological or psychological stress. Cortisol synthesis exerts negative feedback on the HPA axis by regulating CRH neuronal activity at the hypothalamic levels and regulating ACTH at the pituitary levels. This feedback leads to the inhibition of both the expression and secretion of CRH and ACTH (Gjerstad et al., 2018). Through this mechanism, cortisol level is tightly controlled (**Figure 1**)**.**

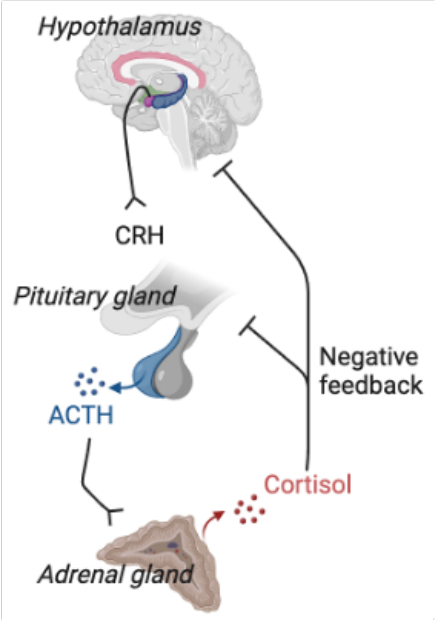

Figure 1: Schematic of the hypothalamic-pituitary-adrenal axis in GCs biosynthesis. CRH secreted from the hypothalamus stimulates the anterior pituitary to secret ACTH into circulation. Primary target tissue of ACTH is the adrenal glands where in ACTH stimulates the synthesis and secretion of cortisol. Cortisol exerts negative feedback on both the hypothalamus and pituitary glands thereby regulating its own secretion (CRH, corticotropin releasing hormone; ACTH, adrenocorticotropin hormone). Figure was created in BioRender.com

b) Glucocorticoid bioavailability

There is no doubt that circulating levels of GCs are important for steroid action; however, GCs activity is determined by several factors that regulate the availability of the steroids to its receptor (Bamberger et al., 1996; Dineen et al., 2019; Marques et al., 2009; Perogamvros et al., 2012; Silverman et al., 2005). Among local factors regulating GCs bioavailability and action are:

(1) Transporter proteins: Corticosterone binding globulin (CBG), and to a lesser extent albumin, transport GCs to their target organs tissues. Only free unbound

GCs are capable of diffusing across the membrane of target tissues (Bae & Kratzsch, 2015).

(2) Cortisol and GCs metabolism: Metabolism of cortisol occurs in some of its target tissues such as the kidney, adipose tissue and the liver (**Figure 2**). In the liver, the degradation of cortisol occurs where it is reduced, oxidized, hydroxylated and then conjugated with sulphates or glucuronic acid before being eliminated in urine (Chapman et al., 2013; Schiffer et al., 2019). Synthetic GCs are also metabolized (Renner et al., 1986; Russell & Wilson, 1994)

(3) Cortisol and GCs transport inside the cell: GCs diffuse passively through membranes but they are also substrates for transporters of the multidrug resistance transporter family. The p-glycoprotein (P-GP) alternatively called the ATP-binding cassette transporter (ABCB1) encoded by the *mdr1* gene is highly expressed in the gut (apical membrane), liver (canalicular membrane), adrenal cortex, kidney (apical membrane of the epithelial cells of proximal tubules), blood brain barrier (luminal membrane of endothelial cells), testis (endothelial cells of capillaries), and placenta (trophoblast). ABCB1 is an ATP-dependent efflux pump that actively transports cortisol and synthetic GCs, but not corticosterone, out of cells, thereby reducing their intracellular availability in target tissues (Chin et al., 1990; Choi, 2005; Karssen et al., 2001; van Kalken et al., 1993).

(4) Glucocorticoid receptor (GR) nuclear translocation and (5) GR interaction with other transcription factors (Marques et al., 2009; Perogamvros et al., 2012; Silverman & Sternberg, 2008).

c) Glucocorticoids metabolism

Predominately expressed in the kidney, the type-2 hydroxysteroid 11β-dehydrogenase (11β-HSD2), inactivates cortisol into cortisone in order to protect the mineralocorticoid receptor from the action of cortisol present in higher concentrations than aldosterone. In the liver and adipose tissue, cortisone is reactivated into cortisol by the type-1 hydroxysteroid 11β-dehydrogenase (11β-HSD1) (Chapman et al., 2013; Schiffer et al., 2019). Reactivation at the splanchnic level contributes to a significant part of daily cortisol production (Andrew et al., 2005). Interestingly, 11β-HSD1 is a bidirectional enzyme that primarily catalyzes the reduction of 11-ketosteroids *in vivo*. This reductase activity is facilitated by the colocalized hexose-6-phosphate dehydrogenase (H6PDH), which regenerates the NADPH required for the conversion of cortisone to cortisol. In contrast, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) functions solely as a dehydrogenase enzyme, inactivating cortisol by converting it to cortisone (Chapman et al., 2013; Schiffer et al., 2019).

The A-ring reduction of GCs is essential for the inactivation of GCs. This reduction is a two-step process irreversible. The first step involves the reduction of the Δ4 double bond, which is catalyzed by either 5α-reductases (SRD5A1 and SRD5A2) or 5β-reductase (AKR1D1). This step yields 5α-dihydro or 5β-dihydro metabolites, respectively (Schiffer et al., 2019). These metabolites are subsequently converted by the 3α -Hydroxysteroid Dehydrogenases into 5α-tetrahydrocortisol (5α-THF) for the 5α-dihydrocortisol (5α-DHF), 5β-tetrahydrocortisol (5β-THF or THF) for the 5β-dihydrocortisol, and 5β-tetrahydrocortisone (THE) for the 5β-dihydrocortisone (Penning, 1997). Among the steroid 5α-reductase enzymes (SRD5A), only SRD5A1 and SRD5A2 function as genuine steroid 5α-reductases. SRD5A1 is primarily expressed in the liver and peripheral tissues, while SRD5A2 is mainly expressed in

male reproductive and genital tissues. SRD5A require NADPH as a cofactor and are unable to utilize NADH. SRD5A enzymes have a high affinity for testosterone, especially the SRD5A2. The affinity for GCs is significantly lower (Nixon et al., 2012).

The 5β-reduction is catalyzed by the aldo-keto-reductase (AKR) family member AKR1D1, which is primarily expressed in the liver. AKR1D1 is the only human enzyme that catalyzes the 5β-reduction of 3-keto-Δ4 steroids and bile acids (Penning et al., 2019). 3α,5α-Reduced and 3α,5β-reduced cortisol metabolites contribute equally to the metabolic clearance of steroids in the liver. Cortisone is a better substrate for 5αRs than cortisol, but its 5α-reduced metabolites are not readily found in urine, possibly due to rapid conversion of 5α-tetrahydrocortisone to 5α-tetrahydrocortisol by 11β-hydroxysteroid dehydrogenase 1. Due to the differential tissue expression patterns of 5αR isoforms and AKR1D1, 5α-reduced metabolites reflect global metabolism, while 5β-reduced metabolites predominantly represent hepatic reduction. The second step of the A-ring reduction is the reduction of the 3-keto group to a hydroxy group, catalyzed by members of the aldo-keto reductase family (AKR1C1, AKR1C2, AKR1C3, and AKR1C4). AKR1C4 is thought to be a liver-specific enzyme that works in concert with AKR1D1. These led to the cortol and cortolone metabolites.

Another enzyme involved in the metabolism of GCs is the cytochrome P450 family 3, subfamily A4 (CYP3A4). The CYP3A4 is the most abundant isozyme of the CYP450 family expressed majorly in the liver and to a lesser extent, the intestines. CYP3A4 is involved in the rapid systemic clearance of ~30% of clinically used drugs including synthetic GCs (Zanger & Schwab, 2013). The CYP3A5 enzyme, a member of the CYP450 family, contributes with the CYP3A4 to irreversibly inactivate glucocorticoids

by converting them to their 6β-hydroxylated forms (Peng et al., 2011; Tomlinson et al., 1997).

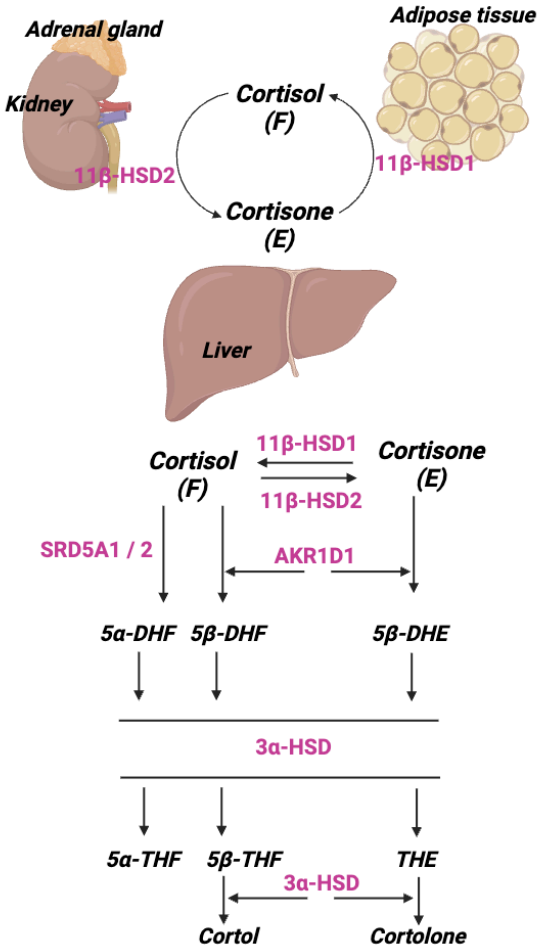

Figure 2: Schematic of the main enzymes involved in GCs metabolism in some tissues. In the kidney, cortisol is inactivated into cortisone by 11β-HSD2. In the liver and adipose tissue, cortisone is reactivated into cortisol by 11β-HSD1. Cortisol and cortisone are degraded in the liver where they are reduced, oxidized, hydroxylated and then conjugated to sulphates or glucuronic acid before being eliminated in the urine. The reduction of cortisol and cortisone is mediated by three enzymes: The SRD5A1/2, which act only on cortisol, metabolizing it, 5α-DHF, and AKR1D1 which metabolizes both cortisol and cortisone into 5β-DHF and 5β-DHE, respectively. The action of 3α-HSD for more downstream metabolites of 5α-DHF into 5α-THF, and 5β-DHF and 5β-DHE into THF and THE respectively. 3α-HSD further metabolizes THF and THE into cortol and cortolone respectively. Figure was created in BioRender.com

1.1.2 Mechanism of glucocorticoids action

Free GCs interact in the target site with the GR and mineralocorticoid receptor (MR) to bring about genomic and non-genomic actions. These actions lead to the direct or indirect regulation of the expression of thousands of genes (Dineen et al., 2019; Timmermans et al., 2019). The GR and MR share a 94% identical, highly conserved DNA-binding domain. The GR is expressed in various tissues, including the brain and immune cells, and primarily bind to GCs, although with a lower affinity compared to the MR (Weikum et al., 2017). The MR exhibits a more localized expression pattern, predominantly found in epithelial tissues such as the kidneys, colon, and salivary glands as well as in specific regions of the central nervous system, particularly the hippocampus. The MR has a similar high affinity for both aldosterone and cortisol (Funder, 2017).

a) The glucocorticoids receptor

The GR, is a member of the nuclear receptor superfamily of transcription factors and is a 97 kDa protein. The GR is encoded by a single gene: nuclear receptor subfamily 3, group C, member 1 (NR3C1), located on chromosome 5 in humans, consisting of 9 exons for which exon 1 forms the 5′ untranslated region (UTR) and exons 2–9 encode the GR protein (Nicolaides et al., 2010). Since the first cloning of GR in 1985, several GR splicing variants have been identified. Alternative splicing at exon 9 (exon 9α and 9β), results in two different but highly homologues GR splice variants, namely the 97kDa GRα or the 94kDa GRβ, and both have been extensively studied (Nicolaides et al., 2010). The human GRα isoform is the predominant isoform, and mediates the classic GC effects with a high affinity for GCs compared to GRβ. GRβ is permanently localized to the nucleus with a dominant negative regulation in the transcriptional

activity of GRα isoform, and regulates gene transcription of non- GRα target genes in an GRα and GCs independent manner (Nicolaides et al., 2010; Timmermans et al., 2019). The human GR protein contains four main distinct domains: The N-terminal domain (amino-terminal A/B region) which contains a major transactivation domain and is ligand-independent; the DNA-binding domain (region C) which contains sequences important for receptor dimerization and nuclear translocation; The hinge region (D) which is also involved in dimerization and confers structural flexibility in the receptor dimmers; And the ligand-binding domain (region E) which contains a second transactivation domain and is ligand dependent. The region E contains sequences important for receptor dimerization, nuclear translocation, binding of the GR to heat shock proteins and interaction with coactivators, (**Figure 3**), (Nicolaides et al., 2010; Weikum et al., 2017).

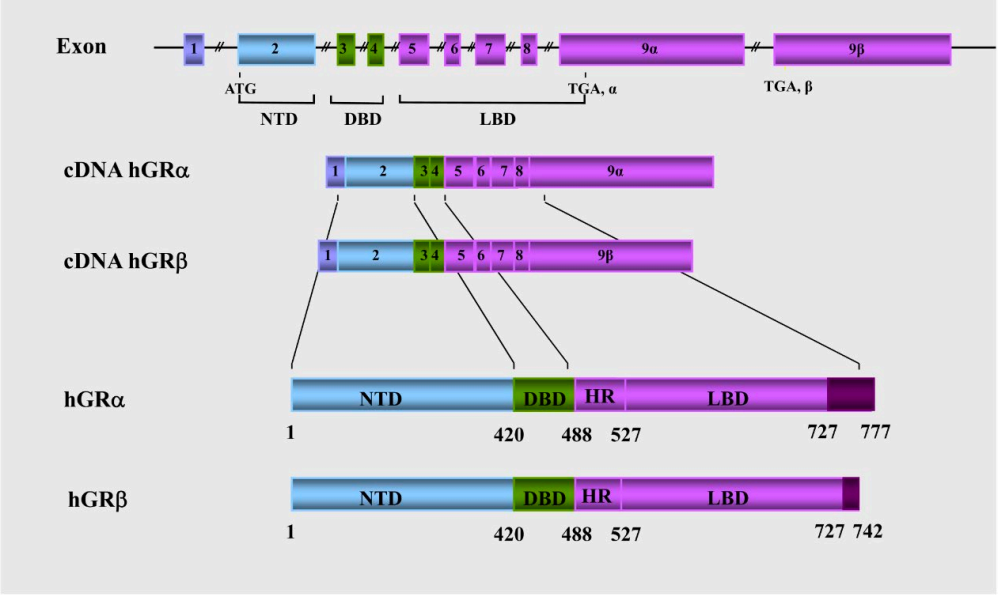

Figure 3: Schematic of the structure of the human GR gene. Alternative splicing of the primary transcript gives rise to the two mRNA and protein isoforms, GRα and GRβ, (NTD, N-terminal domain; DBD, DNA-binding domain; HR, hinge region; LBD, ligand-binding domain). Figure from (Nicolaides et al., 2010).

i. Genomic effect of the glucocorticoid receptor

The genomic action of GCs leads to the modulation of target-genes expression through transcriptional modifications. GCs can also reduce the half-life of certain mRNAs, such as tumour necrosis factor (TNF). These processes involve the classical cytosolic GRα, which translocate to the nucleus after binding to GC and interacts with DNA directly or through protein-protein complexes. In the absence of GCs, GRα exists as a monomer in a chaperone complex with heat shock proteins (HSPs), including HSP90, which maintains its conformation for GC binding while blocking nuclear translocation. GC binding leads to phosphorylation by p38 MAP kinase, causing the dissociation of GRα from the chaperone complex. This phosphorylation exposes the nuclear translocation and dimerization domains, allowing the GRα-GC complex to form homodimers and translocate to the nucleus (Nicolaides et al., 2010; Timmermans et al., 2019).

In the nucleus, the activated GR homodimer can either carry out its regulatory functions by binding to glucocorticoid response elements (GREs) which are composed of a palindromic 15 bp conserved sequence 5'-AGAACAnnnTGTTCT-3' in the promoter region of GC-regulated genes (**Figure 4**). Within the nucleus, the GR functions as a transcription factor (TF), either activating (trans-activation) or repressing (trans-repression) genes, and can also influence the activity of other TFs through tethering. GRα can directly increase gene expression through three mechanisms. GRα can recruit chromatin remodeling complexes to modify nucleosome structures, creating a favourable environment for transcription. When the GRE is near a TATA box, GRα recruits general transcription factors. When the GRE is distant from the TATA box, GRα interacts with co-activators that bridge to the basal transcription machinery. GRα activation of gene transcription is believed to be the main cause of side effects,

including GC-induced diabetes (Galigniana et al., 2010; Nicolaides et al., 2010; Oakley & Cidlowski, 2013; Timmermans et al., 2019).

To initiate transrepression, GRα can bounds directly to other transcription factors, such as NF-κB or activating protein 1, restricting their transcriptional activity. GRα can also interfere with the recruitment of coactivators or other essential proteins required for the transcriptional activation of these factors. This interference can occur through competition for overlapping DNA-binding sites or by sequestering cofactors. The transrepression activity of GR is often considered as the main mechanism responsible for the anti-inflammatory and immunosuppressive effects of GCs (Galigniana et al., 2010; Nicolaides et al., 2010; Oakley & Cidlowski, 2013; Timmermans et al., 2019). After exerting its action, the GR homodimer can be transported back to the cytoplasm where it undergoes phosphorylation by tissue-specific kinases, causing conformational changes that either regulate its transcriptional activity or promote its degradation via ubiquitination (Galigniana et al., 2010; Nicolaides et al., 2010; Oakley & Cidlowski, 2013; Timmermans et al., 2019).

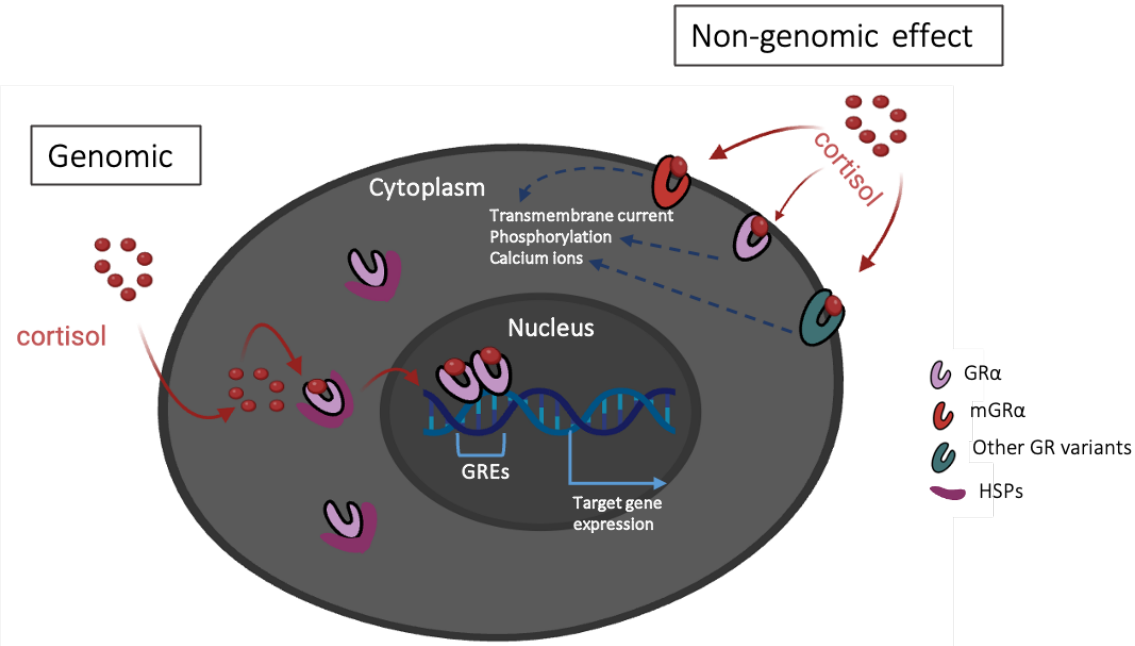

Figure 4: Schematic of the genomic and non-genomic action of the GR. After ligand binding in the cytoplasm of the cells, the GC-GR complex dissociate from the HSPs chaperone protein (heat-shock proteins), and either as monodimer or homodimer with another GC-GR complex translocate to the nucleus where they bind GREs in the promoter region of GCs target genes to regulate their transcription. Additionally, GC can exert non-genomic effects through membrane-bound GRs (mGRα), other non-classical GR variants, or via interactions between cytoplasmic GR (GRα) and other cytoplasmic signaling proteins. Figure was created in BioRender.com

ii. Non-genomic effect of the glucocorticoid receptor

While most GR functions occur within the nucleus, several non-genomic roles have also been identified with some reported to be mediated by cytosolic GR or dependent on membrane-bound GR. These non-genomic effects do not directly alter gene expression but instead trigger rapid responses, such as activating signal transduction pathways, independent of transcription or protein synthesis. Examples include GC-mediated effects through ion channels, particularly involving intracellular Ca2+, or interactions with membrane-bound GR at the plasma membrane. These actions may

be linked to cytoplasmic GRα formed by alternative splicing or a G-protein-coupled receptor that signals via cAMP, potentially modulating pathways like MAPK signalling. Another non-genomic function of GR is its role in mitochondrial activity, as GR can localize to mitochondria and regulate gene transcription from the mitochondrial genome by binding to GRE-like sequences, either alone or in conjunction with other proteins (Czock et al., 2005; Oakley & Cidlowski, 2013; Timmermans et al., 2019).

b) The mineralocorticoid receptor

Aldosterone and GCs have close affinity for the MR. Despite this, the physiological action of aldosterone is largely preserved in mineralocorticoid-sensitive tissues due to the presence of the enzyme 11β-HSD2, which inactivates cortisol to cortisone, as cortisone do not bond the MR. In case of mutation of the enzyme or excessive level of GCs overpassing the enzymatic capacity of 11β-HSD2, GCs can activate the MR in these tissues, leading to hypertension and hypokalemia (Gomez-Sanchez & Gomez-Sanchez, 2014). In tissue where the expression of the 11β-HSD2 is low as the brain, heart, and vasculature, GCs exerts some of its effect through the MR. Therefore, the myriad effects of GC are largely a consequence of transcriptional actions mediated via binding to both the MR and the GR (Czock et al., 2005; Fuller et al., 2000; Gomez-Sanchez & Gomez-Sanchez, 2014). Once activated, MR translocate to the nucleus, where it can regulate gene expression by binding to mineralocorticoid response elements (MREs) in target gene promoters (Chapman et al., 2013; Fuller et al., 2000).

1.1.3 Pharmacodynamics and pharmacokinetics of glucocorticoids

The pharmacokinetics of the various GCs, including their absorption, distribution, and elimination, depends on and are important determinants of their physicochemical properties. These physicochemical properties influence the pharmacodynamics of GC action, which are measured regarding their binding capabilities to transporter proteins, receptor affinities, onset of action, peak effect, duration of effect, and offset of action (**Table 2**).

GCs are lipophilic and are given as prodrugs intravenously, administered either as hydrophilic phosphate or succinate esters or alcoholic solutions which are converted to their active form within 5 – 30 mins of administration. GCs have moderate protein binding and moderate apparent volume of distribution, with a bioavailability of 60-100% after oral administration. The pharmacokinetics of GCs shows a circadian pattern. A GC dose taken in the morning leads to higher average plasma concentrations compared to the same dose taken in the evening. Age, influence of other drugs, dosage regime, sex as well as the female hormone status are known to influence the pharmacokinetics of these drugs. For example, A significantly smaller 50% inhibitory concentration (IC_{50}) value was seen in women as compared to men for suppression of cortisol secretion, indicating increased GC sensitivity among women. It has been reported that prednisolone clearance is lower in females compared to males. Similarly, in the elderly population, the clearance of prednisolone is lower compared to younger adults. As a result, the frequency and severity of adverse effects may be increased in elderly individuals. Finally, GCs are mainly metabolized in the liver and eliminated via the kidney as hydrophilic inactive metabolites after oxidization or hydrogenation, and conjugation (glucuronidation and sulphuration), (Sytske Anne

Bergstra et al., 2023; Czock et al., 2005; Deng et al., 2019; He et al., 2014; Paragliola et al., 2017; Scherholz et al., 2019).

Table 2: Pharmacokinetics of selected three most used synthetic GCs

Synthetic GCs	Dose (mg)	Anti-inflammatory activity (relative to cortisol)	Mineralocorticoid activity (relative to cortisol)	Plasma half-life (hours)	Biological half-life (hours)
Hydrocortisone	20	1	1	1-3	8-12
Prednisolone	5	4	0.3	2-3	12-36
Dexamethasone	0.75	30	0	0.5-2	36-72

Note: Table contains data from (Paragliola et al., 2017)

a) Hydrocortisone

Hydrocortisone (HC) is chemically identical to endogenous cortisol, and can be administered topically, intravenously or orally. Therefore, oral HC is the GCs of choice for the supplementation of adrenal insufficiency. Compared to other GCs, HC is less frequently prescribed, except in cases of adrenal insufficiency where cortisol replacement is needed. HC is well absorbed orally, with 97% bioavailability and a volume of distribution of approximately 0.5 L/kg. In healthy individuals, approximately 90% of circulating cortisol is bound tightly to transcortin, about 5% of cortisol circulates freely in an unbound state in the plasma, and the remaining 5% is either bound to serum albumin or remains unbound. Transcortin binding capacity is saturated at a concentration of about 200-400 µg/L (3.85-7.69 nM), which is attained after administration of >20 mg of HC. HC is a short-acting GC with a plasma clearance of 1-3 hours and a duration of action of 8-12 hours exhibiting a biexponential disposition, (Czock et al., 2005; Deng et al., 2019; Paragliola et al., 2017; Scherholz et al., 2019). For example after oral administration of 20 mg of HC, peak plasma concentrations

reach 841.4 ± 74.4 nM within 1 hour and cleared from plasma between the 10^{th} -11^{th} hour in healthy volunteers (Derendorf et al., 1991). HC exhibits a higher affinity for the mineralocorticoid receptor (MR) than for the GR, however its mineralocorticoid effect is stopped by 11β-HSD2. HC undergoes metabolism primarily in the liver, following the same metabolic pathways as previously described for cortisol. Less than 1% of HC is directly excreted in urine (Czock et al., 2005; Deng et al., 2019; Paragliola et al., 2017; Scherholz et al., 2019).

b) Prednisolone

Prednisolone (PRED) are commonly used to treat a variety of inflammatory and immune disorders, including rheumatoid arthritis and asthma. As HC, PRED has a biexponential disposition with a dose-dependent pharmacokinetics. PRED is usually administered orally with an 80% of oral bioavailability, as inactive prednisone which is activated by the dehydrogenase action of 11β-HSD1. Its mineralocorticoid activity compared to cortisol is 0.8 times lower. However, PRED is 4 times more potent than HC as anti-inflammatory agents. It has a plasma half-life of 2-3 hours (Czock et al., 2005; Deng et al., 2019; Paragliola et al., 2017; Pickup, 1979b; Scherholz et al., 2019). For example peak plasma concentration after oral administration of 5 mg of PRED reaches between 300 - 312 nM between 1 to 2 hours of administration (P. Morrison et al., 1977). PRED and HC's short half-life with no drug accumulation between doses, even when patients are administered multiple daily doses, make these compounds preferable for chronic treatment with short dosing intervals. The biological duration of effect of PRED last for 18-36 hours. As HC, transcortin has a high affinity but low capacity for PRED and albumin has a low affinity but higher capacity. Transcortin becomes fully saturated when plasma PRED concentrations exceed 200 µg/L,

corresponding to a dose of 20 mg of PRED. Therefore, with increasing concentrations from 200 µg/L to 800 µg/L transcortin becomes saturated with PRED, thereby increasing available PRED for binding to albumin or remaining unbound in circulation. Finally, degradation of PRED mainly occurred in the liver via CYP3A4 before renal elimination of its multiple metabolites. SRD5A1 can also metabolized PRED (Czock et al., 2005; Deng et al., 2019; Paragliola et al., 2017; Pickup, 1979b; Scherholz et al., 2019).

c) Dexamethasone

Dexamethasone (DEX), as PRED is used mainly as anti-inflammatory and immunomodulatory drugs. Route of administration for this drug is mainly intravenous and via oral administration. The pharmacokinetics of DEX after intravenous administration are linear and can undergo enterohepatic recirculation which could lead to a second plasma peak (Czock et al., 2005). DEX has a 70-100% oral bioavailability, and 77% total protein binding capacity. Unlike PRED and HC, DEX binds to exclusively to transporter protein albumin. As with both PRED and HC, DEX reaches peak plasma level between 0.5 - 2 hours but in contrast has a much longer biological half-life of 36 to 72 hours. It has an anti-inflammatory potency 25 times relative to HC (Scherholz et al., 2019). For example, Peak plasma concentration after oral administration of 2 mg of DEX reached approximately 30 nM between 1 to 2 hours of administration (Queckenberg et al., 2011). Unlike HC and PRED, DEX has no affinity for the MR but rather an absolute affinity for the GR which is ubiquitously expressed. Unlike both PRED and HC that can be metabolized by SRD5A, DEX is metabolized by CYP3A4. Renal excretion of DEX is less than 10% of total body clearance, and less than 10% of DEX is excreted in the urine (Paragliola et al., 2017; Scherholz et al., 2019).

1.2 Generalities on the pancreas, islets and β-cells

1.2.1 Generalities on the pancreas and insulin secretion

a) Anatomy of the pancreas

The adult pancreas is approximately 14-23 cm long, weighing about 100 grams lying across the posterior wall (retroperitoneal) of the abdomen (Innes & Carey, 1994). The pancreas can be divided into three distinct anatomical parts: the right extremity called the head, followed by a narrow constriction called the body, and a tapering end called the tail, each composed of lobes and smaller 1-10 mm lobules (**Figure 5**). The head lies near the duodenum and the tail extends to the hilum of the spleen. (Innes & Carey, 1994; Longnecker, 2021).

The pancreas consists of vascular cells, neurons, mesenchymal cells, and largely of 95% exocrine and 1-2% endocrine cells population. Interestingly, previous research has shown that all the pancreatic cell types are derived from a common pool of pancreatic progenitors (Edlund, 2002). As part of the gastrointestinal system, it produces and releases alkaline fluids mixed with digestive enzymes into the duodenum of intestine via the exocrine acinar cells and ductal epithelium. The endocrine cells form clusters or groups called islets of Langerhans scattered in the pancreatic parenchyma and lie adjacent to blood vessels. The human pancreas consists of 1-15 million islets. These islets are composed of five different cell types: `alpha (α), beta (β), delta (δ), pancreatic polypeptide (PP), and epsilon (ε) cells, secreting glucagon, insulin, somatostatin, pancreatic polypeptides, and ghrelin hormones, respectively into circulation, all of which play an important role in metabolism. The β-cell mass is the largest (~55%), followed by alpha cells (~33 %), while the other endocrine cells comprise a lesser part of the islets (Baskin, 2015; Edlund, 2002).

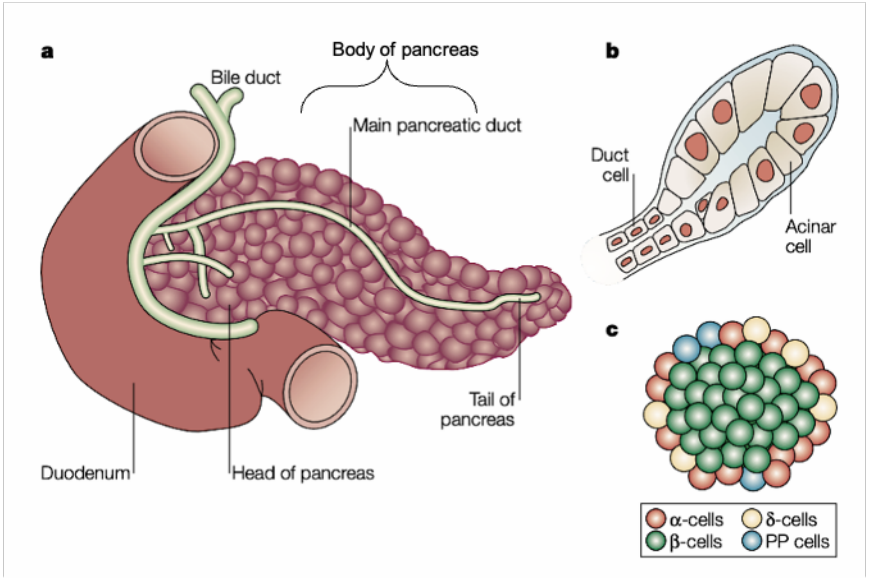

Figure 5: Anatomy of the pancreas. a) The mature human pancreas with three distinct anatomical parts. The head of the pancreas lying near the duodenum of the small intestine, the body, and the tail extending into the hilum of the spleen. b) The exocrine duct and acinar cells of the pancreas. The acinar cells produce and secrete digestive enzymes which are transported via the ductal system to be delivered to the gut. c) The endocrine pancreas consisting of the glucagon secreting α-cells, the insulin secreting β-cells, the somatostatin secreting δ-cells, and the ghrelin secreting ε-cells. Figure was adapted from (Edlund, 2002).

b) Exocrine pancreas

i. The acinar cells

The acinar cell is a highly specialized pyramidal-shaped organ responsible for synthesizing, storing and releasing enzymes and zymogens (pro-enzymes) that aid in digestion of dietary carbohydrate, protein and lipid within the intestinal lumen, such as α-amylase and pancreatic lipase enzymes. These enzymes are stored in zymogen granules within acinar cells. Upon stimulation by neural or hormonal signals triggered by food in the intestine, these enzymes are released into the acinar lumen via exocytosis. The acinar cells also secrete isotonic fluid, mainly composed of chloride

ions. Water and sodium follow the movement of chloride ions out of the acinar cells, forming the overall pancreatic juice (Edlund, 2002; Slack, 1995).

ii. The ductal cells

The ductal cells are the epithelium lining the pancreatic walls and function in the continued transportation of the acinar digestive enzymes to the duodenum. They also secrete bicarbonate rich fluids and isotonic solutions to neutralize gastric acidity and mucins, creating a PH necessary for normal pancreatic function. Adult ductal cells share some similarities with embryonic primitive ducts and may serve as a pool for progenitors for both islet and acinar tissues after birth and into adulthood and hence proposed to be the pancreatic “facultative stem cells” (Bonner-Weir et al., 2004; Edlund, 2002).

c) Endocrine pancreas beside β-cells

i. The alpha cells

Alpha cells (α-cells) are predominantly localized in the body and tail regions of the pancreas, where they secrete the hormone glucagon in response to hypoglycemic conditions (Sutherland & de Duve, 1948). Glucagon plays a critical role in maintaining glucose homeostasis by stimulating glycogenolysis and gluconeogenesis (Miller & Birnbaum, 2016; Muller et al., 2017; Unger, 1985). Some studies suggest that pancreatic α-cells may produce small amounts of GLP-1 and GLP-2 as well (Campbell et al., 2020; Chambers et al., 2017). The regulation of glucagon secretion is influenced by paracrine signals, hormones, and nutrient stimuli. Alpha cells take up glucose primarily through the GLUT1 transporter and possibly via sodium-dependent glucose transporters, SGLT1 and SGLT2. However, SGLTs are expressed at low levels in α-

cells, and their inhibition may have limited effects on glucagon secretion (Chae et al., 2020). Beyond hypoglycemic conditions, certain amino acids, including arginine, alanine, glycine, and proline, strongly stimulate glucagon release (Wewer Albrechtsen et al., 2023).

Glucagon exerts widespread effects across various organs. Glucagon's primary function is the release of glucose from glycogen stores in the liver, particularly during fasting and exercise (Sutherland & de Duve, 1948). It also enhances lipid oxidation while decreasing lipid synthesis in the liver. Glucagon binds to receptors in the brain, promoting satiety and reducing appetite and food intake. It also reduces gastrointestinal motility, increases water reabsorption and glomerular filtration in the kidneys, and promotes lipolysis in adipose tissue. Glucagon also enables autocrine signalling within the islet, acting on glucagon receptors present on α-, β- and δ-cells, (Leibiger et al., 2012). Particularly it has been shown that glucagon signalling enhances insulin secretion, through the glucagon receptors or by activating GLP-1 receptors (Sørensen et al., 2006; Zhu et al., 2019).

ii. The epsilon cells

To date, knowledge of epsilon cells is largely derived from rodent model. The epsilon cells produce ghrelin, a multifunctional hormone known to stimulate growth hormone secretion (Kojima et al., 1999), provoke hunger sensation (Cummings et al., 2004; Horvath et al., 2001), and induce gastric emptying (Horvath et al., 2001). Recently the pancreatic epsilon cell was characterized as a distinct cell type (Andralojc et al., 2009; Wierup et al., 2002), and others found co-localization with the α-cells in mice and humans (Date et al., 2002), or β-cells in humans (Volante et al., 2002). It has been proposed that islets' endogenous ghrelin has insulinostatic function (Dezaki et al.,

2004; Dezaki et al., 2006; Sun et al., 2006). Indeed, pharmacological, immunological and genetic blockade of ghrelin action in pancreatic islets all markedly enhanced GSIS (Dezaki et al., 2004; Dezaki et al., 2006; Sun et al., 2006).

iii. The Pancreatic polypeptide cells

The Pancreatic polypeptide cells are so called because they secretes a polypeptide, pancreatic polypeptide (PP), belonging to the neuropeptide Y family of proteins (NYP) (Larsson et al., 1974). The PP-cells are predominantly in the posterior lobe of the pancreatic head and gut mucosa (Slack, 1995). They function as a feedback inhibitor of pancreatic hormonal secretion after a meal. The release of PP by a meal, occurs in a biphasic manner with up to 6-10 folds increase within 5 minutes of meal, and the stimulation for the first phase mainly due to vagal stimulation, lasts 50-60 minutes (Floyd et al., 1976; Larsson et al., 1974). The more prolonged second phase, lasting up to 5 hours occurs in response to gastro intestinal hormone such as cholecystokinin (CCK) (Floyd et al., 1976; Lonovics, 1981). PP is involved in appetite regulation and induce satiety (Zhu et al., 2023), possesses insulinostatic effect (Bastidas et al., 1990), and inhibits both glucagon (Aragón et al., 2015) and somatostatin secretion (Kim et al., 2014).

iv. The delta cells

The delta cells (δ-cells) secrete somatostatin (SST). δ-cells have been considered as the intra-islet local paracrine regulator of α- and β-cells since SST is a potent and fast inhibitor for both insulin and glucagon secretion preventing large fluctuation of plasma glucose levels (Strowski et al., 2000). In rodent islets, δ-cells are situated in the outer islet mantle closer to α -cells, while in humans they are found scattered throughout the

islets (Cabrera et al., 2006). The δ-cell express a wide range of hormone and neurotransmitter receptors, including glucagon receptor (GCGR), glucagon-like peptide-1 receptor (GLP-1R), glutamate receptor 4 (GluR4) and growth hormone secretagogue receptor (GHSR), which suggest and support the ability of δ-cells to sense paracrine, endocrine, neural and nutritional signals (DiGruccio et al., 2016). Ablation of the paracrine network of the δ-cells can lead to hypoglycemia, impaired islets function and neonatal death (Li et al., 2018). Altered SST secretion could lead to either hypoglycemia or hyperglycemia, on the other hand, over sensitized δ-cells would effectively paralyze hormone secretion from both the α-cells and β-cells (Gao et al., 2021).

1.2.2 Generalities on the β-cells

a) The beta-cells

The beta cells (β-cells) are predominantly localized in the body and tail regions of the pancreas. These cells are responsible for the synthesis, storage and release of insulin. Insulin is a 51-amino-acid hormone discovered by Frederick Banting and Charles Best in 1920 (Hegele & Maltman, 2020). Insulin is secreted in response to elevated blood glucose levels and play a crucial role in human metabolism, regulating plasma glucose levels, as well as carbohydrate and lipid metabolism, and influencing food intake. Insulin secretion is a process that is tightly regulated in response to changes in the body's metabolic status. β-cells receive their regulation from a pancreatic and nonpancreatic environment promoting their function and proliferation (Eberhard & Lammert, 2009). A dense capillary network exists within the islets and facilitates glucose sensing, efficient oxygen, and insulin secretion into peripheral circulation. β-

cell interacts with its capillary network by secreting vascular endothelial growth factor to promote vascular development. In turn the capillary endothelial cells provide a vascular niche comprising of glycoproteins and other growth factors to support β-cells function including insulin secretion, and β-cell proliferation, survival and maturation (Nikolova et al., 2007).

Individual β-cells exhibit significant variability in their secretory activity. Homologous (β-cell with β-cell) and heterologous (β-cell with α-cell) intercellular contacts enhance insulin gene expression and GSIS (Unger & Orci, 1975; Wojtusciszyn et al., 2008). Pancreatic islets are innervated by parasympathetic and sympathetic neurons that play essential roles in regulating insulin secretion and maintaining glucose homeostasis. Parasympathetic innervation and its associated neurotransmitters enhance GSIS by interacting with specific receptors on β-cells. In contrast, sympathetic innervation and its neurotransmitters inhibit insulin secretion, contributing to the physiological maintenance of glucose homeostasis (Ahrén, 2000).
In addition, β-cells also interact with other tissues that are directly or indirectly involved on β-cell differentiation, growth and homeostasis such as the liver, bone, adipose tissue and gut, and the endocrine cells of the intestine (Eberhard & Lammert, 2009). These cells secrete incretins which bind to a G-coupled receptor on the β-cell surface to stimulate insulin secretion and β-cell proliferation (Drucker, 2007).

The other hormone secreted by the β-cell is islets amyloid polypeptide or amylin. Amylin functions to slow the rate of glucose entering the bloodstream. Amylin can be described as a synergistic partner to insulin, where insulin regulates long term food intake and amylin regulates short term food intake (Boyle et al., 2022).

b) Intracellular signaling for insulin biosynthesis and secretion

Humans have one gene coding for insulin (*INS* on chromosome 11) (Steiner et al., 1985), while mouse has in contrast two genes (*Ins1* on chromosome 19, and *Ins2* on chromosome 7) (Irwin, 2021). The Insulin hormone is made up of two peptide chains and three disulfide bridges, the A-chain has 21 amino acids, and the B-chain has 30 amino acids. Of the three disulfide bridges, one is within the A chain and the other two connects the A and B chains. The mature insulin is formed from the post-translation of a single-chain 110 amino acids precursor, preproinsulin (**Figure 6**). The preproinsulin consist of four domains: the amino-terminal signal peptide, the B-chain, the C-peptide and the carboxyl terminal A-chain (Fu et al., 2014).

First, upon translation, the preproinsulin interacts with the Signal Recognition Particles in the cytoplasm which facilitate preproinsulin translocation to the endoplasmic reticulum (ER), where the signal peptide of the preproinsulin is cleaved by signal peptidase to yield proinsulin. Proinsulin is then transported from the ER to the Golgi apparatus. From the Golgi, proinsulin is packaged into immature secretory granules. As these granules mature, proinsulin is cleaved by prohormone convertases 1 (PC1) and 2 (PC2) to yield insulin and C-peptide. Mature insulin, along with C-peptide and amylin, is stored in these secretory granules until stimulation for release into circulation (Campbell & Newgard, 2021; Fu et al., 2014; Liu et al., 2018; Nishi et al., 1990; Patzelt et al., 1978).

Insulin biosynthesis is regulated at both the transcriptional and translational levels. Once synthesized, insulin is stored in the secretory granules. The amount of insulin contained in these granules can vary significantly from one β-cell to another, indicating a high degree of heterogeneity in insulin storage among individual cells. β-cells increase their overall speed of protein translation, including insulin translation, in the

presence of nutrients such as glucose and amino acids. This process is mediated by nutrient-sensing pathways like mTOR. Conversely, insulin levels decrease in response to nutrient deprivation due to reduced glucose metabolism in β-cells. Although multiple factors influence insulin biosynthesis, glucose metabolism is also the primary physiological process that stimulates insulin gene transcription and mRNA translation (Poitout et al., 2006). Insulin production is triggered by a rise in the ATP/ADP ratio generated during the metabolism of glucose in the β-cells of the pancreas, mostly after a systemic rise in postprandial circulating levels of glucose.

Glucose molecules enter the β-cell cytoplasm primarily through facilitated diffusion via the plasma membrane glucose transporter GLUT2 in humans (De Vos et al., 1995; Kennedy et al., 1999). Glucokinase in the β-cell cytoplasm phosphorylates glucose molecules, increasing metabolic flow and resulting in an increased ATP/ADP ratio (De Vos et al., 1995; Kennedy et al., 1999; Malaisse & Sener, 1987; Matschinsky, 2002). The generated ATP then binds to ATP-sensitive potassium (K_{ATP}) channels in the β-cell membrane, causing depolarization and closure of the channels (Ashcroft et al., 1984; Cook & Hales, 1984). Consequently, voltage-gated calcium channels open, allowing Ca2+ influx into the cytoplasm (Rorsman & Ashcroft, 2018; Safayhi et al., 1997; Satin & Cook, 1985). The increased cytoplasmic Ca2+ concentration triggers the exocytosis of insulin granules, resulting in glucose-induced insulin secretion, which is characterized by a biphasic kinetic profile. This process is referred to as the triggering pathway of insulin secretion (**Figure 6**), (Malaisse & Sener, 1987; Olofsson et al., 2002; Rorsman et al., 2000).

Another pathway for GSIS, known as the metabolic amplification pathway, has been described. This involves a K_{ATP} channel-independent pathway that amplifies the signal

generated by the triggering pathway (Gembal et al., 1993). Insulin secretion is also amplified by hormones and neurotransmitters as cAMP, DAG, and IP3 (Seino, 2012).

c) Insulin action

Insulin exerts its effects through a complex series of actions on primary target tissues as the liver, skeletal muscles and adipose tissues. Insulin binds to its tyrosine kinase plasma membrane bound receptor in target cells. Two receptors for insulin have been identified, the insulin receptor A and B (IRA and IRB). The insulin receptors consist of two α- and β- subunits orientated on the plasma membrane with α units in the extracellular compartment where insulin binds, and the β-units that contain the tyrosine kinase protein domains, spans and extends into the cytoplasm of the cells. Binding of insulin to its receptor, activates the IR, leading to its autophosphorylation and phosphorylation of various intracellular proteins causing a cascade on intracellular signalling. The IR activates two main pathways: the RAS/MAPK pathway, primarily mediating cell growth and development, and the PI3K/AKT pathway, mainly responsible for metabolic effects.

Regarding the PI3K/PKB pathway, activated receptor attracts and phosphorylates the insulin substrate 1 (IRS-1) protein which act as an adaptor protein and mediate the receptor tyrosine kinase activity of lipid kinase Phosphoinositide -3 -Kinase, which in turn lead to the phosphorylation of Phosphatidylinositol -2 – Kinase (PIP2) on the plasma membrane to PIP3. The PIP3 then activates PIP3-depdendent protein kinase (PDK-1) which in turn activate the Akt protein kinase-B. Akt, activated by the insulin signalling cascade, phosphorylates key downstream proteins. This phosphorylation leads to the activation of enzymes involved in glucose metabolism and the translocation of glucose transporters to the cell membrane. These molecular actions

form the basis for insulin's various metabolic effects in different tissues. The duration of action of endogenous insulin is short-lived with a half-life of 5-6 minutes, however its metabolic effects can persist up to hours after its secretion (Fu et al., 2014; Matthews et al., 1985; Weiss et al., 2000).

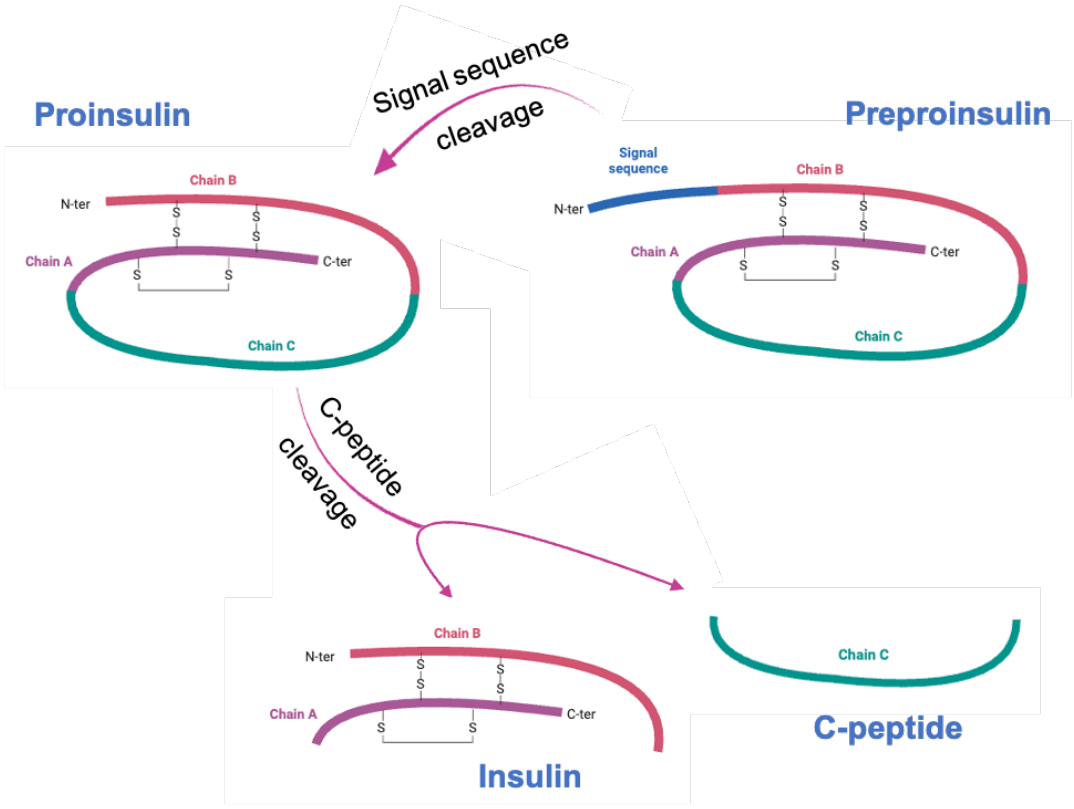

Figure 6: Insulin biosynthesis. Insulin is synthesized in the β-cells from preproinsulin. In the endoplasmic reticulum, signal peptidase cleaves the signal sequence to give proinsulin. Proinsulin is then transported to Golgi apparatus where prohormone convertase 1 and 2 (PC1 and PC2) cleaves the C-peptide from proinsulin to give the insulin hormone consisting of A and B chains with two interconnecting sulfide bonds and one intra sulfide bond within the A chain. Figure was adapted from https://basicmedicalkey.com/endocrine-pancreas-and-pharmacotherapy-of-diabetes-mellitus-and-hypoglycemia/ and created in BioRender.com

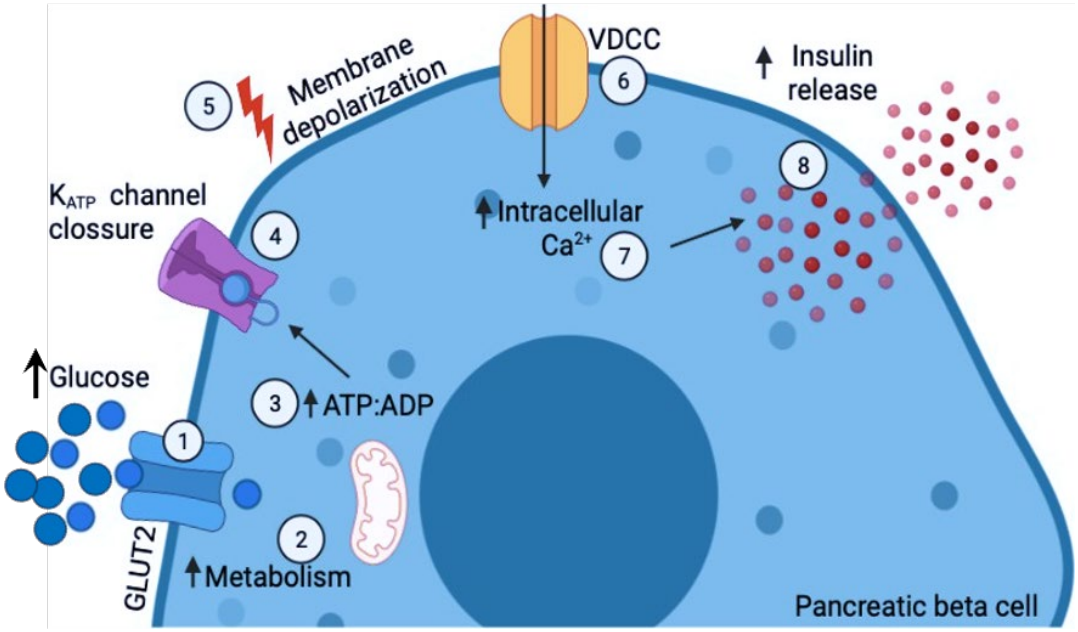

Figure 7: Glucose dependent insulin exocytosis. In high glucose concentrations (1) glucose enters the cells via the GLUT2 transporter and (2) undergoes glycolytic and mitochondrial metabolism, which ultimately has the effect of (3) increasing the ATP:ADP ratio. An increased ATP:ADP ratio leads to (4) the closure of ATP- sensitive KATP channels and to (5) membrane depolarization, which triggers (6) the opening of voltage-dependent Ca2+ channels (VDCCs). (7) The resulting influx of Ca2+ induces (8) the fusion of insulin-containing granules with the plasma membrane and insulin release from the cell. Schema was adapted from (Wang & Thurmond, 2009) and created in BioRender.com

d) Kinetics of insulin secretion

Glucose-stimulated insulin secretion in vivo typically follows a biphasic time course (Cerasi & Luft, 1963; Rorsman et al., 2000) that consists of a brief and fast initial first phase followed by a sustained second phase, *in vivo* in humans (Cerasi & Luft, 1963), and *in vitro* in perfused islets (Curry et al., 1968).

The first phase involves the release of a small pool of granules called the rapidly releasable pool (RRP), accounting for about 5% of the total granules of the β-cells. These granules are already attached to the plasma membrane and release their

contents within 3 – 10 minutes. During this first phase of secretion, the replenishment of the RRP begins by mobilizing, docking, and priming granules that were previously non-docked at the plasma membrane, referred to as the second phase of insulin secretion (Barg et al., 2002; Olofsson et al., 2002; Rorsman et al., 2000).

Continued stimulation with high glucose triggers this second phase which is more prolong, lasting 2 – 4 hours, and involve the release of granules from the reserve pool (Barg et al., 2002; Olofsson et al., 2002; Rorsman et al., 2000). The reserve pool contains the majority of the granules (>95%) and requires chemical modifications or physical translocation through an ATP-dependent process, to become available for release (Parsons et al., 1995).

While the major signal that initiates insulin exocytosis by glucose is a rise in intracellular [Ca2+], the exocytotic capacity of β-cells is enhanced various signaling molecules and pathways. These include activators of protein kinase A (PKA), such as forskolin and GLP-1, as well as other glucose-activated second messengers like cAMP, cGMP, inositol 1,4,5-trisphosphate (IP3), and diacylglycerol (DAG). These factors work in concert to amplify the insulin secretory response (Gromada et al., 1999; Rorsman et al., 2000).

The biphasic insulin secretion response is more complex than a simple two-pool model. The first phase release magnitude can be altered dose-dependently by stimulus intensity, such as glucose concentration. Factors like pre-stimulatory glucose levels and changes in granule properties after docking also influence the response. This biphasic pattern likely results from the integration of multiple signals with varying dynamics at different stimulation levels, rather than just the depletion and replenishment of two distinct granule pools.

1.3 Effect of GCs on metabolism

1.3.1 Glucocorticoid-induced diabetes

The action of GCs on several metabolic-related tissues, under conditions of chronic GCs overexposure, as seen in the Cushing's syndrome or during chronic GCs treatment, lead to important adverse metabolic dyshomeostasis including abdominal obesity, dyslipidemia, bone loss, hepatic steatosis, peripheral insulin resistance, glucose intolerance, and diabetes (Fève & Scheen, 2022; Alex Rafacho et al., 2014; Swarbrick et al., 2021; van Raalte & Diamant, 2014).

a) Glucocorticoid-induced diabetes with GC

i. New-onset GC-induced diabetes

Even a single dose of exogenous GCs can lead to an elevation of glucose level in patients without preexisting diabetes (Hans et al., 2006; Pasternak et al., 2004). Studies have shown that GC use can elevate the risk developing diabetes by two to four times compared to those not exposed (Blackburn et al., 2002; Conn & Poynard, 1994; Gulliford et al., 2006; Gurwitz et al., 1994). Up to 2% of the incident cases of diabetes may be associated with oral GCs therapy in a English primary care population (Gulliford et al., 2006).

Among patients receiving GC treatment for various conditions, including respiratory diseases (Kim et al., 2011), kidney disorders (Uzu et al., 2007), malignancies (Harris et al., 2013), and rheumatoid arthritis (Hoes et al., 2011), the incidence varies from 15% to 40%. In older adults (>65 years), the incidence of diabetes was 4.3% after one year of GC exposure, rising to 11% after three years (Blackburn et al., 2002). The risk is particularly high in certain populations, such as organ transplant recipients, where

10-20% develop diabetes within the first few months of GC treatment and up to 35% after (Dean et al., 2008; Depczynski et al., 2000; Yates et al., 2012). It should be noted that in this particular population, other immunosuppressive therapies also contribute to diabetes development. In hospitalized patients under GCs therapy, the incidence of hyperglycemia reaches 50 to 70% (Burt et al., 2011; Donihi et al., 2006; Fong & Cheung, 2013).

The prevalence of GC-induced diabetes varies also depending on factors such as patient population, GC dose, and duration of treatment. The route of GC administration also affects the risk significantly, with intravenous, oral, and injectable forms conferring a greater risk than inhaled GCs (Suissa et al., 2010). However, even inhaled GCs are associated with a 34% increase in diabetes risk (Suissa et al., 2010). The lack of consensual diagnostic criteria contributes also to the variation in the incidence between studies. Fasting glucose measurements have poor sensitivity in GC-treated patients (Burt et al., 2011; Burt et al., 2012). Although more challenging to assess consistently in clinical practice, postprandial blood glucose monitoring would be more effective for detecting GC-induced hyperglycemia (Burt et al., 2011; Burt et al., 2012).

Finally, risk factors for GC-induced diabetes include: high GC dose and duration of use, advanced age (>60 years), high BMI (>25 kg/m2), abdominal obesity, family history of diabetes, prior impaired glucose tolerance, African-American ethnicity, and specific genetic polymorphisms (Fardet & Feve, 2014; J. X. Li & C. L. Cummins, 2022). The dose is one of the most consistent risks factors observed across the different studies. In a retrospective study of 11,855 patients, the risk of requiring hypoglycemic therapy increased in a dose-dependent manner, with odds ratios around 3, 6, and 10 for patients receiving daily GC doses equivalent to 50, 100, and more than 120 mg of

hydrocortisone (Gurwitz et al., 1994). Two large retrospective studies using the UK Clinical Practice Research Datalink examined diabetes risk in patients receiving a low dose of glucocorticoids therapy. In both studies, diabetes was defined using read codes or any of the following criteria: fasting blood glucose ≥7 mM, HbA1c level ≥ 7%, random glucose level ≥11.1 mM, or glucose tolerance test ≥11.1 mM. In the first study by Movahedi et al. (Movahedi et al., 2016), which included 21,962 UK patients and 12,657 US patients with rheumatoid arthritis from the National Databank for Rheumatoid Disease, no increased risk of hyperglycemia was observed with daily doses <5 mg/d. However, a second study by Wu et al. (Wu et al., 2020), which analyzed 100,722 patients with immune-mediated inflammatory diseases (including 28,365 with rheumatoid arthritis), reported an increased risk of incident diabetes even at the lowest daily dose category (<5 mg/day).

ii. Exacerbation of Pre-existing Diabetes

GC exposure consistently worsens glycemic control in patients with pre-existing diabetes. A study of 80 patients with type 2 diabetes treated with methylprednisolone found that 64% required temporary insulin therapy due to severe hyperglycemia (Feldman-Billard et al., 2005). Another large observational study of 1,066 people with type 2 diabetes revealed that those exposed to GCs had higher HbA1c levels compared to those not exposed (Reynolds et al., 2012). The risk of exacerbation is particularly high in patients with poor baseline glycemic control. After a 3-day pulse of methylprednisolone, all patients with a baseline HbA1c ≥8% required insulin treatment, compared to only 45% of those with HbA1c <8% (Feldman-Billard et al., 2005). The main risk factors for exacerbation of pre-existing diabetes include: poor baseline glycemic control, higher GC doses, longer duration of GC therapy, and patient

characteristics such as older age, higher BMI, and the presence of abdominal obesity (Fardet & Feve, 2014; J. X. Li & C. L. Cummins, 2022; Reynolds et al., 2012).

b) Glucocorticoid-induced diabetes with Cushing Syndrome

The prevalence of Cushing's Syndrome (CS) in patients with type 2 diabetes is rare, estimated below 1% in most studies. Consequently, routine screening for CS is not recommended in type 2 diabetes patients unless additional signs indicative of CS is present (Tabarin et al., 2022). However, the prevalence of impaired glucose tolerance and type 2 diabetes among patients with CS ranges from 15–65% and 10–50%, respectively (Giordano et al., 2014; Hirsch et al., 2018; Roldan-Sarmiento et al., 2021; Schernthaner-Reiter et al., 2019; Valassi et al., 2019). Diabetes is a significant cardiovascular risk factor in CS patients and contributes to increased mortality, even in cases where remission of the disease is achieved (Pivonello et al., 2016; Roldan-Sarmiento et al., 2021; Valassi et al., 2019).

Patients with CS who have a family history of diabetes or metabolic syndrome are more likely to develop diabetes (Giordano et al., 2014). Additionally, metabolic syndrome, age, and waist circumference are associated with the presence of diabetes in CS (Giordano et al., 2014). Interestingly, one study observed a 2.4 time higher prevalence of diabetes in patients with ACTH-dependent Cushing's compared to adrenal CS (Schernthaner-Reiter et al., 2019) while this difference was not observed in another cohort (Hirsch et al., 2018). However, the degree of hypercortisolism was higher in the first cohort in the group of ACTH-dependent CS compared to the adrenal CS while there were no differences in the other study (Hirsch et al., 2018; Schernthaner-Reiter et al., 2019). In a large multicenter cohort of patients with adrenal incidentalomas, nearly 25% of patients with mild autonomous cortisol secretion, who

did not exhibit physical features of CS, were found to have diabetes. In contrast, 18% of patients with non-functioning adenomas presented with diabetes (Deutschbein et al., 2022).

Forty to 56% of patients with CS experience resolution of diabetes following remission of the disease (Roldan-Sarmiento et al., 2021; Schernthaner-Reiter et al., 2019). Patients with higher baseline urinary free cortisol levels are more likely to achieve diabetes remission compared to those with lower levels (Schernthaner-Reiter et al., 2019). For patients who do not achieve remission, it is possible that their diabetes is not solely attributable to hypercortisolism. This may be also explained by the fact that more severe cases of CS tend to be diagnosed earlier, leading to shorter exposure to hypercortisolism. The same association between urinary free cortisol and absence of remission of hypertension was made in the same cohort (Schernthaner-Reiter et al., 2019).

In a series of 140 CS patients, those with diabetes exhibited lower HOMA-β and oral disposition index values, while neither ISI-Matsuda nor HOMA-IR was affected. This suggests that patients with CS and diabetes have a significant defect in insulin secretion without a corresponding decrease in insulin sensitivity compared to CS patients without diabetes (Giordano et al., 2014). This finding was recently confirmed in a Chinese cohort of 60 patients (Gong et al., 2022). Interestingly, in a series including 118 CS patients, the only comorbidity correlated with the level of hypercortisolism was diabetes mellitus, indicating that excess GCs play a crucial role in β-cell failure (Schernthaner-Reiter et al., 2019)

1.3.2 Effect of glucocorticoids on systems involved in metabolism

a) Glucocorticoid action on immune system

Inflammation contributes to β-cell dysfunction in diabetes (Eguchi & Nagai, 2017). GCs modulate both innate and adaptative immunity. Inflammation is a complex process involving several effectors at the molecular and cellular levels (Zen et al., 2011). Three phases in the inflammation response are usually distinguished: the alarm phase where inflammatory mediators are released by macrophages, mast cells present on the injured site; the amplification phase where additional leukocytes are mobilized and finally the resolution phasis, involving the clearance of debris and the secretion of anti-inflammatory cytokines to restore tissue integrity and function (Barnes, 1998; Cain & Cidlowski, 2017).

GCs modulated these three phases, inhibiting the first two phases and, on the contrary, promoting the last phase (Cain & Cidlowski, 2017). Notably, during the first phase, GCs repress toll-like receptor (TLR) signalling essential for the recognition of the pathogens, via the induction of the transcription of immunosuppressive genes as IkBα, dual specificity protein kinase-1 (DUSP1), mitogen activated protein kinase phosphatase-1 (MKP1), and GC-induced leucine zipper (GILZ), which in turn suppress the activation of key transcription factors as AP1, and pathways as the NF-kB pathway and the MAPK cascades.

The inhibition of these pathways, occur through the inactivation of some of their crucial factors as p38, JUN N terminal kinase (JNK), extracellular signal-regulated kinase (ERK), (Cain & Cidlowski, 2017; Taves & Ashwell, 2021; Zen et al., 2011). These mechanisms lead to the suppression of T cell production of inflammatory cytokines, chemokines, and other co-stimulatory molecules such as IL-6, IL-1β, IL-12, TNF-α, CCL2, CXCL1, and TSLP. GCs modulate also T cell activity by suppressing CD4+ T

cell (helper T cells) activation, and by influencing T helper cells programming. GCs acts as well on B-cells development, survival, and decreasing their antibody production, although the mechanisms of these effects are less known (Cain et al., 2020; Cain & Cidlowski, 2017).

Finally, GCs regulate cell death and particularly induce apoptosis in T cells and mature B lymphocytes (Barnes, 1998; Brunetti et al., 1995; Zen et al., 2011). Interestingly, in certain context, GCs may have a pro-inflammatory effect as GCs enhanced pro-inflammatory genes. The timing to GCs exposure appeared crucial for these pro-inflammatory effects. If cells are exposed prior the immune challenge, GCs potentiate the inflammatory responses while when GCs are administrated after, they will exert their anti-inflammatory function (Cruz-Topete & Cidlowski, 2015; Desmet & De Bosscher, 2017).

Interestingly β-cells produce both cytokines and chemokines (e.g., IL-1α, IL-1β, TNF-α, MCP-1) (Burke et al., 2014a; Collier et al., 2021; Lund et al., 2008; Maedler et al., 2002; Piemonti et al., 2002), and their receptors (IL-1R) (Boni-Schnetzler et al., 2018), which are important for their function. GCs exposure can improve β-cells function likely by suppressing inflammatory signals (Boni-Schnetzler et al., 2018; Hult, Ortsater, et al., 2009; Lund et al., 2008). Moreover, local GCs regeneration within the β-cells have been shown to protect against inflammatory β-cells destruction in transgenic mice over expressing 11β-HSD1 exposed to high fat diet (Turban et al., 2012a) or streptozotocin (Liu et al., 2014a).

b) Glucocorticoids action on the central nervous system

The brain regulates glucose homeostasis by sensing blood glucose levels through specialized neurons in the hypothalamus and brainstem. It integrates signals from hormones like insulin and modulates glucose metabolism by influencing the pancreas, liver, and muscle, ensuring stable blood glucose levels. The brain is increasingly recognized as an important factor in metabolic diseases (Alonge et al., 2021; Jais & Bruning, 2017).

The GR is expressed in various cell types within the central nervous system, such as glial cells, neurons, astrocytes, and oligodendrocytes (Vielkind et al., 1990), and in different brain regions, the highest expression being in the hypothalamus and in the hippocampus (Ahima et al., 1991; Ahima & Harlan, 1990). In the hypothalamus and the pituitary, GCs are crucial for the negative feed-back loop of the hypothalamic-pituitary-adrenal axis. GCs generally enhance appetite, particularly during prolonged stress or chronic GC use, which can have unfavorable metabolic consequences. GCs act on hypothalamic nuclei and the ventral tegmental area to dysregulate appetite control, diminishing homeostatic regulation while enhancing hedonic desire for food. This often leads to increased food intake, especially of calorie-dense foods, potentially contributing to metabolic disturbances (Kuckuck et al., 2023).

In the hippocampus, GCs have several genomic and non-genomic effects on brain function as memory, learning, and emotional regulation (Gray et al., 2017). GCs play a role in neural plasticity in the hippocampus, but also the amygdala and the prefrontal cortex (McEwen et al., 2016). They affect neurogenesis and lead to morphological modification in dendritic cells (Gray et al., 2017). They modulate neurotransmitters, particularly the GABA and glutamate (Popoli et al., 2011), but also neuromodulators

and neurotrophic factors (Gray et al., 2017; McEwen, 2007; McEwen et al., 2016). These actions of GCs on the brain are well illustrated by the neurological consequences of GCs overexposure as observed in patients presenting with Cushing's syndrome where neurological symptoms as insomnia, anxiety, cognitive dysfunction, and more rarely psychiatric manifestations as mania can be observed (Pivonello et al., 2015). Morphological and functional changes are also observed with cerebral atrophies, smaller hippocampal volumes and altered functional activity (Andela et al., 2015).

The hypothalamus contains glucose-sensing neurons that play a crucial role in regulating pancreatic insulin secretion. These neurons detect changes in glucose levels, likely through multiple mechanisms including sensing glucose metabolism products such as ATP. The hypothalamus communicates with the pancreas via extensive autonomic innervation, modulating insulin release through various pathways including muscarinic and α-adrenergic signaling (Rodriguez-Diaz et al., 2011). Research by Osundiji et al. (2012) demonstrated this connection in male Sprague-Dawley rats. During an intravenous glucose tolerance test (IVGTT), intracerebroventricular glucose infusion improved glucose handling and enhanced insulin secretion. Conversely, when hypothalamic glucose sensing was inhibited using gelsolin and methylhydrazine, glucose handling was reduced and first-phase insulin secretion was impaired (Osundiji et al., 2012). These findings highlight the significant role of brain glucose sensors in the regulation of pancreatic glucose-stimulated insulin secretion.

c) Glucocorticoids action on bones and growth

Bone mineral, fat tissue, and energy metabolism are closely interconnected. Calcium and phosphorus are involved in insulin secretion and energy production, while fat tissue stores lipids and vitamin D, which influence calcium balance and energy use. Hormones that regulate energy and mineral balance may also affect fat cells. For example, insulin acts on bone-forming cells, and bone-derived factors like osteocalcin help regulate glucose metabolism and insulin secretion (de Paula & Rosen, 2013).

GCs have significant, multifaceted effects on bone cells, including osteoblasts, osteocytes, and osteoclasts, influencing their replication, differentiation, and function both directly by binding to the GR and MR, and indirectly (Beavan et al., 2001; Hardy et al., 2018). Directly, GCs reduce osteocyte mechanosensing, suppress osteoblast-mediated bone formation, and promote osteoclast-driven bone resorption. Indirect effects are mediated through actions on muscle and systemic calcium balance, including increased muscle wasting, reduced muscle loading, decreased intestinal calcium absorption, and impaired renal calcium resorption.

The effects of GCs on bone formation are in fact more complex, differing between *in vivo* and *in vitro* settings, likely due to the complex bone microenvironment and dose-dependent mechanisms. *In vivo*, pharmacological doses of GCs typically inhibit bone formation, while *in vitro* effects can vary based on experimental conditions. This apparent paradox may be explained by the biphasic nature of GC effects, where low doses can stimulate and high doses inhibit osteoblast activity. Several key pathways mediate GC effects on osteoblasts, including RANKL/OPG signalling, Wnt pathways and their inhibitors, microRNAs, IL-11, BMP/Notch signaling, and apoptosis regulation (Frenkel et al., 2015).

High doses of GCs also induce apoptosis of osteocytes while low doses or discontinued treatment show less pronounced effects (Jia et al., 2011). Concerning the osteoclasts, GCs affect their resorption activity and pit formation. High doses of GCs inhibit osteoclast proliferation and disrupt cytoskeletal organization, impairing their resorptive function. GCs at lower doses may be used in culture media to influence osteoclast growth, but their effects on differentiation and activation are dose-dependent and complex (Hardy et al., 2018).

GCs have also dose-dependent effects on mesenchymal precursor cells. At physiological or low doses, GCs can promote osteogenic differentiation of mesenchymal stem cells, while high doses suppress this process and shift differentiation towards the adipocyte lineage. GCs generally slow the proliferation of mature osteoblast-like cells in culture, particularly at high doses (Hardy et al., 2018).

Osteocalcin, a marker of osteoblast activity, is involved in regulating fuel metabolism by influencing insulin production, secretion, and sensitivity (Ferron et al., 2008). The alteration of the osteoblast function induced by GCs lead to a decrease in serum osteocalcin levels (Cooper et al., 2016), and this decrease contribute to the GCs-induced insulin resistance, impaired glucose liver handling, hyperglycemia, and compensatory hyperinsulinemia (Brennan-Speranza et al., 2012; Cooper et al., 2016)

d) Glucocorticoids action on cardiovascular and kidney function

The kidney is the main organ in humans that produces cortisone and plays a significant role in GC metabolism as earlier discussed. 11β-HSD2 activity plays also an important role on the pathophysiology of salt and water retention and a decreased activity or an overexposure overpassing the capacity of the enzyme can lead to hypertension. The

levels of 11β-HSD2 in vasculature is minimal and so the cardiovascular effect of GCs is mediated majorly by 11β-HSD1 activity and or from exogeneous GCs intake (Liu et al., 2019). GCs raise blood glucose in a dose-dependent manner, mainly through increased peripheral vascular resistance (Liu et al., 2019; Oakley & Cidlowski, 2015). They can directly act on vasculature by enhancing angiotensin synthesis (Arlt & Stewart, 2005). Their influence extends to vasoactive factors within the kidney, such as COX-2, affecting renal function (Zhang et al., 1999). Through these mechanisms, GCs increase glomerular filtration rate (GFR) and free water clearance by inhibiting vasopressin's action on the collecting duct (Arlt & Stewart, 2005). Moreover, GCs regulate ion homeostasis and acid/base balance (Hamm et al., 1999), through their effects on different transporters, including the sodium/phosphate co-transporter (Tandowsky, 1949), the sulphate co-transporter (Sagawa et al., 2000), and the sodium/bicarbonate co-transporter (Ali et al., 2000).

e) Glucocorticoids action on the skeletal muscle

The main metabolic action of skeletal muscle is to handle post-meal glucose metabolism. Skeletal muscle captures around 80% of the glucose through insulin-driven glucose disposal, promotes the activation and activity of glycogen synthase, stores up glucose as glycogen, and serves as the largest glycogen reservoir. The entry of glucose from the bloodstream into the muscle cells require glucose transporters GLUT1 and GLUT 4, which are either translocated to the membrane in response to the physiological action of insulin or constitutively expressed on it (DeFronzo et al., 1981; Klip & Paquet, 1990; Merz & Thurmond, 2020; Sylow et al., 2021). In the fasted state, the skeletal muscle is exposed to low insulin levels. In this state, glucose uptake is facilitated via the constitutive transporters GLUT1 (Klip & Paquet, 1990). In the post-prandial state, circulating insulin levels increase and lead to the translocation of

GLUT4 to the membrane via a canonical (involving AKT) and a non-canonical pathway (involving Rac1), both induced by the binding of insulin to its membrane receptor (Merz & Thurmond, 2020). The glucose that enters the cells is mainly stored as glycogen, but glucose oxidation is also increased. Insulin appears to be the stronger regulator of glycogenesis promoting activation of the key enzyme, the glycogen synthase (Sylow et al., 2021).

Several human and animal studies show that GCs impair insulin-dependent glucose uptake and glycogen synthesis (Henriksen et al., 1999). In mouse and rat skeletal muscle cells, DEX treatment decreases the expression and activity of IRS1, leading to a decrease of GLUT4 translocation to the cell membrane (Morgan et al., 2009; Saad et al., 1993). Similarly, in healthy human, treatment with prednisolone for 6 days at 0.8mg/kg, reduced insulin-induce leg glucose uptake (Short et al., 2009). GCs limit the stimulation of GSK-3 phosphorylation by insulin and therefore the enhancement of the glycogen synthase (Ruzzin et al., 2005). Insulin delivery to muscle is a key point to exert its effects (Barrett et al., 2009). Insulin induces an increase in the availability of the capillary surface areas, named capillary recruitment, via the activation of the production of NO by the endothelial cells (Kubota et al., 2011). Interestingly, GCs impaired the insulin-stimulated capillary recruitment, contributing to insulin resistance (van Raalte et al., 2013).

Muscle mass plays a role in regulating glucose homeostasis (Cleasby et al., 2016). GCs overexposure induced myopathy in patients (Khaleeli et al., 1983). Indeed, GCs have catabolic action on protein metabolism, leading to muscular atrophies, and decrease contractibility by the degradation and the decrease of the synthesis of the

myosin heavy chain (Salehian & Kejriwal, 1999). How GCs-induced sarcopenia may favour insulin resistance remains to be studied.

f) Glucocorticoids action on adipose tissue

If adipose tissue accounts for only a small fraction (around 10%) of glucose disposal after food intake, adipocytes play a crucial role on glucose homeostasis. Adipocytes secrete different adipokines including leptin and adiponectin, which both improve muscle and liver insulin sensitivity. Leptin inhibits insulin release while adiponectin enhances glucose-stimulated insulin secretion without change in insulin secretion (Rosen & Spiegelman, 2006). Beside white adipose tissue, brown adipose tissue contributes to glucose homeostasis by favoring glucose and lipids clearance, inducing a negative energy balance. They may also produce molecules that have a hormonal effect impacting glucose tolerance, promoting lipolysis, inhibiting lipolysis, and promoting adipogenesis (Peirce & Vidal-Puig, 2013).

GCs exert pleiotropic effects on adipose tissue. Its impact on adipocytes function and lipid metabolism may appear conflicting depending on the dosage and duration of the exposure (Peckett et al., 2011), on the location of the adipose tissue, and on the nutritional and hormonal status (Lee et al., 2014). GCs promotes lipolysis in mature adipocytes, by increasing transcription and expression of different enzyme as the triacylglycerol lipase, the Hormone Sensitive Lipase and the LipoProtein Lipase (Campbell et al., 2011; Slavin et al., 1994; Xu et al., 2009). This positive effect on lipolysis is mainly observed under stress, after acute exposure to GCs or in the fasting state (Carine Beaupere et al., 2021; Lee et al., 2014). On the other hand, GCs also shows antilipolytic effect (Samra et al., 1998; Vali et al., 2024). GCs may have a permissive effect on catecholamines induced lipolysis (Campbell et al., 2011; Lee et

al., 2014), while on the contrary, one study has shown an inhibitory effect (Ottosson et al., 2000).

GCs have also divergent effects on lipogenesis. In the fasting state, GCs decrease lipids storage while in the feeding state, GCs will act synergistically with insulin to promote lipid storage via the stimulation of the acetyl-CoA carboxylase and the fatty acid synthase (Lee et al., 2014).

As observed in muscle, GCs decrease glucose uptake and metabolism in fasting state or in feeding state, antagonizing the action of insulin (Garvey et al., 1989; Olefsky, 1975). GCs modulate the endocrine function of adipose tissue. They increase the expression of leptin. Acting as an anti-inflammatory drug, they also decrease the production of inflammatory cytokines (Lee et al., 2014; Ouchi et al., 2011).

Finally, GCs promote adipogenesis. GC are known to regulate the maturation of preadipocytes into differentiated adipose cells (Hauner et al., 1989). Interestingly, GCs exposure of pre-adipocytes increase insulin sensitivity and prepare the cells for differentiation (Tomlinson et al., 2010). Circadian GCs rhythm appears also to be crucial to regulate the adipose differentiation with the loss of the physiological circadian oscillations leading to an increase of fat mass (Bahrami-Nejad et al., 2018). This action explains the abnormal fat repartition observed in patients presenting with Cushing's syndrome. Finally, while promoting as well brown preadipocyte differentiation, GCs impair the brown adipose tissue function and promotes its evolution towards a white phenotype (Lee et al., 2014; Soumano et al., 2000; Strack et al., 1995).

g) Glucocorticoids action on the liver

The liver is a central metabolic organ, acting as a metabolic hub, connecting various tissues like skeletal muscle and adipose tissue. It is influenced by hormones such as insulin and glucagon, as well as neuronal signals. Its primary functions include regulating lipid and glucose levels, which it achieves by adjusting its activity based on the body's fed or fasted state to maintain energy balance (Rui, 2014). The hepatocytes are the main cells of the liver constituting about 80%. In the fed state, the hepatocytes take up (and release) glucose via GLUT2 and metabolize glucose through glycolysis and the TCA cycle to produce ATP (Agius, 2008; Rui, 2014; Seyer et al., 2013). Excess glucose is stored as glycogen or used in de novo lipogenesis (DNL) to generate fatty acids. These fatty acids are either stored in lipid droplets, used for cellular membranes, or secreted as very low-density lipoproteins (Rui, 2014; Seyer et al., 2013). During fasting, glucose transport occurs via GLUT1 and the liver sustains blood glucose levels through glycogenolysis and gluconeogenesis. Hepatic gluconeogenesis is the dominant glucose source during prolonged fasting (Rui, 2014; Seyer et al., 2013). The hepatocytes also convert fatty acids from adipose tissue into ketone bodies, providing an alternative energy source for other tissues. Liver energy metabolism is tightly controlled. The sympathetic neuronal system stimulates hepatic gluconeogenesis, while the parasympathetic system suppresses it (Rui, 2014; Stanley et al., 2010). Whereas the action of glucagon augments EGP in the liver, insulin encourages glycolysis and lipogenesis while inhibiting gluconeogenesis. Insulin is the primary driver for hepatic lipogenesis in fed state. These effects of insulin are mediated through insulin receptor signaling (Rui, 2014; Saltiel & Kahn, 2001).

GCs exert significant effects on liver metabolism, particularly in glucose and lipid homeostasis. GCs enhance hepatic glucose production primarily through stimulation

of gluconeogenesis and glycogenolysis. GCs enhance hepatic glucose production through both gluconeogenesis and glycogenolysis. At the molecular level, GCs rapidly stimulate PEPCK gene expression (within 30-60 minutes) through cAMP production, allowing hepatocytes to quickly adapt to metabolic changes. This regulation involves a sophisticated balance where GCs simultaneously promote PEPCK gene expression while decreasing PEPCK mRNA stability, enabling rapid metabolic switching between catabolic and anabolic states (C. Beaupere, A. Liboz, B. Feve, et al., 2021; Hoppner et al., 1986). This is also achieved by increasing the expression of other key enzymes such as glucose-6-phosphatase (G6Pase) (Alex Rafacho et al., 2014).

GCs promote hepatic insulin resistance through multiple mechanisms. In DEX-treated rats, while insulin receptor protein levels remain unchanged, IRS1 and PI3K protein phosphorylation is dramatically decreased, and also PI3K activity (Saad et al., 1993) This inhibition prevents AKT activation and subsequent FOXO1 inhibition, leading to increased expression of gluconeogenic genes (C. Beaupere, A. Liboz, B. Feve, et al., 2021). Some studies have revealed additional mechanisms of GC action. The protein E47 has been identified as a GR modulator in hepatic lipid and glucose metabolism, with E47-invalidated mice showing protection against steroid-induced hyperglycemia and dyslipidemia (Hemmer et al., 2019). GCs also impact mitochondrial function, causing alterations in mitochondrial DNA, increased ROS production, and decreased ATP synthesis, contributing to cellular dysfunction (Luan et al., 2019) (Luan et al., Molecules, 2019).

Regarding lipid metabolism, GCs promote hepatic lipid accumulation through multiple pathways. They enhance de novo lipogenesis by increasing the expression of lipogenic enzymes and sterol regulatory element-binding protein 1c (SREBP-1c),

while simultaneously enhancing very low-density lipoprotein (VLDL) secretion and decreasing hepatic fatty acid β-oxidation (Woods et al., 2015). GCs also increase lipolysis in visceral adipose tissue, leading to elevated levels of non-esterified fatty acids (NEFAs) in circulation. These NEFAs can activate inflammatory pathways, exacerbate insulin resistance, and promote hepatic lipid accumulation, collectively contributing to fatty liver diseases (Vegiopoulos & Herzig, 2007). This pro-steatotic effect of GCs involves both direct actions on hepatic lipid metabolism and indirect effects through altered adipose tissue function (Xu et al., 2009). Furthermore, GCs modulate inflammatory responses in the liver through regulation of pro- and anti-inflammatory mediators, which can impact overall metabolic function (Cain & Cidlowski, 2017; J.-X. Li & C. L. Cummins, 2022).

LXRβ has emerged as a crucial mediator, as LXRβ-knockout mice are protected from GC-induced hepatic steatosis. The LXRβ pathway intersects with GC signaling by modulating GR translocation and co-activator recruitment to metabolic gene promoters (Patel et al., 2017). GCs also activate AMPK, which paradoxically promotes hepatic lipogenesis, as demonstrated by the suppression of DEX-induced lipogenesis through AMPK inhibition (Hu et al., 2019) (Hu et al., Comp Biochem Physiol, 2019).

1.3.3 Effect of glucocorticoids on islet function

a) Impact of glucocorticoids exposure on β-cell function *in vitro*

A large body of literature has shown that GCs inhibit insulin release *in vitro* in primary rodents' cell models or immortalized rodents cell lines and more rarely immortalized human cells lines or human islets (**Table 3**) Inhibited insulin secretion has been observed with different GC compounds at both short term (minutes) (G. Barseghian &

Rachmiel Levine, 1980; Barseghian et al., 1982b; B. Billaudel & B. Ch Sutter, 1979), or prolonged exposure (hours to days) (Brunstedt & Nielsen, 1981; Sandrine Gremlich et al., 1997; I.-K. Jeong et al., 2001; Lambillotte et al., 1997b). Several mechanisms contribute to this GC-induced reduction in GSIS:

- DEX promotes posttranslational degradation of GLUT2 in isolated rat pancreatic islets (Sandrine Gremlich et al., 1997).
- DEX inhibits glucokinase gene expression in a dose-dependent and time-dependent manner in RIN 1046-38 cells, a rat insulinoma cell line (Borboni et al., 1996)
- GCs reduce the expression of pancreatic and duodenal homeobox 1 (Pdx1), a transcription factor crucial for pancreatic development and β cell maturation (Jonathan L. S. Esguerra et al., 2020; M. M. L. Linssen et al., 2011; Sharma et al., 1997; Zhang et al., 2009). One of the pathways leading to the reduction of PDX1 expression characterized In RINm5F cells and rat islets is the increase of active FoxO1 (Zhang et al., 2009).
- DEX suppress insulin gene expression in mouse islets (Lambillotte et al., 1997b), and also in HIT-T15-CRL-1777 β-cell line isolated from Syrian golden hamster (Goodman et al., 1996), and via a GCs negative regulatory elements in the promotor region of the INS gene (Goodman et al., 1996).
- GCs suppress the voltage-dependent Ca^{2+} channel function and the Ca^{2+} flux (Nicholas H. F. Fine et al., 2018; Lambillotte et al., 1997b; Ullrich et al., 2005). This effect is likely mediated by the upregulation of SGK1 (Jonathan L. S. Esguerra et al., 2020; Ullrich et al., 2005) which in turns increase the transcription of the voltage-gated potassium channel Kv1.5 leading to a decrease of insulin release in the INS-1 rat pancreatic β cell line (Ullrich et al.,

2005). The Phospholipase C/protein kinase C signaling pathway, involved also in the function of the β-cell voltage-activated Ca2+ channels, is also impaired after GCs exposure (Zawalich et al., 2006). Interestingly, physiological concentration of corticosterone also suppress the voltage-dependent Ca^{2+} channel function, but without altering the insulin secretion thanks to the upregulation of parallel amplifying cAMP signals and an increase in the number of membrane-docked insulin secretory granules (Nicholas H. F. Fine et al., 2018).

- PRED induces unfolded protein response, an endoplasmic reticulum stress response, resulting also on an impairment of insulin biosynthesis and release (M. M. L. Linssen et al., 2011).

These effects are mediated via the GR as treatment with GR antagonist mifepristone, compound RU486, inhibit them (Jonathan L. S. Esguerra et al., 2020; Sandrine Gremlich et al., 1997; Lambillotte et al., 1997b; M. M. L. Linssen et al., 2011; Zawalich et al., 2006). Interestingly, Glycogen Synthase Kinase 3 (GSK3), a kinase inhibiting Glycogen Synthase in the insulin signaling pathway, regulates the expression of the GR and contributes to the β-cell dysfunction induced by GCs (Delangre et al., 2021).

Several studies showed that GCs treatment induce apoptosis (Delangre et al., 2021; Jonathan L. S. Esguerra et al., 2020; Guo et al., 2016; Schmidt et al., 2004). GCs activate several key pathways that contribute to β-cell apoptosis. Notably, GCs activate GSK-3β (Guo et al., 2016), which plays a crucial role in β-cell death (Delangre et al., 2021; Guo et al., 2016). This activation is associated with increased NOX4-derived reactive oxygen species (ROS) generation, highlighting the role of oxidative stress in GC-mediated β-cell damage (Guo et al., 2016). This finding is complemented by the identification of the induction of thioredoxin-interacting protein (TXNIP) by GCs

another mediator of GC-induced apoptosis (Reich et al., 2012). Recent research has identified imatinib, a tyrosine kinase inhibitor, as a potential protective agent against GC-induced β-cell damage. Indeed, Imatinib prevents GC-induced β-cell apoptosis by increasing glutathione S-transferase P1 (GSTP1) expression and reducing oxidative stress (Semprasert et al., 2024).

GCs also modulate various signaling pathways crucial for β-cell survival and function. They inhibit the phosphorylation of IRS-2, PKB, and ERK, promoting apoptosis (Diana Avram et al., 2008). Additionally, GCs activate mitogen-activated protein kinase (MAPK) pathway (Fransson et al., 2014; Reich et al., 2012). The induction of TXNIP by the GCs dependent on p38 MAPK activation (Reich et al., 2012). The endoplasmic reticulum (ER) stress induced by GCs is another mechanism of β-cell apoptosis. Prednisolone increases the expression of ER stress markers and induces apoptosis in INS-1E cells (M. M. L. Linssen et al., 2011). Finally, at the mitochondrial level, GC-induced apoptosis involves downregulation of Bcl-2, activation of calcineurin, dephosphorylation of BAD, and mitochondrial depolarization (Felicia Ranta et al., 2006). Exendin-4, a glucagon-like peptide 1 analog, protects against GC-induced apoptosis through a cAMP-dependent protein kinase (PKA) pathway, suggesting potential therapeutic approaches.

Collectively, these studies highlight the intricate network of signaling pathways involved in GC-induced β-cell apoptosis and dysfunction. The interplay between ER stress, oxidative stress, MAPK signaling, and alterations in pro-and anti-apoptotic protein expression underscores the complexity of GC effects on β-cells.

b) Impact of GCs exposure on β-cell function *in vivo*

i. Impact of acute exposure of GCs on β-cell function

Despite ongoing research, the exact mechanisms by which GCs affect β-cell function in vivo remain unclear due to the difficulty in distinguishing between the direct effects of GCs on β-cells and the indirect effects of GC-induced peripheral insulin resistance. Oral intake of a single dose of PRED 15 mg (S. C. Kalhan & P. A. J. Adam, 1975) or 75 mg (Daniël H. van Raalte et al., 2010), DEX 1 mg (Schneiter & Tappy, 1998a), HC 100 mg (Plat et al., 1996) acutely impaired insulin secretion and/or decreased insulinogenic index in healthy volunteers during glucose infusion, meal, OGTT or dextrose infusion respectively.

In mice, acute intraperitoneal administration of 300 mg/kg of HC suppressed insulinogenic index and decreased insulin secretion, induced by an intravenous glucose challenge (Longano & Fletcher, 1983). This effect has been observed between 30min to 4h after the exposure. Interestingly, an intravenous bolus of HC (0.6 mg/kg) in healthy volunteers led to enhanced insulin secretion, then lower glucose levels within 15 minutes of a subsequent glucose bolus. This rapid effect suggests a different non-genomic effect of GCs in glucose homeostasis (Vila et al., 2010).

In other conditions as adult rat following 6h administration of DEX (10 µg/day), the acute genomic inhibitory effect of GCs on insulin secretion was not observed instead a state of insulin resistance characterized by increased total hepatic glucose production despite higher insulin levels (Stojanovska et al., 1990). Similarly, a single acute exposure to HC or methylprednisolone in healthy volunteers induces alterations in glucose tolerance within 12 hours when typically, insulin resistance should develop within one to two days of GCs treatment (Bruno et al., 1994).

ii. Impact of chronic exposure of GCs on β-cell function

In healthy volunteers, more prolonged exposure to oral DEX or PRED 3 to 40 mg equivalent PRED dose for 2 to 15 days resulted to fasting hyperinsulinemia while fasting glycemia remained normal or mildly increased (**Table 1**) (Ahren, 2008; Beard et al., 1984; Henriksen et al., 1997; Hollingdal et al., 2002; Larsson & Ahren, 1999; Matsumoto et al., 1996; Nicod et al., 2003; Schneiter & Tappy, 1998a; Daniël H. van Raalte et al., 2010; Wajngot et al., 1992; Willi et al., 2002). Hyperinsulinemia was also observed during glucose challenging with a hyperglycaemic-clamp (Beard et al., 1984; Nicod et al., 2003), or an OGTT (Hollingdal et al., 2002; Schneiter & Tappy, 1998a; Willi et al., 2002), or IV glucose challenge (Petersons et al., 2013). In addition, an increased in the plasma C-peptide values can be also observed after PRED treatment at basal (Hollingdal et al., 2002), and during a meal tolerance test (Daniël H. van Raalte et al., 2010). This enhanced β-cell function was also observed in adult rats treated between 1 to 26 days with DEX (0.125–5.0 mg/kg) as these led to hyperinsulinaemia at basal state (Karlsson et al., 2001; Novelli et al., 1999; Ogawa et al., 1992b; Rafacho et al., 2008), or after glucose challenging (Rafacho et al., 2011; Rafacho et al., 2008). This augmented β-cell function occurred in a dose- (Rafacho et al., 2008) and time-dependent manner (Rafacho et al., 2011). In normal adult mice, administration of DEX 2.5 mg/kg/day for 10 days (Thomas et al., 1998) or corticosterone (25 or 100 µg/mL in drinking water) for up to 6 weeks also resulted in basal hyperinsulinaemia (Emilie Courty et al., 2019; Fransson et al., 2013). This effect is observed from the first week of treatment for corticosterone (Fransson et al., 2013). Ex vivo analysis, i.e., GSIS study in isolated islets from rat or mice treated by GCs, confirmed that GCs treatment in vivo enhanced insulin secretion (Karlsson et al., 2001; Novelli et al., 1999; Rafacho et al., 2011; Rafacho et al., 2008; Rafacho et al., 2010).

This hyperinsulinemia allows healthy subjects to compensate for GC-induced insulin resistance. Consequently, the product of insulin secretion and insulin sensitivity, also called the disposition index, remained constant. However, in susceptible populations, including normoglycemic individuals with a reduced insulin sensitivity (Larsson & Ahrén, 1999; Wajngot et al., 1992), before treatment with GCs, or healthy, first-degree relatives of patients with T2DM (Alford et al., 1997), and in obese women (Besse et al., 2005), this compensation failed, resulting in hyperglycaemia. Similar results were obtained in rodent models of obesity and insulin resistance.

In Zucker fatty rats (fa/fa) (Grill & Rundfeldt, 1986a; Ogawa et al., 1992a), and ob/ob mice (Khan et al., 1992). DEX readily induced hyperglycemia and markedly reduced or completely abrogated GSIS. In rats with streptozocin-induced diabetes, DEX further increased fasting hyperglycemia and diminished GSIS (Grill & Rundfeldt, 1986a). The combination of GC exposure and high-fat diet in young rats can initially increase insulin secretion and β-cell mass through proliferation. However, when acting synergistically, they may promote severe insulin resistance beyond the adaptive capacity of β-cells, leading to impaired insulin response to glucose and hyperglycemia in the long run (Beaudry et al., 2013).

It also seems that enhanced GC sensitivity, induced by the overexpression of the GR in β-cells, can worsen aged-induced insulin resistance and promote the development of diabetes in one-year-old mice (Davani et al., 2004). Moreover, these mice present impaired glucose tolerance at three months, progressing to a diabetic state at 12 months of age, suggesting that GC exert a strong and direct diabetogenic effect on β-cells, potentially through the regulation of insulin secretion via the α2-adrenergic receptor (Davani et al., 2004). Enhanced GC sensitivity, induced by the

overexpression of the GR in β-cells, can also worsen age-induced insulin resistance and promote the development of diabetes in mice (Davani et al., 2004).

iii. Mechanism involved in β-cell adaptation in vivo

Numerous studies in rodents have demonstrated β-cells adaptation through proliferation in response to various states of insulin resistance, including pregnancy (Sorenson & Brelje, 1997), high-fat diet (Golson et al., 2010), and partial pancreatectomy (Bonner-Weir et al., 1993). Different mechanisms are involved in this GC-induced β-cell adaptation: higher glucose responsiveness described by higher insulin secretion at both low and stimulatory glucose with similar levels of insulin content (Karlsson et al., 2001; Rafacho et al., 2008); increased glucose sensitivity, (Rafacho et al., 2008); oxidative metabolism indicated by enhanced mitochondrion function and increased generation of NAD(P)H (Rafacho et al., 2010); higher Ca^{2+} handling characterized by increased glucose stimulated Ca^{2+} signalling (Rafacho et al., 2010); and higher response to cholinergic signals via increased activity of the PLC/DAG/$InsP_3$/PAC pathway (Angelini et al., 2010; Rafacho et al., 2010).

Further, structural changes have been also observed, with β-cell mass increasing in a time- and dose-dependent manner, corresponding to the degree of insulin resistance (Rafacho et al., 2011; Rafacho et al., 2009). Recent studies have proposed that β-cell mass may adapt to GC-induced insulin resistance through neogenesis and the formation of new β-cells from precursors (Emilie Courty et al., 2019). In a model of chronic corticosterone treatment in mice, Courty et al. observed a massive increase in β-cell mass due to augmented cell proliferation and increased islet density, suggesting β-cell neogenesis. This was accompanied by an increase in the expression of genes involved in β-cell neogenesis, such as NGN3. Interestingly, newly formed β-cell did not

appear to derive from SOX9 ductal progenitors' cells. In addition, β-cell neogenesis was found to be an indirect effect of GCs, mediated by proteogenic factors present in the serum of GC-treated mice (Emilie Courty et al., 2019). On the other hand, this adaptive mechanism of corticosterone is lost when combined with HFD in Wister rats. Increased hyperinsulinemia, hyperglycemia *in vivo*, and a depletion in insulin content *ex vivo* during a GSIS analysis, all of which are characteristic of IR (Beaudry et al., 2013).

In summary, GCs induce complex adaptive responses in pancreatic β-cells, involving both proliferation and neogenesis, to counteract the insulin resistance resulting from GC exposure. Whether the same mechanisms occur in humans has not been confirmed yet. The adaptative β-cells responses lead to hyperinsulinemia and the maintenance of basal and post-prandial euglycemia. Patients with altered insulin secretion capacity will develop glucose intolerance or diabetes.

iv. Impact of prenatal GCs overexposure on β-cell function in vivo

While GR overexpression in mature β-cells does not affect proliferation, it impairs insulin secretion. In contrast, GR overexpression in precursor cells reduces the adult β-cell fraction without affecting insulin secretion or glucose tolerance (Blondeau et al., 2012). Deletion of GR in pancreatic precursor cells results in an increased β-cell mass, with a greater number and size of islets (Gesina et al., 2004). Overexposure to GCs during early fetal life is also associated with reduced insulin secretion in adulthood, which can lead to abnormal glucose tolerance later in life. This has been observed in contexts of maternal food restriction, where rodent models have shown an association between elevated corticosterone levels and the role of GR in the development of reduced β-cell mass in offspring (Blondeau et al., 2001; Valtat et al., 2011).

Furthermore, this connection between fetal GCs overexposure and impaired insulin secretion has also been observed in humans exposed prenatally to dexamethasone (DEX) for the prevention of female fetal virilization when the mother is affected by congenital adrenal hyperplasia due to 21-hydroxylase deficiency (Riveline et al., 2020).

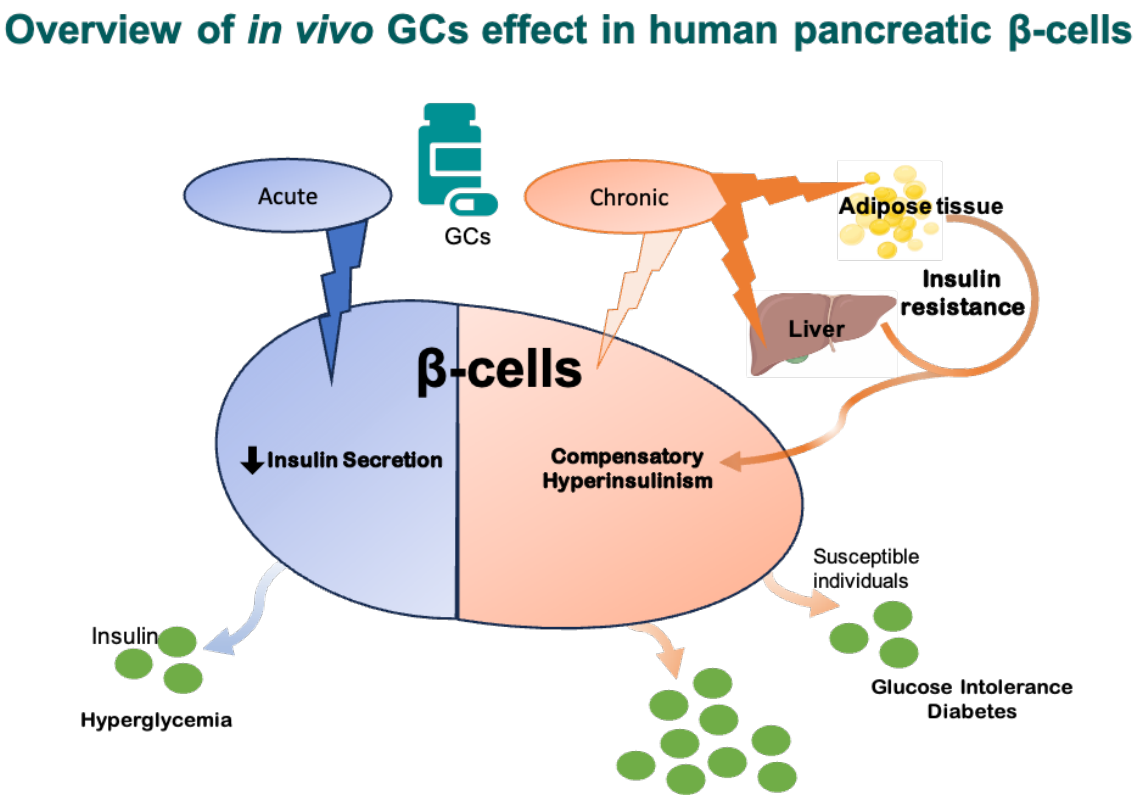

Figure 8: Schematic of the *in vivo* GCs effect in human pancreatic β-cells. An acute single dose of oral GCs inhibits GSIS and leads to β-cells apoptosis. On the other hand, chronic use of GCs leads to peripheral insulin resistance and compensatory hyperinsulinemia with increased β-cell mass. Patients with altered insulin secretion would be more prone to develop diabetes.

Table 3: summary of main studies investigating the *in vivo* impact of GCs on human islets

human subjects	GC / dose / no of times /duration	Glucose test performed	Outcome of the study	Ref
6 healthy volunteers	Dexamethasone / 0.5 mg/d / 4X / 2days and 0.5mg/day / 1X / 1 day	OGTT	↑post-load glucose level, ↓metabolic clearance of glucose, no effect on total glucose turnover, total and exogenous glucose oxidation, suppression of EGP, ↓glucose metabolic clearance, ↑insulin concentration.	(Schneiter & Tappy, 1998a)
6 healthy male volunteers	Prednisolone / 75mg/d / 1X / 1 day	Standardized meal test, OGIS,	↑AUC $_{\text{post-prandial glucose}}$, ↓β-cell responsivity (AUC$_{\text{C-peptide}}$ / AUC $_{\text{glucose}}$), ↓insulinogenic index, ↓glucose sensitivity, ↓potentiation factor ratio, ↓OGIS, ↓AUC$_{\text{ISR}}$/AUC $_{\text{glucose}}$	(D. H. van Raalte et al., 2010)
23 healthy male volunteers	Prednisolone / 30mg/d / 1X / 15 days		↑FBG, ↑HOMA-IR, ↓OGIS, ↑AUC glucose, ↑AUC C-peptide	
	Hydrocortisone / 100 mg/d /1X / 1 day	10% dextrose infusion at 5 g/kg / day for 26h	0-4h: ↓AUC $_{\text{ISR}}$, ↓AUC $_{\text{insulin}}$, ↓ISR, ↓insulin 4-11h: ↑AUC $_{\text{ISR}}$, ↑AUC $_{\text{insulin}}$, ↑AUC $_{\text{glucose}}$, ↑ISR, ↑insulin, ↑glucose 11-15h: ↑AUC $_{\text{ISR}}$, ↑AUC $_{\text{insulin}}$, ↑AUC $_{\text{glucose}}$	(Plat et al., 1996)
8 healthy male volunteers	IV bolus of Corticotropin-releasing hormone / 25 µg / IX / 1 day	10% dextrose infusion at 5 g/kg /day for 37h	Immediate response: ↓AUC $_{\text{ISR}}$	
10 healthy male volunteers	IV bolus of hydrocortisone / 0.6 mg/kg / IX / 1 day	IVGTT	0-15mins: ↓ AUC $_{\text{glucose}}$, ↑AUC $_{\text{insulin}}$, ↑AUC $_{\text{C-peptide}}$ 0-60 mins: ↓AUC $_{\text{glucose}}$, ↑ AUC $_{\text{insulin}}$, 120-180 mins: ↑ AUC $_{\text{glucose}}$, ↓AUC $_{\text{C-peptide}}$,	(Vila et al., 2010)
9 healthy female volunteers	Dexamethasone / 15 mg / 1X / 3 days	IV of 5 g of arginine before glucose stimulation	↑fasting insulin, ↑insulin secretion,	(Ahren, 2008)
6 low insulin responders **6 high insulin responders**	Dexamethasone / 15 mg / 1X / 2 days	OGTT, hyperglycemic clamp	↑glucose, ↑insulin secretion, diabetic OGTT, ↑hepatic EGP, ↑glucose output, ↑glucose cycling, ↓basal glucose metabolic clearance, unable to compensate for dexamethasone-induced insulin resistance ↑insulin response, ↑↑insulin secretion,	(Wajngot et al., 1992)
20- 1st degree relative of NIDMM	Dexamethasone / 4mg / 1X / 5 days	OGTT, IVGTT	Impaired glucose regulation, ↑glucose, ↓first phase insulin secretion, ↓insulin sensitivity index, unable to compensate for dexamethasone-induced insulin resistance	(Henriksen et al., 1997)
10 women with normal glucose tolerance **5 women with high insulin sensitivity**	Dexamethasone / 3mg / 2X / 2.5 days	IV of 5 g of arginine before glucose stimulation	↓insulin sensitivity, ↑ fasting glucose, ↓insulin sensitivity, ↑fasting insulin, ↑plasma insulin post-glucose, ↓glucagon response to arginine	(Larsson & Ahren, 1999)
10 healthy men	Dexamethasone / 2mg / 1X / 3 days	IVGTT	↓insulin sensitivity,	(Matsumoto et al., 1996)
10 healthy men	Dexamethasone / 6mg / 1X / 3 days		↓glucose clearance rate, ↓↓insulin sensitivity, ↓glucose uptake, ↑EGP	
human subjects	GC / dose / no of times /duration	Glucose test performed	Outcome of the study	Ref

8 healthy men	Prednisolone / 30 mg / 1X / 2 µM / 6 days	,	↑HOMA-S, ↑↑1st phase insulin secretion, AUC $_{glucose}$, ↓AUC $_{C\text{-}peptide}$,	(Hollingdal et al., 2002)
8 healthy non-obese volunteers	Dexamethasone /2 mg/ 1X / 2 days	↓two-step hyperglycemic clamp	↓insulin sensitivity, ↑ 1st phase insulin secretion, ↑ insulin concentration, fasting glucose, Hyperinsulinemia,	(Nicod et al., 2003)

Table 4: summary of main studies investigating the *in vitro* impact of GCs on human islets and/ or experimental models

Experimental model / human islets	GC / dose/duration	Outcome (GC treated vs control)	Limitation of studies	Ref
Male mice whole pancreas	Corticosterone / 100 nM / 5 or 50 mins	↑ glucagon secretion, ↓GSIS	No normalization to islets protein contents. No data on islets viability	(G. Barseghian & Rachmiel Levine, 1980)
Rat islets	Dexamethasone / 1 or 10 or 100 nM / IH or 6H	Islet viability was not affected. No dexamethasone effect at 1H, Effect observed from 3H, dose and duration-dependent ↓insulin content, dose-dependent ↓GSIS and ↓ pre-proinsulin levels at 6H	No normalization to islets protein contents GC effect on α-cells not characterized	(I.-K. Jeong et al., 2001)
Mice islets	Dexamethasone / 0.01 or 0.1 or 1 µM & 250 nM / 18H	no effect at 1H incubation time ↓GSIS in a dose-dependent manner, ↓1st and 2nd phases GSIS, ↑ glucose oxidation at 1 µM conc, ↑ insulin content was observed 250 nM had a reversible effect on ↓GSIS	No normalization to islets protein contents Supra pharmacological dose at 1µM. GC effect on α-cells not characterized, No data on islets viability	(Lambillotte et al., 1997b)
Rat islets	Dexamethasone / 1 µM / 48H	↓proinsulin mRNA, ↓insulin content, ↓GSIS	Supra pharmacological dose No normalization to islets protein contents, No data on islets viability / apoptosis	(Sandrine Gremlich et al., 1997)
Rat pancreas	Cortisone or cortisol / 1 or 10 or 100 µM. / 10 or 50 mins	Cortisol has no effect on GSIS Cortisone 10 and 100 µM dose dependently ↓GSIS, Cortisol and Cortisone at 100 µ M ↓basal glucagon secretion	Supra pharmacological doses No data on islets viability / apoptosis	(Barseghian et al., 1982b)
Rat Insulinoma cells, glucagonoma cells, mice islets	Dexamethasone / 1 or 10 or 100 or 500 nM / 24 or 48 or 72H Methylprednisolone / 167 nM / 24 or 48 or 72H	Dexamethasone, ↓β-cells viability in a dose and duration dependent manner, Dexamethasone ↑ β-cells apoptosis, no effect on glucagonoma cell viability Methylprednisolone ↓insulinoma cells viability in a duration and dose-dependent manner	No normalization to islets protein contents	(Reich et al., 2012)
Mice islets	Dexamethasone / 1 µM / 1 or 3H	no effect at 1H, ↓GSIS, no difference in insulin content and glucose utilization	Supra pharmacological dose	(Zawalich et al., 2006)
Rat islets	Dexamethasone / 6.3 µM / 1 or 2H or 48H, prednisolone and hydrocortisone at equimolar concentrations	↓GSIS, no difference in insulin content, ↓1st and 2nd phases GSIS	Supra pharmacological dose, no normalization to islet protein content	(Pierluissi et al., 1986)
Human islets	Cortisol / 20 nM / 48H Cortisone / 200 nM / 48H	↑ca2+ but both GCs have no effect on GSIS	No reflect of alpha cells function	(Nicholas H. F. Fine et al., 2018)
Human immortalized EndoC-βH1 - β-cells	Dexamethasone /100nM / 2,8,24, and 48H,	↓GSIS, ↓insulin content	No normalization to insulin content	(Alexandros Karagiannopoulos et al., 2023)
Human immortalized EndoC-βH1 - β-cells, human islets	Dexamethasone / 2 µM / 24H	↓GSIS, ↑β-cells apoptosis,	Supra pharmacological dose No reflect of alpha cells function	(Jonathan L. S. Esguerra et al., 2020)

1.4 Cortisol metabolism and metabolic diseases

1.4.1 Overall cortisol metabolism in metabolic diseases

Patients exposed to an excess of GCs overexposure present, even without obesity, similar metabolic impact than obesity. Cortisol exposure has been also suggested to be abnormal in obese patients. However, several studies in obese subjects have found discordant results, with some showing increased (Anagnostis et al.) and others decreased (Rask et al.) plasma or urinary cortisol levels. Collectively, these studies suggest that circulating cortisol levels do not appear to be significantly altered in obese patients (Aldhahi and Goldfine).

It has also been hypothesized that tissue exposure to GCs may contribute to the metabolic complications of obesity. In 1997, Bujalska et al. demonstrated that cultured cells from human omental adipose tissue could convert cortisone to cortisol, leading them to propose the concept of "Cushing's disease of the omentum." (Bujalska et al., 1997). A few years later, Andrew et al. (Andrew et al., 2002), provided strong experimental evidence using a novel tracer infusion method that conversion of cortisone to cortisol in humans occurs and contributes significantly to the daily production of cortisol. They demonstrated that the infusion of D4 cortisol in fasting, nondiabetic individuals resulted in measurable amounts of plasma D3 cortisol, meaning that D4 cortisol has been transformed in D3 cortisone and D3 cortisol by subsequently the 11β-HSD2 and 11β-HSD1. Basu et al. (Basu et al., 2004), combined the same tracer infusion method with hepatic venous and leg catheterization techniques to determine the site(s) of cortisone to cortisol conversion. Their studies revealed that in healthy nondiabetic individuals, splanchnic cortisol production rates

were equal to or even exceeded those produced by extrasplanchnic tissues, such as the adrenals. However, due to the simultaneous uptake of cortisol within the splanchnic bed, only a small net amount of cortisol was released. Andrew et al. (Andrew et al., 2005), further estimated that approximately two-thirds of splanchnic cortisol production occurs in visceral fat, while the liver accounts for the remaining one-third. Finally, using the same methods but including direct measurement of visceral and liver cortisol production obtained in obese patients during bariatric surgery, Basu et al demonstrated that liver is responsible for nearly all splanchnic cortisol production (Basu et al., 2009), observation supported by further study by Walker's team (Stimson et al., 2009). Although HSD11B1 gene expression is present in visceral fat, albeit at much lower levels than in the liver, the viscera do not appear to release cortisol into the portal vein. Conversely, despite the very low expression of HSD11B2 mRNA in visceral fat, the viscera releases cortisone into the portal vein, thus providing a substrate for intrahepatic cortisol production (Basu et al., 2009). In addition, it was shown that 11β-HSD1 generates significant cortisol in subcutaneous adipose tissue likely acting primarily through local (intracrine/paracrine) mechanisms rather than contributing to systemic circulation, since hypothalamic-pituitary-adrenal axis regulation maintains homeostatic cortisol levels (Stimson et al., 2009).

These findings prompted extensive research into cortisol metabolism in obesity and diabetes. Studies using different catheterization techniques and tracer methods consistently demonstrated that hepatic and splanchnic cortisol production rates were not different between lean and obese subjects, regardless of diabetes status (Basu et al., 2005; Dube et al., 2014; Stimson et al., 2011). However, obesity, but not diabetes, was associated with increased splanchnic cortisol uptake (Basu et al., 2005). Whole-body 11β-HSD1 activity was shown to be increased in obese men with type 2 diabetes

(Stimson et al., 2011), while showing only a trend toward increase in non-diabetic obese men (Anderson et al., 2021). In adipose tissue specifically, 11β-reductase activity was detected only in obese individuals, not in lean subjects (Anderson et al., 2021).

1.4.2 11β-HSD and metabolic diseases

a) 11β-HSD1 alterations in obesity

Urinary total cortisol metabolites excretion assessed in a 24h urines collection is increased in patients presenting with obesity (Andrew et al., 1998; Kim et al., 2021; Stewart et al., 1999; Westerbacka et al., 2003) The ratio THF + αTHF/THE reflecting the global 11β-HSD1 has been shown to be decrease in patients with obesity (Kim et al., 2021; Rask et al., 2001; Stewart et al., 1999). Several studies have investigated *HSD11B1* expression and activity of 11β-HSD1 in liver, subcutaneous adipose tissue (SAT) or visceral adipose tissue (VAT) of obese patients. Increased *HSD11B1* expression in SAT of obese patients compared to lean controls was reported in several studies (Engeli et al., 2004; Methlie et al., 2013; Pardina et al., 2015; Paulmyer-Lacroix et al., 2002; Rask et al., 2001; Woods et al., 2015). Additionally, SAT *HSD11B1* expression and 11β-HSD1 activity were found to correlate positively with BMI, percentage body fat, and waist circumference in some cohorts (Kannisto et al., 2004; Lindsay et al., 2003; Methlie et al., 2013; Munoz et al., 2009; Wake et al., 2003; Westerbacka et al., 2003). However, Tomlinson et al. (Tomlinson et al., 2002), did not observe a correlation between SAT *HSD11B1* expression and BMI in a cohort of non-obese women. SAT *HSD11B1* expression was higher in patients with metabolic syndrome (MetS+) compared to those without (MetS-) in one cohort of 62 obese patients (Alberti et al., 2007), while two other cohorts including 37 and 50 morbidly

obese patients did not found significant difference (Constantinopoulos et al., 2015; Torrecilla et al., 2012).

Regarding VAT, increased *HSD11B1* expression in obese patients compared to controls was also reported (Mariniello et al., 2006; Pardina et al., 2015; Woods et al., 2015). Constantinopoulos et al. (Constantinopoulos et al., 2015), found that VAT *HSD11B1* expression was higher in obese patients with MetS+ compared to those without MetS, and a positive correlation with waist circumference was observed. However, two other teams (Alberti et al., 2007; Torrecilla et al., 2012), did not find significant differences in VAT *HSD11B1* expression between MetS+ and MetS- obese patients. Liver *HSD11B1* expression was found to be correlated with BMI in some studies (René Baudrand et al., 2011; Constantinopoulos et al., 2015; Pardina et al., 2015), while (Baudrand et al., 2010), reported a negative correlation between liver HSD11B1 expression and BMI. Torrecilla et al. (Torrecilla et al., 2012) observed increased liver *HSD11B1* expression in MetS+ obese patients compared to MetS-, but this finding was not replicated by Baudrand et al. (Baudrand et al., 2010), or Pardina et al. (Pardina et al., 2015). Finally, impaired 11β-HSD1 liver activity, assessed by the conversion of oral cortisone to cortisol, was also reported in obese patients (Rask et al., 2001; Rask et al., 2002; Stewart et al., 1999).

In summary, while several studies have reported increased *HSD11B1* expression in SAT and VAT of obese patients, findings regarding liver *HSD11B1* expression and its correlation with BMI have been inconsistent. Severe obesity may lead to a decreased *HSD11B1* expression in the liver as a protective mechanism from the increase omental cortisol generation (Chapman et al., 2013). While based on these data and on animal models (see below) it is hypothesized that increase cortisol regeneration secondary to

11β-HSD1 expression/activity alteration led to local cortisol overexposure and deleterious metabolic effects due to the activation of the GC, there is no data in human highlighting the 11β-HSD1 alteration and the activation of some GC-target genes. However, the relationship between GR and *HSD11B1* has been investigated. Torrecilla et al. (Torrecilla et al., 2012), found that liver GR mRNA expression was higher in the liver of morbidly obese patients with metabolic syndrome and positively correlated with *HSD11B1* expression. This observation is consistent with some mice models of obesity where *Hsd11b1* and GR expression are associated (Liu et al., 2005; Morton et al., 2005). Constantinopoulos et al., reported that liver *HSD11B1* mRNA levels were negatively correlated with the liver GR mRNA levels in severely obese patients without metabolic syndrome, suggesting that this negative correlation could represent a compensatory mechanism preventing the appearance of metabolic syndrome in severely obese patients (Constantinopoulos et al., 2015).

Interestingly, adipose leptin synthesis or secretion are enhanced by GCs (Leal-Cerro et al., 2001), and an increase in leptin mRNA expression or serum leptin level is observed in mice with overexpression conditional to adipocytes (Masuzaki et al., 2001). In a cohort of 32 individuals, adipose leptin mRNA levels were positively associated with 11β-HSD1 activity and *HSD11B1* mRNA levels, as well as BMI and fasting insulin levels (Wake et al., 2003). The enhancement of leptin may also mitigate some detrimental effects of cortisol overexposure. Interestingly, *HSD11B1* expression in preadipocytes seems to play a role their differentiation and may also contribute to visceral fat accumulation in obesity. *HSD11B1* expression or reductase activity is high in omental preadipocytes compared to subcutaneous preadipocytes in control women or in mice (De Sousa Peixoto et al., 2008; Tomlinson et al., 2002). Its expression is strongly enhanced by GCs in omental compared to subcutaneous preadipocytes

(Bujalska et al., 2006). However, Tomlinson et al. (Tomlinson et al., 2002) found that *HSD11B1* expression and 11β-HSD1 activity is decreased in omental preadipocytes pool of obese patients, and inversely correlated with BMI. They propose that enhanced preadipocyte proliferation within omental adipose tissue, as a consequence of decreased 11β-HSD1 activity, may contribute to increases in visceral adipose tissue mass in obese patients.

The relationship between 11β-HSD1 and weight change has been investigated in several studies. In a cohort of 20 volunteers, SAT *HSD11B1* mRNA expression, but not activity, was positively associated with weight gain during follow-up (Koska et al., 2006). The impact of weight loss on SAT *HSD11B1* expression has yielded conflicting results. After a 5% weight loss achieved through diet and exercise, no modification of SAT HSD11B1 gene expression was observed (Engeli et al., 2004). However, another diet protocol led to a decrease in SAT *HSD11B1* mRNA expression (Purnell et al., 2009) In contrast, Tomlinson et al. (Tomlinson et al., 2004), reported an increase in SAT *HSD11B1* expression 10 weeks after a weight loss of >10% achieved through a very low-calorie diet. In the same study, hepatic 11β-HSD1 activity remained unchanged. Consistent with this finding, they observed increased cortisol availability within adipose tissue interstitial fluid after weight loss (Jeremy W. Tomlinson et al., 2008). Interestingly, when weight loss was induced by bariatric surgery, SAT *HSD11B1* expression decreased to levels comparable to those of control subjects (Leyvraz et al., 2012; Methlie et al., 2013; Pardina et al., 2015; Woods et al., 2015). An assessment of cortisol generation from cortisone showed that liver 11β-HSD1 activity increased, while subcutaneous adipose tissue activity decreased following bariatric surgery (Woods et al., 2015).

b) 11β-HSD1 alterations in T2D

In human pancreatic islets, HSD11B1 is expressed and active. While several studies have demonstrated positive associations between *HSD11B1* expression and 11β-HSD1 activity in SAT, VAT, and liver with various glucose tolerance parameters, some conflicting results have been reported, possibly due to differences in the studied populations and statistic power. In SAT, 11β-HSD1 activity was positively associated with fasting glucose, fasting insulin, and/or insulin resistance in a cohort of Caucasian and Pima Indian individuals (Lindsay et al., 2003), in a cohort of men and women from a population-base study (Wake et al., 2003) and in a cohort of obese patients (Alberti et al., 2007). Similarly, increased *HSD11B1* expression in SAT was associated with the HOMA index in non-diabetic postmenopausal white women (Engeli et al., 2004). In a study of monozygotic twin pairs, intrapair differences in SAT *HSD11B1* mRNA levels correlated positively with serum fasting insulin (Kannisto et al., 2004). In 66 obese women with impaired glucose tolerance (IGT), SAT *HSD11B1* expression was increased and correlated with glucose levels across the OGTT, but this was not observed in the 35 men (Jeremy W. Tomlinson et al., 2008). Regarding visceral adipose tissue (VAT), *HSD11B1* expression was positively correlated with fasting insulin in a cohort of patients undergoing surgery for obesity (Baudrand et al., 2010). In another study, VAT *HSD11B1* expression was positively correlated with fasting glucose and insulin (R. Baudrand et al., 2011). However, Constantinopoulos et al. found no correlation between VAT *HSD11B1* expression and glucose levels in severely obese patients (Constantinopoulos et al., 2015). In contrast to the above findings, Munoz et al., observed no correlation between SAT or VAT *HSD11B1* expression and fasting glucose in a cohort of 32 morbidly obese patients (Munoz et al., 2009). Liver HSD11B1 expression was positively correlated with fasting glucose in a cohort of

patients undergoing surgery for obesity (Baudrand et al., 2010), and in severely obese patients (Constantinopoulos et al., 2015). However, Baudrand et al. (Metabolism, 2011) did not find a correlation between hepatic *HSD11B1* expression and glucose or insulin levels in another cohort of patients undergoing surgery for obesity (R. Baudrand et al., 2011). Interestingly, a cohort study of 65 obese patients revealed that changes in SAT HSD11B1 expression were associated with changes in glucose tolerance after 5 years follow-up. Patients with deteriorating glucose tolerance showed decreased *HSD11B1* expression, while those with improving glucose tolerance exhibited increased expression. The decrease in *HSD11B1* expression also correlated with the glucose AUC during the OGTT (Crowley et al., 2019). In a cohort of volunteers, SAT *HSD11B1* expression and activity was associated with changes in HOMA-IR during a follow-up period of 0.8 to 5.3 years (Koska et al., 2006). In the cohort of obese patients, there was no change overtime in the global 11β-HSD1 activity assessed by the urinary ratio THF + 5αTHF/THE. Basal 11β-HSD1 activity was not associated with HOMA measurement at the last assessment (Crowley et al., 2014).

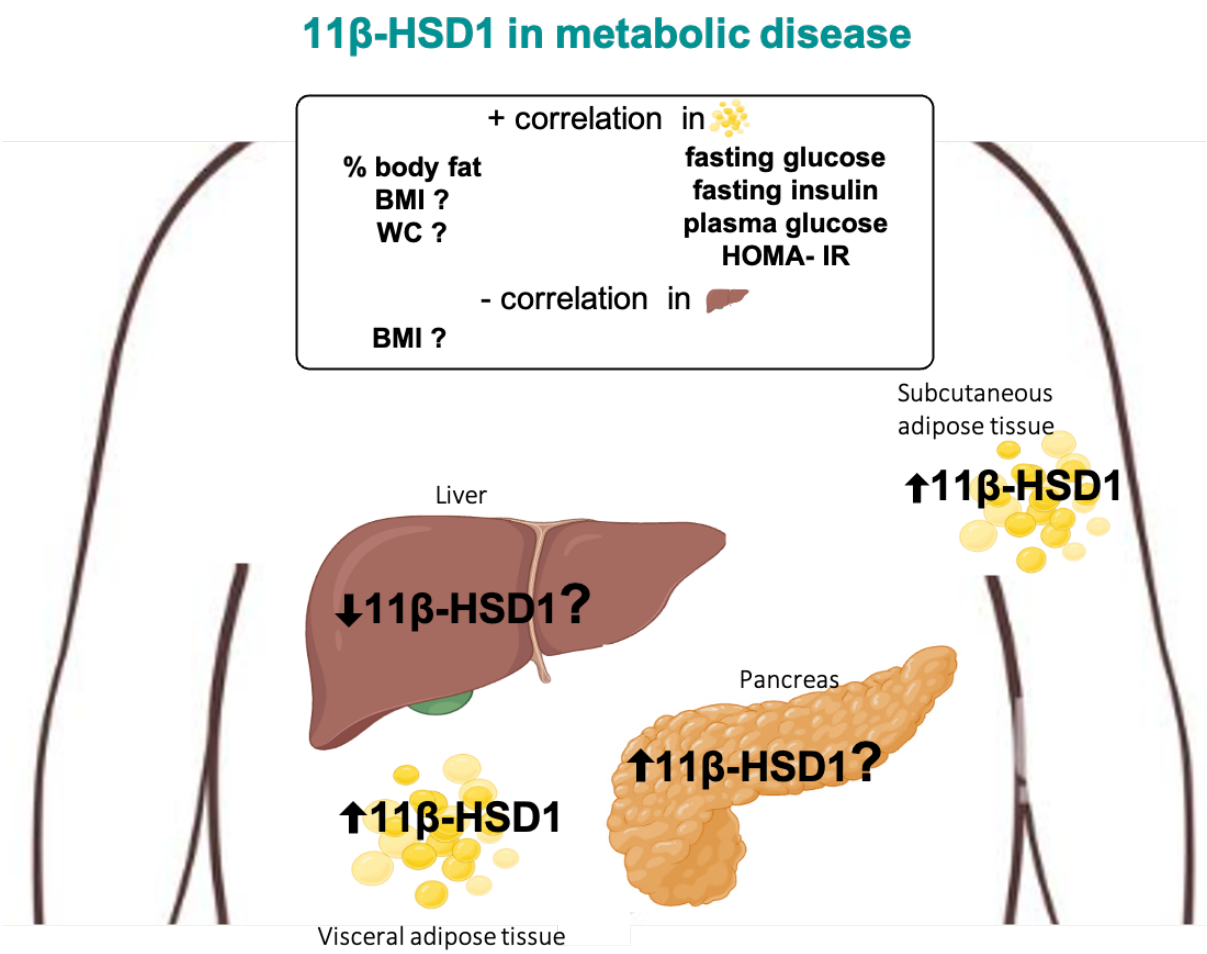

Figure 9: Schematic of tissue-specific dysregulation of 11β-HSD1 expression and activity in metabolic disease. 11β-HSD1 expression or activity is increased in subcutaneous and visceral adipose tissue and pancreas, while it is decreased in the liver, although conflicting data exist regarding liver and pancreatic expression and activity. Adipose tissue *HSD11B1* mRNA expression positively correlates with % body fat, fasting glucose, insulin, plasma glucose, and HOMA-IR. Conflicting results are reported for the correlation between adipose tissue or liver mRNA expression and BMI or WC.
BMI – body mass index, WC – weight circumference.

c) 11β-HSD1 alterations in animal models of metabolic phenotypes

In mice, *Hsd11b1* expression and activity mirror human patterns in metabolic conditions. Moderate obesity leads to increased expression in adipose tissue and pancreas. Severe obesity causes a greater increase in these tissues, while liver expression decreases to limit GC regeneration (Chapman et al., 2013). Several studies have investigated the effects of modulating the *Hsd11b1* gene in mice, either through knockout or overexpression to provide insights into the role of 11β-HSD1 in metabolic disorders.

Masuzaki et al., demonstrated that transgenic mice overexpressing *Hsd11b1* in adipose tissue developed visceral obesity, insulin resistance, hyperlipidemia, and hypertension (Masuzaki et al., 2001). These findings were supported by Paterson et al., who showed that adipose-specific overexpression of *Hsd11b1* in mice led to increased adipose mass, insulin resistance, and hypertension (Paterson et al., 2004). These mice models with adipose-specific overexpression of *Hsd11b1* also have high arterial blood pressure with increased sensitivity to dietary salt and increased plasma levels of angiotensinogen, angiotensin II, and aldosterone (Masuzaki et al., 2003). Liver-specific overexpression of *Hsd11b1* has also been investigated. Paterson et al., reported that liver-specific overexpression in mice resulted in fatty liver, dyslipidemia, and insulin resistance without obesity (Paterson et al., 2004). Similarly, Wang et al., found that liver-specific overexpression of *Hsd11b1* led to fatty liver, dyslipidemia, insulin resistance, and hypertension (Wang, endocrinology, 2006).

On the other hand, a global knockout of the *Hsd11b1* gene in mice fed high-fat diet, led to a phenotype characterized by improved glucose tolerance, attenuated GC-inducible responses, and increased insulin sensitivity (Kotelevtsev et al., 1997).

Similarly, Morton *et al*., found that a general knockout of *Hsd11b1* resulted in protection against high-fat diet-induced obesity, improved insulin sensitivity and glucose tolerance, and reduced intra-abdominal fat accumulation (Morton et al., 2001). Morgan et al., reported that in the presence of excess circulating GC, a global knockdown of *Hsd11b1* in mice, protect mice from the glucose intolerance, hyperinsulinemia, hepatic steatosis, adiposity, hypertension, myopathy, and dermal atrophy of Cushing syndrome (Morgan et al., 2014). Conditional knockout studies have also been conducted to investigate the tissue-specific roles of *Hsd11b1*. Morgan et al, reported that while liver specific knockdown of *Hsd11b1* led mice to develop a full cushingoid syndrome, adipose specific knockdown protected them from hepatic steatosis and circulating fatty acids excess syndrome (Morgan et al., 2014). Lavery et al., reported that liver-specific knockout of *Hsd11b1* in mice fed high-fat diet, led to improved lipid profiles and reduced hepatic lipid accumulation (Lavery et al., 2012). In contrast, Harno et al., found that brain-specific knockout of *Hsd11b1* had no effect on glucose homeostasis or body weight in either control or high-fat diet-fed mice (Harno et al., 2013).

In summary, these studies demonstrate that modulation of the *Hsd11b1* gene in mice, either through general or tissue-specific knockout or overexpression, can have significant effects on metabolic phenotypes, including obesity, insulin resistance, glucose intolerance, and dyslipidemia. These findings highlight the important role of 11β-HSD1 in the regulation of metabolic homeostasis and suggest that targeting this enzyme may have therapeutic potential in the treatment of metabolic disorders.

d) 11β-HSD1 as a therapeutic target for metabolic diseases

Natural endogenous compounds as steroids and their metabolites (Latif et al., 2005), as well as bile acid (Diederich et al., 2000), extract from vegetable and fruits as flavone (Atanasov et al., 2006), polyphenols from tea and herbs have inhibitory properties on 11β-HSD1. Compounds derived from the licorice plant (Glycyrrhiza glabra), such as glycyrrhetinic acid (18α-glycyrrhetinic acid, the isoform β inhibiting preferentially the 11β-HSD2) and its lab-made variant carbenoxolone, are powerful inhibitors of 11β-HSD1. These substances can block the enzyme's activity at very low concentrations (with an IC50 in the nanomolar range) when tested in laboratory conditions. However, their inhibitory effects are not limited to just this enzyme (Monder et al., 1989). Aside carbenoxolone, several others selective 11β-HSD1 compound have been produced and tested in vitro, in mice models and in phase 1 or phase 2 trials in humans. None has progressed to phase 3 clinical trials for their metabolic effects so far.

Preclinical studies with selective 11β-HSD1 inhibitors showed promising results in various mouse models. BVT.2733 demonstrated significant metabolic improvements, including reduced glucose and insulin levels, decreased hepatic gluconeogenic enzyme expression, and improved lipid profiles in diabetic and obese mice (Alberts et al., 2002; Alberts et al., 2003; Barf et al., 2002). Additionally, BVT.2733 reduced inflammation markers and macrophage infiltration in adipose tissue, while improving glucose tolerance in diet-induced obese mice (Wang et al., 2012) Similarly, Merck compound 544 improved glucose homeostasis, insulin sensitivity, and lipid profiles in diet-induced obese mice, with additional atheroprotective effects in ApoE-/- mice (Hermanowski-Vosatka et al., 2005). Despite these encouraging preclinical results, neither compound has progressed to human trials.

The carbenoxolone inhibitors have been extensively used in preclinical models (Gregory et al., 2020). It has been also tested in Human. Seven days use of 100 mg of carbenoxolone every 8 hours in seven lean healthy subjects (Walker et al., 1995), and in six lean T2D patients with matched healthy non-T2D (Andrews et al., 2003), showed improved insulin sensitivity attributable to reduced hepatic glucose production and not peripheral glucose uptake. This improvement in insulin sensitivity was however in a study on six lean and six obese human subjects (Sandeep et al., 2005). Another study in elderly group of 10 healthy elderly men and twelve T2D patients on 100 mg/day (3x/day) of carbenoxolone for 4 and 6 weeks respectively, show also no improvement in insulin sensitivity with no change in glycemic control or lipid profile, although increased cognitive function and verbal fluency was reported (Sandeep et al., 2004).

Several phase II clinical trials have evaluated 11β-HSD1 inhibitors in metabolic conditions, with varying degrees of success. The most promising results were observed with INCB13739 in type 2 diabetes, which demonstrated significant improvements in both primary and secondary endpoints, including reduced HbA1c, fasting plasma glucose, and HOMA-IR levels (Rosenstock et al., 2010). Other compounds (RO-151, RO-838, MK-0916) showed mixed results. While they failed to meet primary endpoints in diabetes trials (such as changes in mean daily glucose or fasting plasma glucose), some secondary benefits were observed at higher doses, including reductions in body weight, HbA1c, and blood pressure (Feig et al., 2011; Heise et al., 2014). However, MK-0916 was associated with increased LDL cholesterol. In obesity trials, MK-0736 did not achieve its primary endpoint of reducing systolic blood pressure, though some secondary benefits in body weight and lipid profiles were noted, albeit considered non-significant by the investigators (Shah et al.,

2011). Notably, using 11β-HSD1 inhibitors as adjunct therapy to metformin in type 2 diabetes showed no clear benefits. In a randomized controlled trial of 32 healthy men, co-administration of the 11β-HSD1 inhibitor AZD4017 with prednisolone did not significantly improve overall glucose disposal but prevented the deterioration in hepatic insulin sensitivity observed in the placebo group (Othonos et al., 2023). Despite some encouraging results, particularly with INCB13739, none of these compounds appear to have progressed beyond phase II trials, suggesting challenges in developing these agents as viable therapeutic options.

The limited therapeutic success of 11β-HSD1 inhibitors likely stems from multiple challenges in targeting cortisol metabolism. It has been suggested that the lack of specificity for the reductase activity could be a limitation but regarding the weak dehydrogenase activity, this should not be an issue (Anderson et al., 2021). The complex tissue-specific regulation of 11β-HSD1, with opposing changes in liver and adipose tissue in obesity, makes it difficult to achieve optimal therapeutic effects through systemic inhibition. Furthermore, the body's compensatory mechanisms, particularly through the hypothalamic-pituitary-adrenal axis, may counteract local cortisol reduction. The timing of intervention may also be crucial, as targeting a single pathway might be insufficient once metabolic dysfunction is established. Additionally, pharmacological limitations and patient heterogeneity suggest that these inhibitors might be more effective in specific patient subgroups rather than as a broad therapeutic approach (Stomby et al., 2014).

1.4.3 SRD5A1 and metabolic diseases

a) SRD5A1 alterations in obesity

Urinary steroid profiles reveal that increased 5α-reduced and 5β-reduced metabolites contribute significantly to the elevated total cortisol metabolites observed in obese subjects (Stewart et al., 1999; Westerbacka et al., 2003). While the excretion of 5α-THF does not differ based on BMI in control individuals (Rask et al., 2001), excretion is higher in patients with greater body fat content (Westerbacka et al., 2003). Some studies suggest that 5β-reduction is more enhanced than 5α-reduction in obesity (Stewart et al., 1999) , while others find no such difference (Andrew et al., 1998; Rask et al., 2001). The tissular expression of *SRD5A1* has not been specifically studied in the context of obesity. Weight loss induced by low-fat diet results in decreased SRD5A1 activity, with this decrease being more pronounced compared to 5β-reduced metabolites (J. W. Tomlinson et al., 2008). Similarly, after Roux-en-Y Gastric Bypass (RYGB), SRD5A1 activity decreases (Rask et al., 2013; Woods et al., 2015), and the decreased of the 5α-reduced metabolites is more marked than the decreased of the 5b-reduced activity (Rask et al., 2013).

b) SRD5A1 alterations in diabetes

In 25 lean patients with T2D or impaired glucose tolerance, the proportion of cortisol excreted as 5α- and 5β-reduced metabolites is increased compared to controls, suggesting an association between SRD5A1 and diabetes independent of obesity (Andrews et al., 2003). This suggests an independent association from obesity between SRD5A1 and diabetes. SRD5A1 activity also positively correlates with insulin resistance parameters in 101 obese subjects (Jeremy W. Tomlinson et al., 2008). However, in the same cohort, no difference in metabolite excretion or subcutaneous

adipose tissue *SRD5A1* expression was observed between patients with and without glucose intolerance or diabetes. *SRD5A1* expression positively correlates with fasting insulinemia in 41 obese patients, but not significantly with serum glucose (René Baudrand et al., 2011).

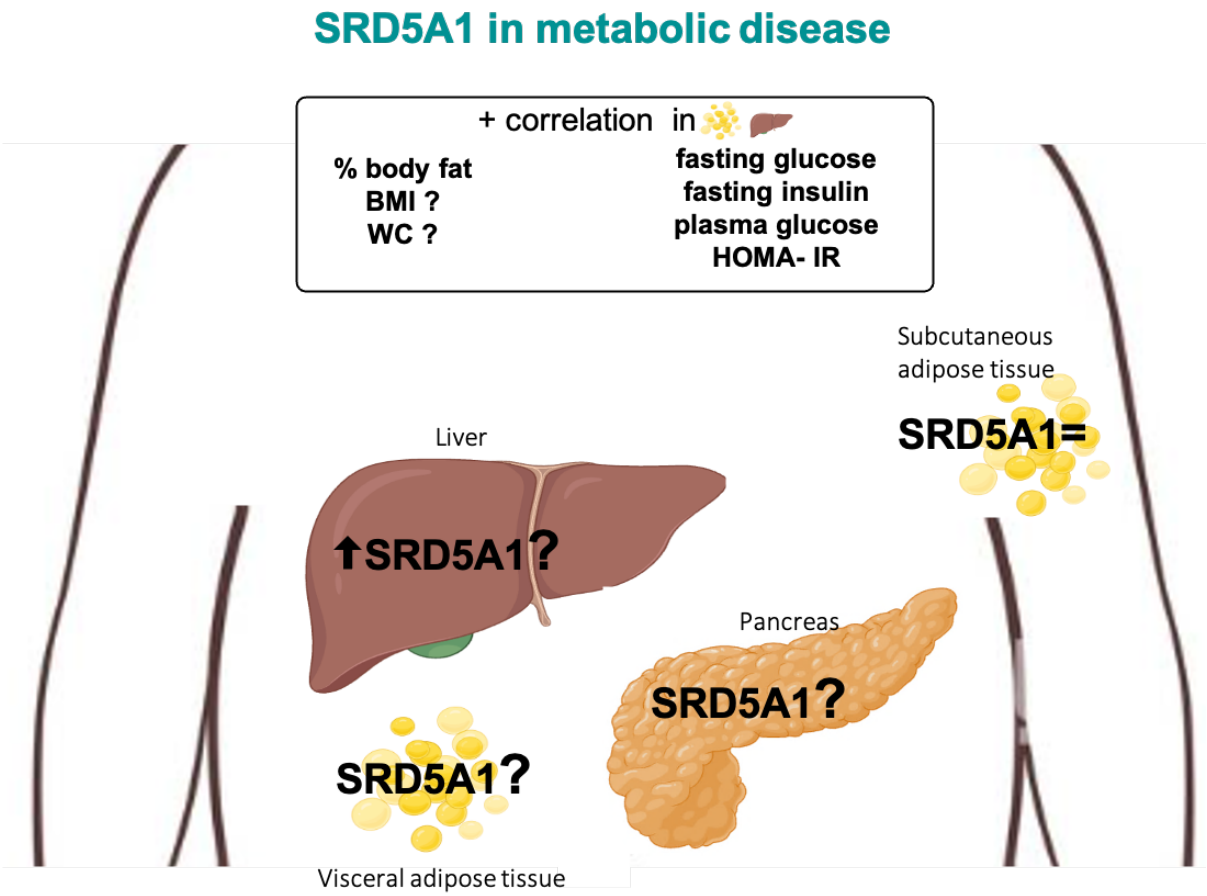

Figure 10: Schematic of tissue specific dysregulation of SRD5A1 expression and activity in metabolic disease. SRD5A1 activity is increased in the liver. Regarding the subcutaneous and visceral adipose tissue, conflicting data exists. Nothing is known about SRD5A1 activity in the pancreas. Liver and adipose tissue *SRD5A1* mRNA expression positively correlates with % body fat, fasting glucose, insulin, plasma glucose and HOMA-IR. Conflicting results is reported when adipose tissue or liver mRNA expression is correlated with BMI and WC. BMI – body mass index, WC – weight circumference.

c) Models of SRD5A1 inhibition

Inhibition of SRD5A may raise GCs bioavailability, potentially exacerbating their deleterious metabolic effects. Inhibitors of SR5A2 alone (finasteride) or SRD5A1 and SRD5A1 (dutasteride) are available. There are used in clinic for patients presenting for prostate hyperplasia. A study based on UK and Taiwanese health databases, which included more than 50,000 men, revealed an increased risk of diabetes in patients treated with dutasteride (Wei et al., 2019). Intervention studies in 46 healthy male volunteers demonstrate that dutasteride at 0.5 mg/ day treatment for 3 months, but not finasteride 5 mg/day, increase body fat, decrease insulin sensitivity (Upreti et al., 2014), and also led to increase hepatic lipid accumulation and decrease adipose lipid mobilization in 12 healthy male volunteers on a 3 weeks intervention study (Hazlehurst et al., 2016). In another intervention study on 12 healthy male volunteer given prednisolone alone at 10 mg/day (n= 6), or co-administered prednisolone at 10 mg/day with dutasteride at 0.5 mg/day (n=6), for 7 days, dutasteride exacerbate prednisolone deleterious effect on peripheral, hepatic, and adipose tissue insulin sensitivity. Dutasteride increased circulating level of prednisolone, amplifying prednisolone-induced peripheral insulin resistance and glucose oxidation, impaired adipose tissue insulin sensitivity in suppressing circulating NEFAs, and reduced glucose disposal and utilization in these subjects (Othonos et al., 2020).

Animal studies support these observations. In male obese Zucker rats, finasteride, which acts as a dual SRD5A inhibitor in rat compared to human, induces insulin resistance, hepatic steatosis, independently from androgens as castration did not modify the phenotype (Livingstone et al., 2015). Similarly, *Srd5a1* knockout mice challenged with high fat-high sucrose diet had more body weight, developed insulin resistance, hepatic steatosis compared to wild-type mice. In addition, the knockout

predisposes to hepatic fibrosis (Livingstone et al., 2015; Livingstone et al., 2017) with the female *Srd5a1* knockout mice exhibiting exaggerated predisposition to metabolic disorders at 6 months compared to male mice and eventually progress to obese at 12 months (Livingstone et al., 2017). On ALIOS diet, *Srd5a1* knockdown presented with acceleration of the MAFLD and developed greater hepatic steatosis than WT mice. Hepatic mRNA expression of genes involved in insulin signaling was also decreased. Interestingly, the mice were protected from the development of hepatocellular carcinoma (Dowman et al., 2013). The absence of this phenotype in mice with *Srd5a2* deletion supports the role of GCs on the MAFLD rather than a role of androgens (Dowman et al., 2013). In *Srd5a1* knock-out mice or mice treated with dutasteride at 1.8 mg/kg/day for 4 weeks (SRD5A dual inhibitor in mice) and presenting with metabolic phenotype including increased body weighty gain, increased fasting plasma insulin levels, insulin resistance following glucose tolerance test, and increased hepatic triglycerides level, treatment with A-348441, a liver-selective hepatic GR antagonist, improved insulin sensitivity and attenuate weight gain while observed hepatic steatosis was unresponsive to hepatic GR antagonism. This supports the role of GCs on the metabolic impact of SRD5A1 inhibition albeit additional factors may be involved (Mak et al., 2019).

1.4.4 AKR1D1 and metabolic diseases

AKR1D1 is involved in the synthesis of bile acids, specifically in the production of the primary bile acids chenodeoxycholic acid and cholic acid. Bile acid dysregulation can drive metabolic diseases (Fleishman & Kumar, 2024). However, data on AKR1D1 and cortisol metabolism are relatively scarce. AKR1D1 activity, assessed by the excretion of its metabolites THF and THE, seems to increase in patients with higher fat body mass (Westerbacka et al., 2003), but does not correlate with BMI (Rask et al., 2001) in cohorts of healthy subjects. After weight loss induced by low-fat diet, the excretion of these metabolites is not significantly modified (J. W. Tomlinson et al., 2008; Tomlinson et al., 2004). However, after RYGB, the metabolites were significantly decreased in a cohort of obese patients (Woods et al., 2015). Liver expression of *AKR1D1* was decreased in patients with type 2 diabetes compared to controls, with nearly 80% of this cohort having MAFLD. This decreased activity may be secondary to the activation of PPARα (Valanejad et al., 2017).

In healthy individuals, ratios reflecting AKR1D1 activity (5β-THF/cortisol and 5β-THE/cortisone) are strongly associated with liver fat content (Westerbacka et al., 2003). AKR1D1 activity is significantly increased in female patients with fatty liver or NASH compared to healthy controls (Konopelska et al., 2009). However, in obese patients with NASH, *AKR1D1* liver expression negatively correlates with fibrosis, steatosis, and the NAFLD activity score (Nikolaou et al., 2019). As with SRD5A1, Ahmed et al. observed increased AKR1D1 activity in simple steatosis compared to controls suggesting also a protective role against liver fat accumulation, while activity in NASH was similar to controls (Ahmed et al., 2012). Patients with loss-of-function mutations in *AKR1D1* exhibit altered GC and bile acid metabolism, with urinary bile acids being nearly absent. These patients develop neonatal cholestasis, likely due to

the accumulation of bile acid precursors and 5α-reduced bile acids. In one patient, the absence of 5β-reduced cortisol and cortisone metabolites was observed, while 5α-reduced metabolites were increased (Palermo et al., 2008). A general *Akr1d1* knockout mouse model has been generated. At 30 weeks, male mice challenged with a high-fat diet have reduced fat mass, improved insulin tolerance, and reduced lipid accumulation in the liver and adipose tissue compared to controls. However, they exhibit hypertriglyceridemia and increased muscular triacylglycerol. Circulating GC levels, GC-regulated gene expression, and adrenal mass were unchanged (Gathercole et al., 2022). This suggests a less prominent role of AKR1D1 in metabolism compared to 11β-HSD1 or SRD5A1.

1.5 Hypothesis and Aims of the Thesis

While insulin resistance is well established as a primary driver of GCID, GCs also directly affect β-cell function, particularly insulin secretion. However, the impact of low-dose GCs on GSIS and whether different GCs with varying kinetic profiles differentially affect GSIS remains understudied. **We also hypothesize that even at lower doses, GCs impact GSIS, and that DEX, HC, and PRED at anti-inflammatory equipotent doses may differentially affect GSIS.**

GC metabolism modulates GC bioavailability, and alterations in GC metabolism can lead to excess GC exposure. While islets are key regulators of glucose homeostasis, data on cortisol metabolism within islets remains scarce. Although 11β-HSD1 expression and activity have been extensively studied in metabolic diseases, its pancreatic expression has not been characterized in human cohorts. SRD5A1 has recently emerged as another potential mediator of cortisol metabolism dysregulation in obesity and diabetes, but its expression pattern, including in pancreatic tissue, remains poorly characterized in metabolic diseases. **Here, we hypothesize that the expression of both *HSD11B1* and *SRD5A1* is altered in islets during metabolic disease.**

SRD5A1 inhibition decreases both cortisol and prednisolone degradation, potentially exacerbating the metabolic impact of local GC overexposure (as seen in obesity) or systemic overexposure (as observed in patients receiving synthetic GC therapy). The inhibition of SRD5A1 contributes to the development of diabetes. **Conversely, we hypothesize that SRD5A1 overexpression could mitigate the impact of GCs on**

islets (focusing on GSIS) by enhancing GC degradation and decreasing their bioavailability.

Therefore, the objectives of this thesis are:

Chapter 2

To investigate the impact of low-dose PRED on GSIS and compare its effects with other GCs (DEX and HC) at anti-inflammatory equipotent doses.

Chapter 3

To characterize the expression profile of key GC metabolism genes including *HSD11B1* and *SRD5A1* in human islets

To study endogenous GC metabolism in islets.

Chapter 4

To determine whether SRD5A1 overexpression in islets modulates the effects of HC and PRED on GSIS.

CHAPTER 2

Impact of therapeutic doses of prednisolone and other glucocorticoids on insulin secretion from human islets

IMPACT OF THERAPEUTIC DOSES OF PREDNISOLONE AND OTHER GLUCOCORTICOIDS ON INSULIN SECRETION FROM HUMAN ISLETS

2.1 Introduction

Glucocorticoids (GCs) are widely prescribed medications used to treat various conditions, including inflammatory diseases, autoimmune disorders, cancer, and organ transplant rejection. At any given time, 1-3% of the general population receives long-term GC therapy (Laugesen et al., 2019; van Staa et al., 2000). While GCs are known for their anti-inflammatory properties, they also significantly affect glucose and lipid homeostasis. As a consequence, 2 to 40% of patients develop GC-induced diabetes (GCID) as a side effect (Descours & Rigalleau, 2023; Feve & Scheen, 2022; J. X. Li & C. L. Cummins, 2022). Notably, GCID risk is elevated regardless of the route of GC administration, whether oral, inhaled, or topical (J. X. Li & C. L. Cummins, 2022). This metabolic impact is further evidenced in Cushing syndrome, a condition characterized by endogenous cortisol excess, where 7-64% of affected patients develop impaired glucose tolerance or diabetes (Pivonello et al., 2016).

Insulin resistance is the primary mechanism underlying GCID (Ogawa et al., 1992b; Plat et al., 1996; D. H. van Raalte et al., 2010; Yasuda et al., 1982). Short-term administration (2-15 days) of dexamethasone (DEX, 3-5 mg) or prednisolone (PRED, 30 mg) in healthy volunteers, as well as chronic high-dose corticosterone exposure in mice, enhances insulin secretion and increases β-cell function and mass. These changes represent compensatory responses to GC-induced insulin resistance (E. Courty et al., 2019; Larsson & Ahren, 1999; D. H. van Raalte et al., 2010; Wajngot et

al., 1992). This compensatory nature makes studying the direct effects of GCs on β-cell function *in vivo* particularly challenging. However, acute GC administration demonstrates distinct effects on insulin secretion. In interventional studies with healthy volunteers, a single oral dose of PRED (15-75 mg) or DEX (1 mg) directly impaired insulin secretion during glucose infusion, meal tests, or oral glucose tolerance tests (S. C. Kalhan & P. A. Adam, 1975; Schneiter & Tappy, 1998b; D. H. van Raalte et al., 2010). These findings are further supported by *in vitro* studies using rat or mouse-perfused pancreas and murine or human cell lines treated with DEX, hydrocortisone (HC), or corticosterone. Both acute and chronic GC exposure affects β-cell function, specifically by inhibiting glucose-stimulated insulin secretion (GSIS) in a time- and dose-dependent manner ((G. Barseghian & R. Levine, 1980; Barseghian et al., 1982a; J. L. S. Esguerra et al., 2020; S. Gremlich et al., 1997; I. K. Jeong et al., 2001; A. Karagiannopoulos et al., 2023; Lambillotte et al., 1997a; Zawalich et al., 2006). Studies on human islets have demonstrated that GSIS remains unimpaired at physiological GC concentrations (20 nM cortisol or 200 nM cortisone) (N. H. F. Fine et al., 2018). However, GSIS impairment becomes evident at suprapharmacological concentrations, such as 2 µM dexamethasone (DEX) (J. L. S. Esguerra et al., 2020; A. Karagiannopoulos et al., 2023).

Translating these experimental findings to clinical practice presents several challenges. Although prednisolone (PRED) is the most commonly prescribed GCs (van Staa et al., 2000), with low-dose therapy (≤7.5 mg/day) recommended for chronic treatment (Buttgereit et al., 2002), most *in vitro* studies have utilized DEX at concentrations exceeding clinical relevance. Pharmacokinetic data indicate that oral PRED doses of 5-10 mg achieve peak plasma concentrations of 300-700 nM (equivalent to 45.6-106.4 nM of DEX) (Pickup, 1979a). To bridge the gap between

laboratory research and clinical practice, our study had two primary aims. First, we evaluated the impact of clinically relevant, low-dose prednisolone (PRED, 250 nM) on GSIS in human islets. Second, given that GCs possess distinct pharmacokinetic profiles, we compared the effects of PRED, DEX, and HC on GSIS at anti-inflammatory equipotent doses.

2.2 Materials and Methods

2.2.1 Human islet isolation

Human islets were isolated from brain-dead donors using a modified Ricordi method, as previously described (Kerr-Conte et al., 2010; Ricordi et al., 1988). Following purification and washing, the endocrine fraction was cultured for 18-36 hours prior to experimentation in complete CMRL media containing 5.5 mM glucose (Gibco, Life Technologies, Paris, France) and supplemented with 0.625% human serum albumin (HSA) and 100 U/ml penicillin-streptomycin (Gibco, Cat #15140122). All experimental procedures complied with French regulations and were approved by the Institutional Ethical Committee of the University of Lille and CHU Lille (France). Only islets exhibiting >90% viability and >70% purity (endocrine to exocrine ratio) were selected for experiments.

2.2.2 GCs treatment

Human islets (300 islet equivalents) were washed twice in DPBS 1X (Gibco, Cat #14040141) and cultured in a 5.5 mM glucose media (Gibco, Life Technologies, Paris, France), supplemented with 0.625% human serum albumin (HAS), 100 U/ml penicillin-streptomycin (Gibco, Cat #15140148). The media contained either GCs (prednisolone [PRED], hydrocortisone [HC], dexamethasone [DEX]) or 0.001% methanol/ethanol (control). Clinical oral PRED dosing ranges from 5-90 mg/day for acute treatment, while chronic therapy typically uses ≤7.5 mg/day, (Buttgereit et al., 2002), corresponding to approximately 300 to 700nM (Pickup, 1979a), (**Table 5**). To investigate PRED's impact on GSIS, Islets were treated with PRED (Sigma-Aldrich, Cat # P6004) at 250 nM, 500 nM, and 1 µM for 24h. Based on anti-inflammatory

equipotent conversion factors (where 5 mg PRED equals 20 mg HC or 0.76 mg DEX) (Buttgereit et al., 2002; Meikle & Tyler, 1977), (**Table 2**). HC was used at 1 µM (Sigma-Aldrich, Cat # H0888) and DEX at 38 nM (Sigma-Aldrich, Cat# D2915).

Table 5: PRED peak plasma concentration in healthy volunteers following oral administration of PRED

Drug treatment	Number of volunteers	Clinical Dose (mg)	Peak plasma concentration (nM)	REF
Prednisolone	8	10	624	(Ferry et al., 1988)
Prednisolone alcohol	6	15	386	(English et al., 1975)
Prednisolone metasulphobenzoate	6	15	436 ± 44	
Prednisolone	32	20	1400	(Magee et al., 2001)
Prednisolone	14	20	1760	(Bashar et al., 2018)
Prednisolone	6	90	3700	(D'Arcy et al., 1971)
Prednisolone	16	20	699	(Powell & Axelsen, 1972)
Prednisolone	12	10	674	(Sullivan et al., 1976)
Prednisolone	10	20	232	(Tembo et al., 1977)
Prednisolone	4	5	314	(P. J. Morrison et al., 1977)
Prednisolone	8	10	712	
Prednisolone	10	10	322	(Davis et al., 1978)

2.2.3 Glucose Stimulated Insulin Secretion (GSIS) Assessment Using Dynamic Islet Perifusion

For each perifusion experiment, 300 islet equivalents were loaded into individual chambers and perfused at 1 ml/min with continuously gassed KREBS solution containing 1 mg/ml BSA (pH 7.3) (Henquin et al., 2015). Islets underwent a 50-minute preincubation period at low glucose (3 mM) before sample collection began. Effluent fractions were then collected at 2 minutes intervals for 20 minutes during sequential

exposure to low (3 mM) and high (15 mM) glucose concentrations. Flow rate, oxygen levels, temperature, and pressure were maintained constant throughout the experiment. Following perifusion, islets were recovered from each chamber and transferred to 500 µl of acid-ethanol solution (1.5% HCl, 70% EtOH, 28.5% ddH2O) for insulin extraction (Detimary et al., 1996). Insulin secretion rates were normalized to total insulin content and expressed as percentage per minute. GSIS was quantified by analyzing the area under the curve (AUC) and evaluating both first-second-phase insulin secretion (calculated as the average of the first 10 minutes and last 10 minutes of 15 mmol/L glucose stimulation). Further analysis of the GSIS was done by calculating the % of decrease in AUC, first-second-phase insulin secretion as:

$$\frac{(vehicle\ treated\ islets\ -\ GCs\ treated\ islets)}{(vehicle\ treated\ islets)} X\ 100$$

2.2.4 Donor's Characteristics

Clinical and biological characteristics of all donors are summarized in **Table 6.** Donors were categorized according to body mass index (BMI) as follows: lean (BMI < 25 kg/m²), overweight (BMI > 25 kg/m² to <30 kg/m²), obese (BMI 30-34.9 kg/m²), and morbidly obese (BMI ≥ 35 kg/m²). Glycemic status was classified based on HbA1c levels: normal glycemia (HbA1c < 5.7%) and glucose intolerant (HbA1c 5.7- 6.4%)

2.2.5 Statistical Analysis

Statistical analyses were performed with GraphPad Prism 10.2.0 (GraphPad Software, La Jolla, California, USA). Data are expressed as mean ± SEM. Comparison between control and GC treatment was performed using Wilcoxon paired and non-parametric t-tests. Comparison between groups was performed using Wilcoxon

unpaired and non-parametric t-tests. Comparison between the different doses of PRED and GCs at equipotent dose were performed using one-way ANOVA with Sidak's multiple comparison *post hoc* tests, and two-way ANOVA and nonparametric tests with Sidak's *post hoc* tests for multiple comparisons where statistically applicable. Differences were considered significant at $p < 0.05$. Significance was expressed as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ and **** $p < 0.0001$.

2.3 Results

2.3.1 Low Therapeutic Dose of Prednisolone Inhibits Biphasic Insulin Secretion in Human Islets Across Diverse Metabolic Phenotypes.

In our dose-response study, prednisolone (PRED) treatment at 250 nM, 500 nM, and 1 μM significantly reduced both first- and second-phase insulin secretion compared to untreated control islets, with comparable inhibition across all doses (**Figure 11A, B**). Insulin content remained unchanged (*n* = 4), (**Figure 11C**). Based on these findings, we selected the lowest dose (250 nM) for subsequent experiments. In a follow-up study using seven additional islet preparations from eleven donors, 24-hour treatment with 250 nM PRED consistently decreased both phases of insulin secretion compared to controls (**Figure 11D, E**), while maintaining comparable insulin content between groups (**Figure 11F**).

2.3.2 Prednisolone Induced Inhibition of Glucose-Stimulated Insulin Secretion Is Consistent Across Donor Demographics.

The magnitude of PRED-mediated GSIS inhibition varied considerably among donors (**Figure 12A-K**)**.** with reductions in insulin secretion ranging from 11.2% to 67.6% for total AUC, 17.3% to 79.9% for first-phase insulin secretion, and 5.2% to 52.9% for the second- phase secretion **(Table 6)**. Despite this variability, PRED's inhibitory effect showed no statistically significant differences when stratified by: BMI (lean (*n*=5) *vs.* overweight (*n*=4); **Figure 13A-C**), sex (male (*n*=7) *vs.* female (*n*=4); **Figure 13D-F**), age (<50 years (*n*=4) *vs.* >50 years (*n*=7); **Figure 13-I**), or glycemia (HbA1c: <5.7

(*n*=6) *vs.* ≥5.7 (*n*=5); **Figure 13J-L**. However, islets from overweight and male donors showed a trend toward greater PRED-mediated inhibition (**Figure 13A-F**).

2.3.3 Comparative Effects of Equipotent Anti-inflammatory Doses of GCs on Biphasic Insulin Secretion in Human Islets.

In clinical practice, low therapeutic oral GC doses are defined as ≤7.5 mg/day prednisolone (PRED) equivalent, corresponding to ≤30 mg/day hydrocortisone (HC) and ≤1.2 mg/day dexamethasone (DEX) (Buttgereit et al., 2002). We compared these equipotent doses using islet preparations from five donors. DEX demonstrated significantly greater inhibition of insulin secretion compared to PRED and HC (**Figure 14A)** with more pronounced impairment of both first- and second-phase insulin secretion (**Figure 14B**). Islet insulin content remained comparable across all treatment groups (**Figure 14C**). Analysis of percentage inhibition revealed greater DEX-mediated reduction in total insulin secretion (AUC) compared to HC and PRED (**Figure 14D**). While inhibition of first-phase secretion was comparable across all GCs (**Figure 14E**), DEX induced significantly greater suppression of second-phase secretion compared to HC and PRED (**Figure 14F**).

Table 6: Clinical and biological characteristics of the islet donors, and individual donor islets' heterogeneity in GSIS response to 250 nM of PRED.

Donor ID	Age	Sex	BMI	HbA1c	Effect of PRED 250 nM on GSIS (% decrease compared to control islets)		
					AUC	First phase IS	Second phase IS
D1	49	M	20.9	5.5	54	57.5	48.2
D2	67	F	22	5	27.3	38.9	14.2
D3	58	F	24.2	5.3	25.9	16.3	32.7
D4	55	M	24.5	5.5	20.5	22.5	45.6
D5	58	M	24.5	5.6	11.2	17.3	5.2
D6	70	M	26.6	5.6	64.2	65	61.4
D7	62	M	25.9	5.7	67.6	79.9	52.9
D8	55	F	29.1	5.7	35.7	44.6	30.8
D9	49	M	29.2	5.7	17.8	21.7	14.7
D10	47	F	30.1	5.9	39.2	39.1	39
D11	29	M	43.3	5.8	63.3	66.6	57.4

IS = Insulin Secretion

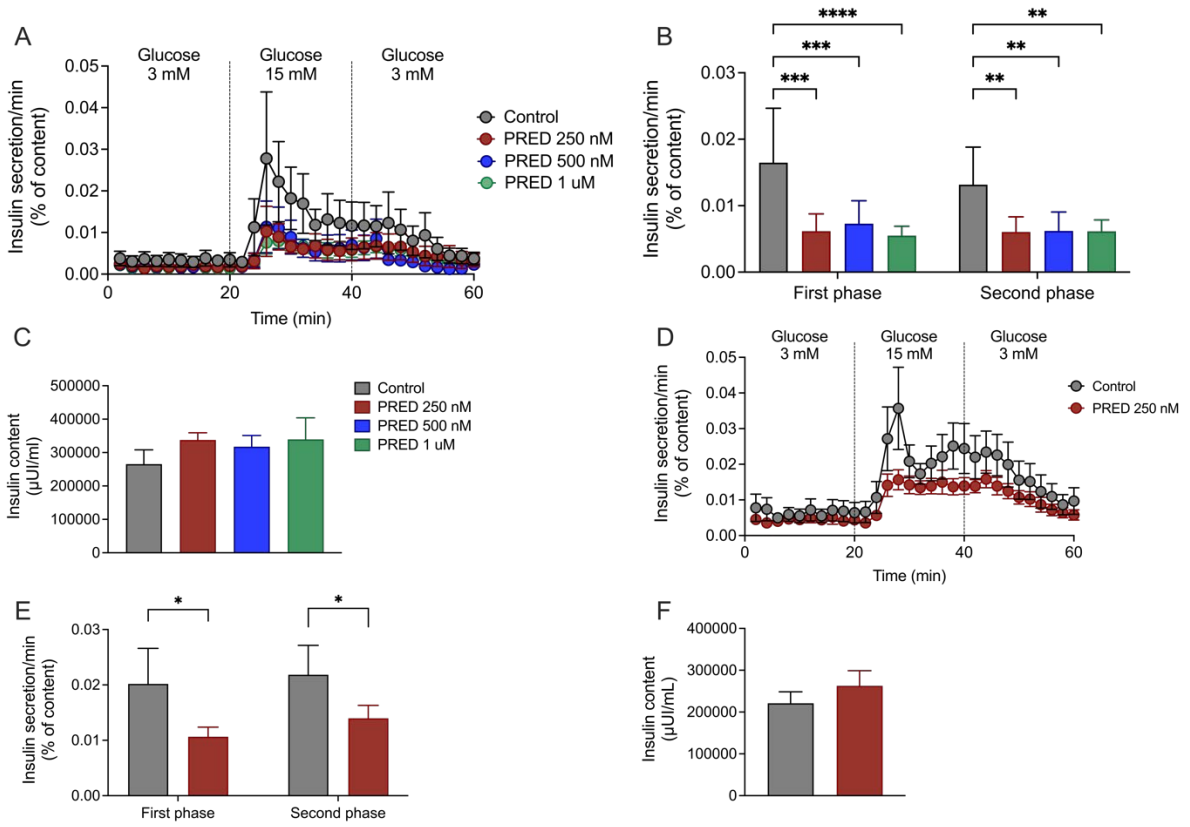

Figure 11: Low Therapeutic Dose of Prednisolone Inhibits Biphasic Insulin Secretion in Human Islets Across Diverse Metabolic Phenotype.

Glucose-stimulated insulin secretion assessed by dynamic perifusion in human islet cultures treated with vehicle (grey), 250 nM (red), 500 nM (blue), or 1 µM (green) of PRED for 24h. **(A)** Islets were perfused with 3 mmol/L, and 15 mmol/L glucose concentrations, and insulin secretion was normalized to % of insulin content (*n* = 4). **(B)** The first and second phases of insulin secretion were calculated as the first 10 minutes and the last 10 minutes of 15 mmol/L glucose stimulation, respectively. Comparison between the control vehicle-treated islets and PRED-treated islets on the first and second phase of insulin secretion was performed using Wilcoxon paired and non-parametric t-tests; ****p* = 0.0001, for PRED 250 nM *vs*. control, ****p* = 0.0003, for PRED 500 nM *vs*. control, *****p* < 0.0001, for PRED 1 µM *vs*. control, for the first phase of insulin secretion; **p = 0.0016, for PRED 250 nM *vs*. control, **p = 0.0019, for PRED 500 nM *vs*. control, **p = 0.0018, for PRED 1 µM *vs*. control, for the second phase of insulin secretion. **(C)** Insulin content of human islets at the end of dynamic perifusion experiments (*n* = 4). Comparison between the controls and PRED-treated islets was performed using Wilcoxon paired and non-parametric t-tests. **(D)** After 24h treatment with vehicle (grey) or 250 nM PRED (red), islets were perfused with 3 mmol/L and 15 mmol/L glucose concentrations, and insulin secretion was normalized to % of insulin content (*n* = 7). **(E)** The first and second phases of insulin secretion were calculated as

the first 10 minutes and the last 10 minutes of 15 mmol/L glucose stimulation, respectively. Comparison between the control islets and PRED treatment, on the first and second phases of insulin secretion, was performed using Wilcoxon paired and non-parametric t-tests; *p = 0.0156. **(F)** Insulin content of human islets at the end of dynamic perifusion experiments (*n* = 7). Comparison between the controls and PRED-treated islets was performed using Wilcoxon paired and non-parametric t-tests.

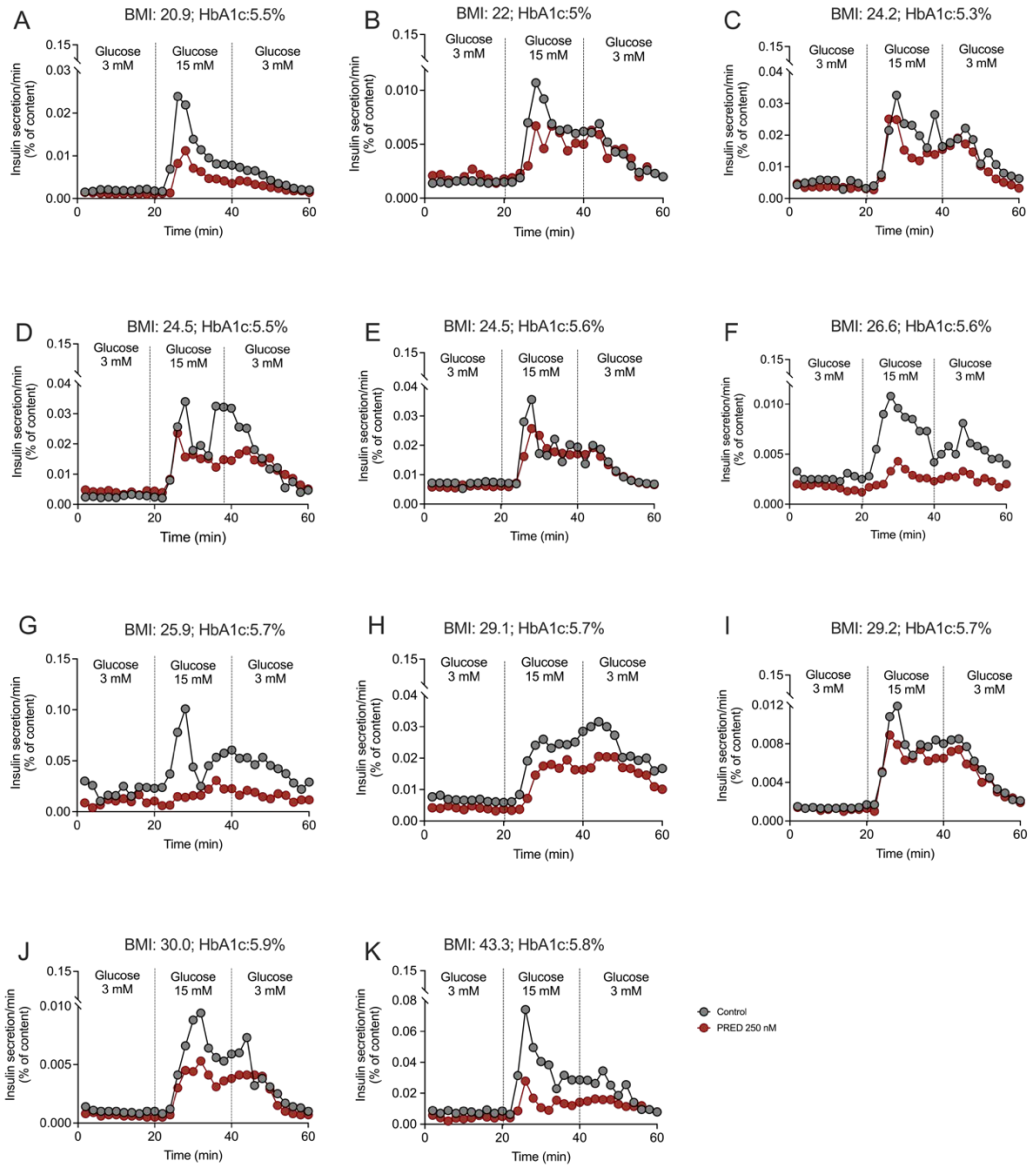

Figure 12: Prednisolone Induced Inhibition of Glucose-Stimulated Insulin Secretion is Heterogenous Across Donors.

Glucose-stimulated insulin secretion assessed by dynamic perifusion in human islet cultures treated with vehicle (grey), 250 nM (red) of PRED for 24h. Islets were perfused with 3 mmol/L and 15 mmol/L glucose concentrations, and insulin secretion was normalized to % of insulin content (*n* = 11). Human islets were isolated from *n* = 5 lean normoglycemic (BMI: < 25 kg/m^2, HbA1c: <5 .7%) **(A-E),** *n* = 1 normoglycemic donor with overweight (BMI: ≥ 25 kg/m^2 to < 30 kg/m^2, HbA1c: < 5.7 %) **(F),** *n* = 3 donors with overweight and glucose intolerance (BMI: ≥ 25 kg/m^2 to < 30 kg/m^2, HbA1c: ≥ 5 .7% to < 6.5%) **(G–I),** *n* = 1 donor with obesity and glucose intolerance (BMI: ≥ 30

kg/m^2 to < 35 kg/m^2, HbA1c: ≥ 5 .7% to < 6.5%) **(J)**, and *n* = 1 donor with morbid obesity and glucose intolerance (BMI: ≥ 35 kg/m^2, HbA1c: ≥ 5 .7% to < 6.5%) **(K)**.

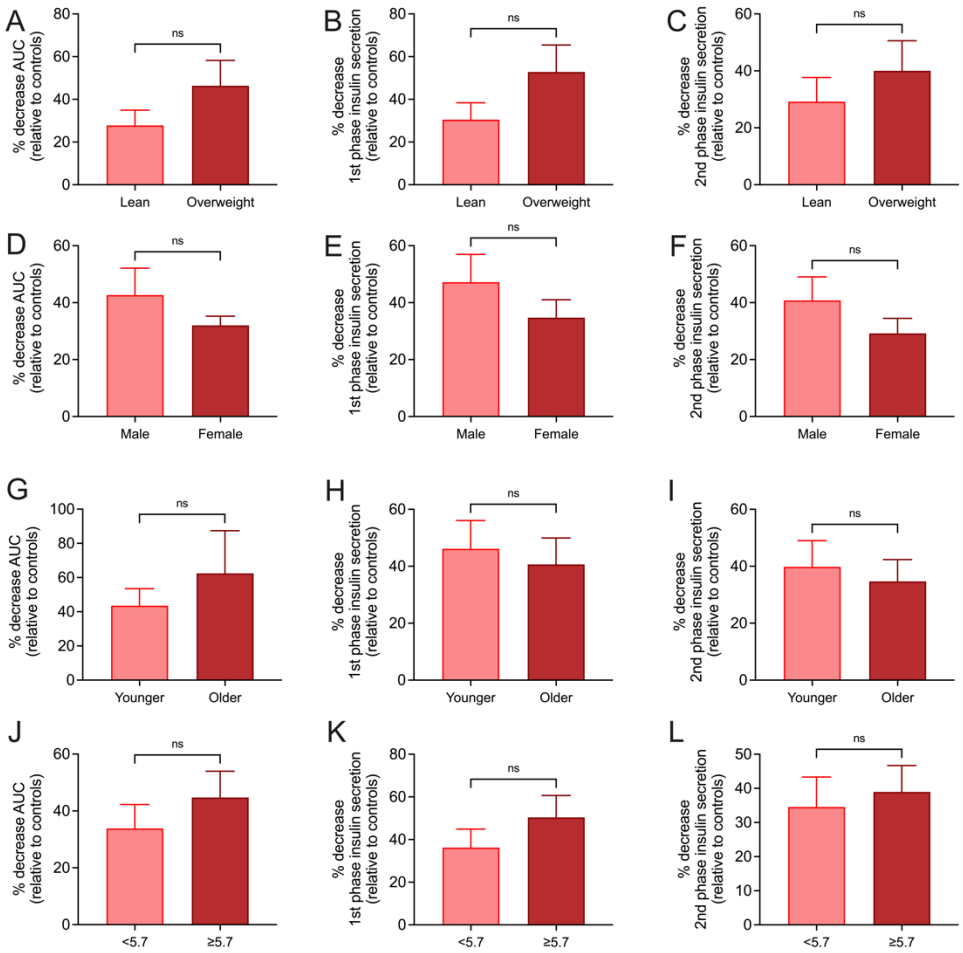

Figure 13: Prednisolone Induced Inhibition of Glucose-Stimulated Insulin Secretion is Consistent Across Donor Demographics.

Percentage of decrease of the area under the curve (AUC), the first phase or second phase of insulin secretion from a glucose-stimulated insulin secretion on human islets treated with vehicle or 250 nM PRED for 24h.

(A - C) Human donors stratified by BMI into lean (< 25 kg/m^2, light red bars, *n* = 5) and overweight (>25 kg/m² to <30 kg/m², dark red bars, *n* = 4). **(D - F)** Human donors stratified by sex into males (light red bars, *n* = 7) and females (dark red bars, *n* = 4). **(G - I)** Human donors stratified by age into younger (< 50 years, light red bars, *n* = 4) and older (>50 years, dark red bars, *n* = 7). **(G - I)** Human donors stratified by HbA1c into normoglycemic (<5.7, light red bars, *n* = 6) and glucose intolerant (≥5.7, dark red bars, *n* = 6). All data are expressed as means ± SEM. Statistical analysis was performed using Wilcoxon paired and non-parametric t-tests.

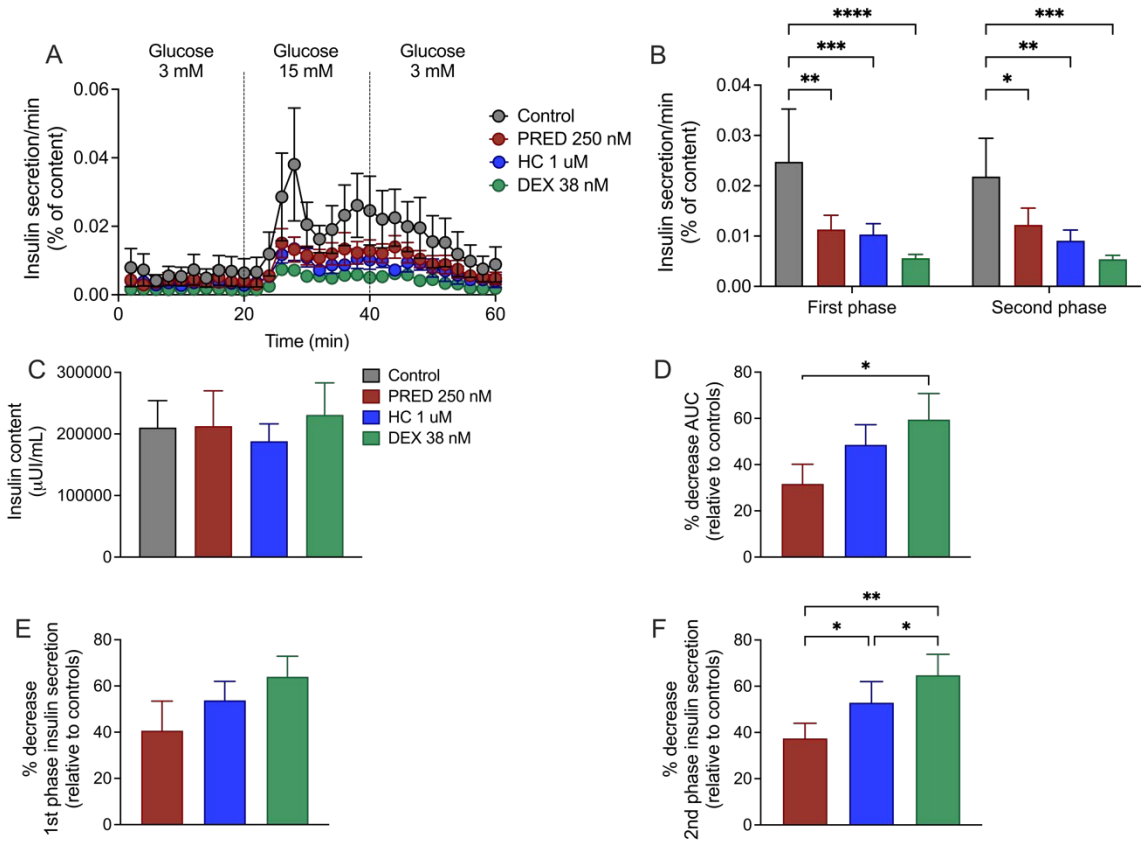

Figure 14: Comparative Effects of Equipotent Anti-inflammatory Doses of GCs on Biphasic Insulin Secretion in Human Islets.

Glucose-stimulated insulin secretion assessed by dynamic perifusion in human islet cultures treated with vehicle (grey), 250 nM PRED (red), 1 µM HC (blue), or 38 nM DEX (green) for 24h. Human islets were isolated from *n* = 3 normoglycemic donors, and *n* = 2 donors with overweight and glucose intolerance. **(A)** Islets were perfused with 3 mmol/L, and 15 mmol/L glucose concentrations, and insulin secretion was normalized to % of insulin content (*n* = 5). **(B)** The first and second phases of insulin secretion were calculated as the first 10 minutes and the last 10 minutes of 15 mmol/L glucose stimulation, respectively. Comparison between the control vehicle-treated islets and GCs-treated islets, on first and second phase of insulin secretion was performed using two-way ANOVA with Sidak's multiple comparison *post hoc* tests; first phase: **p = 0.0012, for PRED, ***p = 0.0007, for HC, ****p < 0.0001, for DEX; second phase: *p = 0.0164, for PRED, **p = 0.0019, for HC, ***p = 0.0002, for DEX, relative to control vehicle-treated islets. **(C)** Insulin content of human islets at the end of dynamic perifusion experiments (*n* = 5). Comparison between the controls and GCs-treated islets was performed using one-way ANOVA and nonparametric tests with Sidak's *post hoc* test for multiple comparisons.

(D-F) Percentage of decrease of the area under the curve (AUC), the first phase or second phase of insulin secretion from a glucose-stimulated insulin secretion on human islets treated

with vehicle (grey), 250 nM PRED (red), 1 μM HC (blue), or 38 nM DEX (green) for 24h. All data are expressed as means ± SEM. Comparison between the controls and GCs-treated islets was performed using one-way ANOVA and nonparametric tests with Sidak's *post hoc* test for multiple comparisons. AUC: *p = 0.00117, for PRED *vs*. DEX. Second phase: *p = 0.0230, for PRED *vs*. HC, *p = 0.0363, for HC *vs*. DEX, **p = 0.0013, for PRED *vs*. DEX.

2.4 Discussion

Our study demonstrates that low-dose GC treatment significantly impairs insulin secretion *in vitro*, with DEX exhibiting stronger inhibitory effects compared to PRED and HC at clinically relevant concentrations (Derendorf et al., 1991; Pickup, 1979a). Notably, we observed substantial variability in GC-mediated effects on GSIS among individual donor islet preparations.

While daily and cumulative GC doses are established risk factors for GCID (S. A. Bergstra et al., 2023; Buttgereit et al., 2002; Deutsch et al., 2023; Fardet & Feve, 2014; Pofi et al., 2023), the relationship between treatment duration and GCID risk remains inconsistent (Deutsch et al., 2023; Fardet & Feve, 2014; Pofi et al., 2023). Previous *in vitro* studies have shown dose-dependent GSIS inhibition by DEX (I. K. Jeong et al., 2001; Lambillotte et al., 1997a). However, these pharmacological findings have limited clinical relevance, as long-term therapy typically targets daily doses ≤7.5 mg/day PRED equivalent (Buttgereit, 2020). Earlier studies examining human islets used 2 µM DEX (J. L. S. Esguerra et al., 2020; A. Karagiannopoulos et al., 2023), equivalent to approximately 13 µM PRED, a concentration achievable with 260 mg oral PRED (Buttgereit et al., 2002; Meikle & Tyler, 1977). Our findings extend this knowledge by demonstrating that even low doses (250 nM PRED, 1 µM HC, and 38 nM DEX) significantly inhibit GSIS.

The established anti-inflammatory equipotency ratios relative to HC 4:1 for PRED and 25:1 for DEX (Buttgereit et al., 2002). At equipotent concentrations equivalent to <5 mg oral PRED, DEX showed markedly stronger inhibition of biphasic insulin secretion compared to PRED and HC, which exhibited similar effects. This observation aligns

with previous reports of dissociation between anti-inflammatory and hyperglycemic effects among different GCs (Kendall et al., 1963). This finding further supports the disconnect between anti-inflammatory efficacy and metabolic potency. A recent retrospective study corroborates these observations, showing that DEX treatment resulted in higher capillary glucose levels compared to HC and PRED, which demonstrated similar effects (Limbachia et al., 2024). These differences likely stem from the distinct pharmacological properties among GCs, including their receptor binding affinities, half-lives, and duration of action, factors that influence their genomic effects (Buttgereit et al., 2002).

Age consistently emerges as the primary risk factor for GCID (Deutsch et al., 2023; Fardet & Feve, 2014; Pofi et al., 2023), while associations with ethnicity, BMI, and familial diabetes history show less consistency across studies (Deutsch et al., 2023; Fardet & Feve, 2014; Pofi et al., 2023). Additional factors, including kidney disease and reduced glomerular filtration rate, also contribute to GCID risk (Deutsch et al., 2023; Katsuyama et al., 2015). While our study found no significant differences in PRED's effects on GSIS when stratified by BMI, sex, or age, these findings warrant cautious interpretation due to limited donor numbers and potential stress effects from islet isolation. Moreover, our analysis lacked data on ethnicity and baseline kidney function. Recent research has identified a type 2 diabetes (T2D)-associated polygenic score that correlates with GCID susceptibility (Deutsch et al., 2023). Studies on polygenic scores highlight the crucial role of genetic variants affecting β-cell function and insulin secretion in diabetes pathogenesis (Udler et al., 2019). The marked variability in GC-mediated inhibition of insulin secretion among donors suggests that genetic factors modulating β-cell response to GCs likely represent a critical determinant of GCID risk.

The precise mechanisms underlying GCID and GC-induced β-cell dysfunction are not fully understood. Studies in mice have shown that GCs receptor (GR) overexpression significantly reduces acute insulin response to glucose, suggesting direct GR-mediated inhibition of insulin release (Delaunay et al., 1997). Insulin secretion exhibits a characteristic biphasic pattern: an initial phase occurring within 5 minutes of glucose exposure, followed by a sustained second phase lasting 2-4 hours under persistent glucose elevation. Our perifusion analysis enabled examination of both phases. Among five previous studies using dynamic perifusion to investigate GC effects on GSIS (B. Billaudel & B. C. Sutter, 1979; I. K. Jeong et al., 2001; Lambillotte et al., 1997a; Pierluissi et al., 1986; Zawalich et al., 2006), one study using rats islets and high doses of DEX (6.3 μM), reported effects on both phases (Pierluissi et al., 1986). Impaired first-phase insulin secretion typically represents an early T2D marker and plays a crucial role in suppressing endogenous glucose production, while the second phase mediates peripheral glucose uptake (Park et al., 2021). Our findings demonstrate that GCs affect both phases of insulin secretion, consistent with the critical role of intracellular Ca2+ signaling in both acute and sustained insulin release and the observation that reduced Ca2+ efficiency contributes to post-GC treatment secretory defects (G. Barseghian & R. Levine, 1980; Barseghian et al., 1982a; J. L. S. Esguerra et al., 2020; S. Gremlich et al., 1997; I. K. Jeong et al., 2001; A. Karagiannopoulos et al., 2023; Lambillotte et al., 1997a; Zawalich et al., 2006). *In vivo* studies have shown that short-term DEX treatment in insulin-sensitive individuals induces compensatory increases in insulin secretion in response to global insulin resistance (Larsson & Ahren, 1999). However, this compensatory response diminishes with prolonged treatment in obese and streptozocin-induced diabetic rats, leading to

sustained insulin resistance (Grill & Rundfeldt, 1986b; Ogawa et al., 1992b), potentially contributing to GCID development.

A key strength of our study lies in using human islets, as most previous research relied on rodent models or immortalized cell lines (A. Rafacho et al., 2014). This distinction is crucial given that GR affinity differs between mice and humans, limiting the clinical relevance of earlier findings (Giannopoulos & Keichline, 1981). However, several limitations merit consideration: First, limited islet availability prevented molecular analyses of pathways such as p38 MAPK/TXNI (D. Avram et al., 2008; C. Beaupere, A. Liboz, B. Feve, et al., 2021; F. Ranta et al., 2006), insulin biosynthesis, and signaling cascades (J. L. S. Esguerra et al., 2020; S. Gremlich et al., 1997; I. K. Jeong et al., 2001; A. Karagiannopoulos et al., 2023; M. M. Linssen et al., 2011). Second, while considered chronic for in vitro analysis, our 24-hour treatment duration was relatively short, and longer exposures might reveal β-cell dedifferentiation into exocrine cells (Russ et al., 2009). Third, although we visually confirmed islet viability before each experiment, we did not specifically assess viability and apoptosis, despite GCs' known potential to induce β-cell apoptosis (A. Rafacho et al., 2014).

In conclusion, our study demonstrates that low-dose PRED treatment inhibits GSIS *in vitro*. However, the extent to which this direct inhibitory effect counterbalances insulin resistance-induced hyperinsulinemia *in vivo* requires further investigation. Our findings support previous evidence suggesting that interindividual β-cell susceptibility to GCs may predispose certain individuals to GCID. Furthermore, DEX has a more detrimental effect on GSIS, underscoring that anti-inflammatory equipotency does not equate to metabolic potency. Therefore, our findings reinforce that PRED should remain the GC

of choice in most clinical settings, while emphasizing the importance of bearing in mind potential metabolic effects even at low doses.

CHAPTER 3

Cortisol metabolism in the human pancreatic islets

CORTISOL METABOLISM IN THE HUMAN PANCREATIC ISLETS

3.1 Introduction

Tissue-specific GC action is regulated through multiple mechanisms modulating its bioavailability, including local metabolism. The 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), predominantly expressed in kidney, reversibly inactivates cortisol to cortisone, protecting mineralocorticoid receptors from cortisol's effects. Conversely, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) reactivates cortisone to cortisol in tissue as the liver and adipose tissue (Chapman et al., 2013). A-ring reduction by 5α-reductases (SRD5A1 and SRD5A2) or 5β-reductase (AKR1D1) contributes to irreversible GC inactivation in the liver (Schiffer et al., 2019). While GCs directly impact islet function, the expression and activity of cortisol-metabolizing enzymes in the pancreas remain poorly characterized.

In pancreatic tissue, *HSD11B1* mRNA or protein expression has been confirmed in rat INS-1 cells, primary rat islets, ob/ob mice islets, and human islets (Davani et al., 2000; Schmid et al., 2011). However, its cellular localization remains controversial (Chapman et al., 2013; Liu et al., 2011). Some studies report *HSD11B1* in β-cells (Davani et al., 2000; Schmid et al., 2011), while others demonstrate predominant expression in α-cells and pancreatic polypeptide cells (Swali et al., 2008). *HSD11B2* expression has been detected in pancreas (Albiston et al., 1994; Smith et al., 1997), including rat INS-1 cells, rat and human primary islets (Schmid et al., 2011), though its cellular distribution remains to be studied. Regarding A-ring reductases, *SRD5A1* is expressed in islets and has been localized in β-cell while *SRD5A2* is not expressed

(Xu et al., 2020a). The expression profile of *AKR1D1* in pancreatic islets has not yet been described in published work.

Regarding the expression or activity of these enzymes in islets, in Zucker Diabetic Fatty (ZDF) rats, islet *Hsd11b1* expression increases progressively with diabetes severity, showing a 16-fold increase in severely diabetic rats compared to controls (Duplomb et al., 2004). However, the expression of *HSD11B1* in human islets under metabolic conditions remains largely unexplored. On the other hand, the tissue-specific expression of *SRD5A1* has not been well studied in the context of obesity. A study on 41 obese subjects shows a positive correlation of *SRD5A1* expression with fasting insulinemia but not significantly with serum glucose (René Baudrand et al., 2011).

Given the importance of local GC metabolism and the limited understanding of these pathways in pancreas, we first investigated the expression of genes involved in cortisol metabolism in human pancreatic tissue at the cellular level using RNAscope in situ hybridization. Second, we studied if the expression of these genes is altered in obesity or diabetes in a large cohort of human islets.

3.2 Materials and Methods

3.2.1 Human Islet Isolation

Confer **Chapter 2**. The information on the clinical and biological characteristics of the donors used in this thesis are listed in **Supplementary Table 1.**

3.2.2 GC treatment

Human islets (300 islet equivalents) were washed twice in DPBS 1X (Gibco, Cat #14040141) and cultured in a 5.5 mM glucose media (Gibco, Life Technologies, Paris, France), supplemented with 0.625% human serum albumin (HAS), 100 U/ml penicillin-streptomycin (Gibco, Cat #15140148). The media contained either GCs (hydrocortisone [HC], or cortisone or 0.001% methanol/ethanol (control)). To characterize endogenous cortisol metabolism in human islets (peak average physiological level at 8 am in healthy humans (Kraan et al., 1998), islets were treated with 500 nM of HC (Sigma-Aldrich, Cat # H0888) or 500 nM of cortisone (Sigma-Aldrich, Cat# D2915), for 24hours.

3.2.3 RNA extraction

Total RNA was extracted from human islets (500 IEQ) using the RNeasy Mini Kit (QIAGEN, Cat No. 79216, Germany). Islets were resuspended in 350 µL of RNA lysis buffer (Qiagen, Cat No 74106, Germany) supplemented with 3.5 µL of β-mercaptoethanol (BME) (Sigma, Cat No. M6250, Germany) before being stored at -20ºC. Upon defrosting, 70% ethanol (350 µL) was added to the sample. Then, the mix (700 µL) was transferred to the RNA extraction column and centrifuged at 13000 rpm for 1 minute at 4ºC. The collector tube was emptied and 700 µL of RW1 buffer was

added before another centrifugation step at 13000 rpm for 1 minute at 4ºC. The columns were washed twice with 500 µL of RPE buffer each and centrifuged at 13000 rpm for 1 minute at 4ºC. The collector tube was empty, and columns were centrifuged for at 13000 rpm for 3 minutes at 4ºC. RNA was resuspended by adding 30 - 35 µL of DNase/RNAse-free distilled water to the column and collected in a new Eppendorf after 1 minute centrifugation. RNA samples were kept on ice during the RNA quantification measurement by ND-1000 Spectrophotometer V3.8 (Thermo Scientific, Cat No F924, USA) and stored at -80 °C.

3.2.4 Reverse transcription for cDNA generation

First-strand cDNA synthesis was performed with the Superscript IV reverse transcriptase (Life Technologies, Cat No 18090050, Lithuania) according to the manufacturer's instructions. A total of 0.5 mg RNA of each sample was diluted in DNase/RNase-free distilled water up to 11 µL, and 0.5 µL of Oligo d(T) (50 µM) and 0.5 µL random hexamers (50 ng/µL) (Thermo Scientific, Cat No SO142, Lithuania) were added per sample. The mix was incubated at 65 °C for 5 minutes and then placed on ice for 1 minute. Then, SSIV buffer 5X (4 µL), 10 mmol/L DTT (1 µL), RNAse inhibitors (1 µL), and Superscript IV reverse transcriptase (200 U/ Fisher Scientific l) were added to the reaction tube. The reaction mix was incubated at 23°C for 10 minutes, 55°C for 10 minutes, and 80°C for 10 minutes. cDNA was stored at -80°C until the quantitative real-time PCR experiments.

3.2.5 Real time PCR

Quantitative RT-PCR was conducted using the Bio-Rad MyiQ Single-Color Real-Time PCR Detection System and the Bio-Rad SYBR Green Supermix (Bio-Rad Laboratories, Cat No 172-5274, USA). Primers were used at a concentration of 500 nmol/L (250 nmol/L forward + 250 nmol/L reverse).and the cDNA was diluted 1:10. The mix consisted of 1 µL of cDNA diluted, 5 µL of SYBR Green, and 3 µL of DNase/RNase free distilled water, and 0.5 µL of each primer. The Bio-Rad CFW Connect Real-Time thermal cycler was used. The thermal cycle was 95°C for 3 minutes, followed by 40 cycles of 95°C for 10 s (denaturation) and 60°C for 30 s (annealing and extension). Melting curve analysis was performed to determine the specificity of the amplification, and gene expression was normalized to the s18 rRNA housekeeping gene. Primers were designed using IDT Primer Quest tool (https://www.idtdna.com/pages/tools/primerquest) and synthesized in IDT technologies. Primer's specificities were corroborated using the Basic Local Alignment Search Tool (BLAST) and tested for efficiency and specificity before use. **Table 7** detail the primer sequence used for gene expression analysis in humans by RT-qPCR.

Table 7: Human primer sequence used in RT-PCR technique

Gene (Protein)	Forward sequence (5' – 3')	Reverse sequence (5' – 3')
AKR1D1 – 002 (AKR1D1)	caaagacgctggcttggtga	gctgggtgaaatacggatgg
AKR1D1 – 001 (AKR1D1)	gcaagtcaccgcatacctct	ggccccatcaatatgtcggt
AKR1C4 (3α-HSD)	tgggaggtcatggagaagtg	tgagtcctggcttgttgagg
AKR1C3 (3α-HSD)	cctcaacaagccaggactca	ggtccacccatcgtttgtct
AKR1C2 (3α-HSD)	tggtcacttcatgcctgtcc	caatcttgcttcggatggcc
SRD5A2 (SRD5A2)	ggaagcctggagaaatcagc	ctcgcagcccaaggaaaca
SRD5A1 (SRD5A1)	gcttgtggttaacgggcatg	gcatagccacaccactccat
HSD11B2 (11β-HSD2)	gctgtgaactccttccctgg	cttgcgcttttcccactgac
HSD11B1 (11β-HSD1)	cagaccagagatgctccaagg	ggtgccagcaatgtagtgtg
18S rRNA (18S rRNA)	ggccgttcttagttggtgga	tcaatctcgggtggctgaac

3.2.6 RNAscope in situ Hybridization

Human pancreas tissue sections were fixed in 4% PFA for 32 hours. The samples were then transferred to 70% ethanol and embedded in paraffin blocks. Human islets (1000 IEQ) were washed twice with 1X PBS before being fixed with 4% PFA-PBS for 1 hour. Following fixation, the islets were washed twice with 1X PBS and preserved in 80-100 µL of pre-heated histogel (Thermofisher Scientific, Cat No. HG-4000-012, UK). The histogel containing human islets was then transferred to 70% ethanol and embedded in paraffin blocks.

RNA In Situ Hybridization

RNA *in situ* hybridization was performed using RNAscope® Multiplex Fluorescent Reagent Kit v2 specifically (Advanced Cell Diagnostics, Cat. No. 323100-USM, USA) following the manufacturer's guidelines. In this experiment, human probes used

include HSD11B1 probe (Cat No. 432331), HSD11B2 probe (Cat No. 432351-C2), SRD5A1 probe (Cat No. 1210721-C1).

Slide Preparation

Tissue sections of 5 µm were baked for 1 hour in a HybEZ hybridization oven (Advanced Cell Diagnostics, Cat No. 321720, USA). The slides were deparaffinized in xylene twice (5 minutes each), then dehydrated in 100% ethanol (2 times, 5 minutes each). Tissue sections were treated with hydrogen peroxide for 10 minutes before being washed with distilled water. For antigen retrieval, slides were immersed in the kit solution 1X for 8 minutes for human islets in histogel, and 15 minutes for human pancreas at a boiling temperature (99 ºC) using a steamer (Braun, Cat No. FS3000). The samples were then rinsed in deionized water for 15 s and immediately incubated with ethanol 100% for 3 minutes at room temperature. A hydrophobic barrier was drawn with a Dako pen (Agilent Dako, Cat No. S2002, Denmark), and tissue sections were treated for 8 minutes with protease plus and washed with distilled water. The slides were incubated at 40°C in a HybZ hybridization oven in the following order: target probes for 2 hours, amplifier 1 for 30 minutes, amplifier 2 for 30 minutes, amplifier 3 for 15 minutes and HRP-C1/2/3 for 15 minutes. After each hybridization step, slides were washed with 1X wash buffer twice for 2 minutes each at room temperature. Finally, tissues were incubated with the fluorophore TSA Plus Cyanine 3 fluorophore (Akoya, Cat No. NEL744001KT, USA) diluted 1:1500 in TSA buffer (Advanced Cell diagnostics, Cat No. 322809, USA) for 30 minutes at 40°C. Before proceeding to immunofluorescence, the slides were incubated with the blocker for 15 minutes.

Controls and Imaging

Assays using archival FFPE specimens included parallel processing with positive and negative controls (positive control for human – Advanced Cell Diagnostics, Cat No. 320861, negative control for human– Advanced Cell Diagnostics, Cat No. 320881, USA), to ensure interpretable results.

3.2.7 Immunofluorescence technique

Slices were cut (10 um) and after deparaffinized following the standard protocol: xylene for 5 minutes, xylene for 10 minutes, ETOH 100% for 5 minutes, ETOH 90% for 5 minutes, ETOH 80% for 5 minutes, ETOH 70% for 5 minutes, ETOH 50% for 5 minutes, H_2O for 5 minutes, PBS for 5 minutes. Heated-mediated antigen retrieval was performed under specific conditions for each primary antibody detailed in **Table 8**. Tissues were incubated with serum-free protein block (Dako, Cat No. X0909, USA) for 15 minutes at room temperature. Single or double immunofluorescence staining was performed with the primary antibodies (details of antibodies and dilutions are specified in **Table 8**). Primary antibodies were incubated overnight at 4ºC, and after washing the slides three times with PBS for 5 minutes each, secondary antibodies were incubated for 1 hour at room temperature (**Table 9**). Finally, nuclei were counterstained with DAPI (Vectashield, Vector Laboratories, Cat No. H-1200-10, USA) for 10 minutes with a dilution 1:1000, and mounted with Dako Fluorescence Mounting Medium (Dako, cat No. 53023, USA). Images were acquired using the Zeiss LSM 710 confocal microscope with the Airyscan super-resolution module (Zeiss, Germany) + rotating disk and Zeiss Spinning Disk confocal microscope. Images were obtained using a 40X objective (Jena, Germany) with immersion oil. The images were processed and adjusted using ImageJ, version 1.8.0_172/1.53q99 (https://imagej.nih.gov/ij).

Table 8 lists the primary antibodies used in Western Blot (WB) and Immunofluorescence (IFI) techniques. **Table 9** lists the secondary antibodies used. Antibodies for WB were diluted in 5% Bovine Serum Albumin in 1X Tris-Buffered Saline, 0.1% Tween® 20 Detergent (TBST), or 5% milk-TBST (Aldrich, Cat No. A9418, USA). Antibodies for IFI were diluted in PBS supplemented with 0.3% triton (Sigma Aldrich, Cat No. X100, USA) and 0.2% donkey serum (Sigma-Aldrich, Cat No. D9663, USA).

Table 8: Primary antibodies for IFI and WB

N⁰	Antibody	Manufacturer	Catalogue number	Species / Type	Technique	Dilution	Antigen Retrieval / preincubation
1	Insulin	Abcam	Ab181547	Rabbit Monoclonal	IFI	1:500	RNAscope antigen retrieval
2	Glucagon	Sigma	G2654	Mouse Monoclonal	IFI	1:1000	RNAscope antigen retrieval
3	Somatostatin	Merck Millipore	MAB354	Rat Monoclonal	IFI	1:100	RNAscope antigen retrieval
4	SRD5A1	Novus Biologicals	NB100-1491	Goat Polyclonal	WB	1:500	N/A
5	SRD5A1	Protein Tech	66329-1-lg	Mouse Monoclonal	WB	1:500	N/A
7	eGFP	Invitrogen (Thermofisher)	MAI-952	Mouse Monoclonal	WB	1:1000	N/A
8	B-actin	Sigma	A5441	Mouse Monoclonal	WB	1:10000	N/A

Table 9: Secondary antibodies for IFI and WB

N⁰	Antibody	Manufacturer	Catalogue number	Technique	Dilution
1	Goat anti-mouse	Invitrogen	A11032	IFI	1:800
2	Goat anti-rat	Invitrogen	A11007	IFI	1:800
3	Donkey anti-rabbit	Invitrogen	A31573	IFI	1:800
4	anti-mouse HRP	General Electric	NXA931V	WB	1:10000
5	anti-mouse HRP	bio-techne	HAF017	WB	1:1000

3.2.8 Cortisol metabolite measurement by HPLC-MS/MS

Cortisol and its metabolites were measured in the supernatant and lysate using HPLC-MS/MS. Cells/islets were lysed in 250 µl of cell lytic (Sigma-Aldrich, Lot# 018M4125V) supplemented with anti-protease (1 on 50 dilution; PhosSTOP, Roche) and anti-phosphatase (1 on10 dilution; Sigma-Aldrich Product #P8340). After lysing, cells were sonicated, and centrifuged at 13000 rpm for 12 mins at 4^0C. After which, cell lysates were collected, and cell pellets discarded. Experiments was performed for each donor in duplicate. First, we check for the possible matrix effect (Mikkaichi et al., 2004) in the cell/islets supernatant and lysate, and was confirmed by insignificance to interfere with steroid measurement. Samples were prepared by adding 100 µl of an internal standard (IS) of known concentration of deuterated steroids (Fd4 for cortisol, ED8 for cortisone, THF-d5 for THF, THF, a-DHF and DHF, THE-D5 for THE, DHE, a-DHE) in 500 µl of cell substrate or in the whole volume of cell lysate. We produced a linear curve using an increased concentration range of standard steroids used for quantification of steroids. The culture medium associated to the IS were purified by extractions in C18 columns and filtered under nitrogen pressure using a Positive Pressure Manifold 96 Processor (PPM-96, Agilent, USA). In total, 2 extractions were performed separated by enzymatic hydrolysis with β-gluconidase overnight. Samples were evaporated under nitrogen at 40°C after each extraction. The samples were resolubilized in assay mobile phase (55% of water, 45% of Methanol), loaded into vials and analyzed by UHPLC-MS/MS (Waters, ACQUITY UPLC H-Class PLUS, XEVO TQD, USA) in negative mode of ionization. Limit of detection were quantified for each metabolite: F; 0.55 nM, E; 0.42 nM, DHF and THF; 7.9 nM, THE; 6.03 nM, 5α-DHF and THF; 3.3 nM. As existing protocol for the measurement of dihydrocortisol (a-DHF and DHF), and dihydrocortisone (a-DHE and DHE) did not exist in the lab, we set up and optimized

separation conditions for these compounds with the HPLC – MS/MS program (**Table 10**), (**Figure 15**).

Table 10: Parameters defined for compounds after successful optimization of HPLC/MC program

Compounds	Molecular weight (g/mol)	Mother ion	Collison voltage	Daughter ion	Retention time
5α- DHF	362.15	363.15	15	345.1	4.09
5α- DHE	342.15	365.15	10	347.1	4.43
DHF	362.15	363.15	20	251.07	5.08
DHE	342.15	343.15	40	335.4	5.15

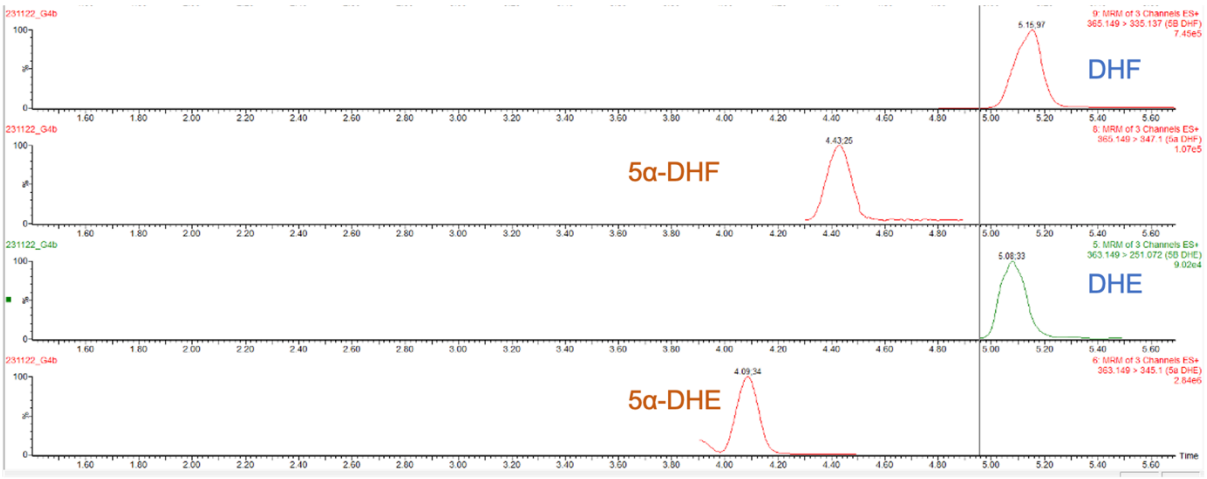

Figure 15: Calibration of the HPLC-MS/MS with 5α-DHF, 5α-DHE, DHF and DHE, 5α-DHF. Figure showing Chromatograph separation of 5α-DHF, 5α-DHE, DHF and DHE, 5α-DHF by UHPLC-MS/MS after optimization of the HPLC-MS/MS program.

3.2.9 Statistical Analysis

Statistical analyses were performed with GraphPad Prism 10.2.0 (GraphPad Software, La Jolla, California, USA). Data are expressed as mean ± SEM. Comparison between control and GC treatment was performed using Wilcoxon paired and non-parametric t-tests. Comparison between groups was performed using Wilcoxon unpaired and non-parametric t-tests. Comparison between the different doses of PRED and GCs at equipotent dose were performed using one-way ANOVA with Sidak's multiple comparison *post hoc* tests, and two-way ANOVA and nonparametric tests with Sidak's *post hoc* tests for multiple comparisons where statistically applicable. Differences were considered significant at $p < 0.05$. Significance was expressed as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ and **** $p < 0.0001$.

3.3 Results

3.3.1 *HSD11B1* and *SRD5A1* are expressed in the β-cells

Using qPCR, we studied the expression of *HSD11B1*, *HSD11B2*, *SRD5A1*, *SRD5A2*, *AKR1D1* in eight human islets. We could not detect *AKR1D1* and *SRD5A2* gene expression. We observed that *HSD11B1*, *HSD11B2*, and *SRD5A1* were expressed (**Figure 16A**). Islets are suspected to transdifferentiate into exocrine cells with increased duration of culture, which could potentially affect gene expression. To address this concern, we investigated whether the expression of *HSD11B1*, *HSD11B2*, and *SRD5A1* is impacted by the duration of islet culture. Our analysis revealed no significant differences in the expression of these genes after 7 days of culture compared to freshly isolated islets (**Figure 16B**).

To confirm the specific localization of *HSD11B1*, *HSD11B2*, and *SRD5A1* mRNA, we performed RNAscope in situ hybridization combined with immunofluorescence to co-localize these transcripts with insulin-secreting β-cells or glucagon-secreting α-cells in pancreatic sections from 3 lean normoglycemic donors (BMI: <25, HbA1c: <5.7%). We observed robust expression of both *HSD11B1* and *SRD5A1* in islets, while *HSD11B2* expression was barely detectable. *HSD11B1* and *SRD5A1* showed predominant expression in β-cells with minimal expression in α-cells. Conversely, *HSD11B2* was primarily expressed in the surrounding exocrine tissue of the lean normoglycemic donors, where *HSD11B1* and *SRD5A1* expression were rarely observed (**Figure 17; Figures 20A-C, 21A-FC 22A-C**; **Supplementary Figures 1A-F, 3A-F, 5A-F**).

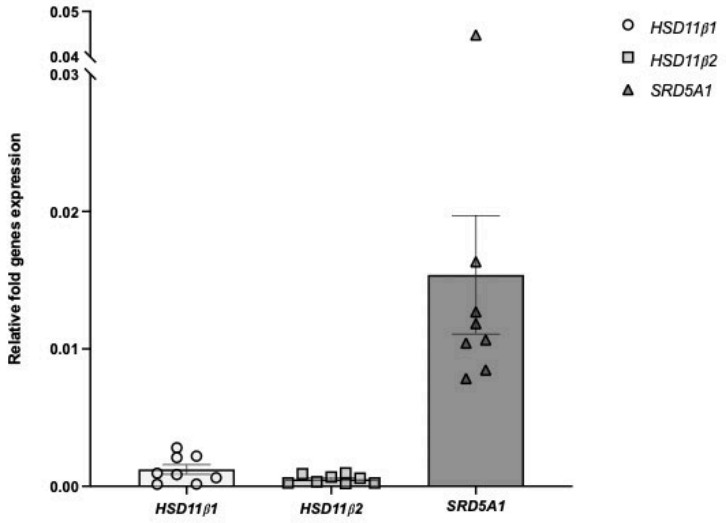

Figure 16A: Expression of genes involved in cortisol metabolism. mRNA expression of genes normalized to the housekeeping gene *GAPDH*. Human islets (*n*=8), expressed *HSD11B1*, *HSD11B2*, *SRD5A1*, while *SRD5A2* and *AKR1D1* are not expressed.

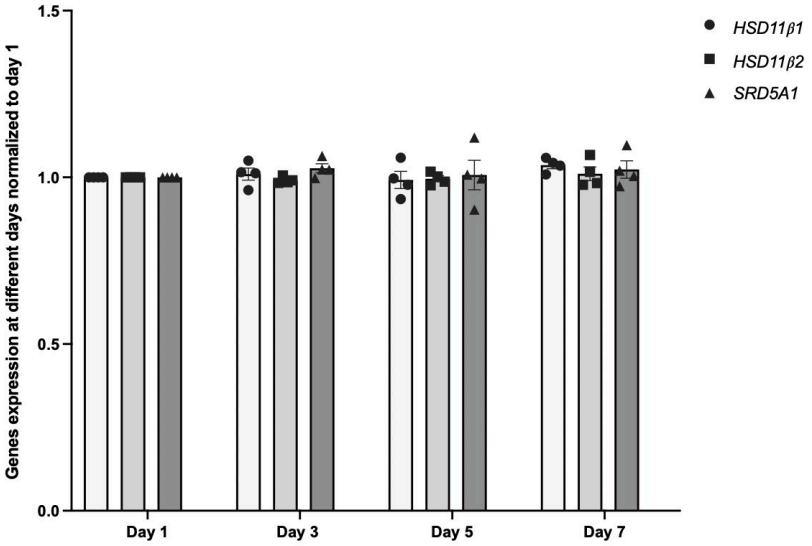

Figure 16B: Expression of *HSD11B1*, *HSD11B2*, and *SRD5A1* genes in the human islets after different days of culture. Islets from 3 donors were cultured for one to seven days in basal glucose level (5.6 mM). The mRNA expression fold change of HSD11B1, *HSD11B2* and *SRD5A1*, remain stable irrespective to the number of days in culture after isolation.

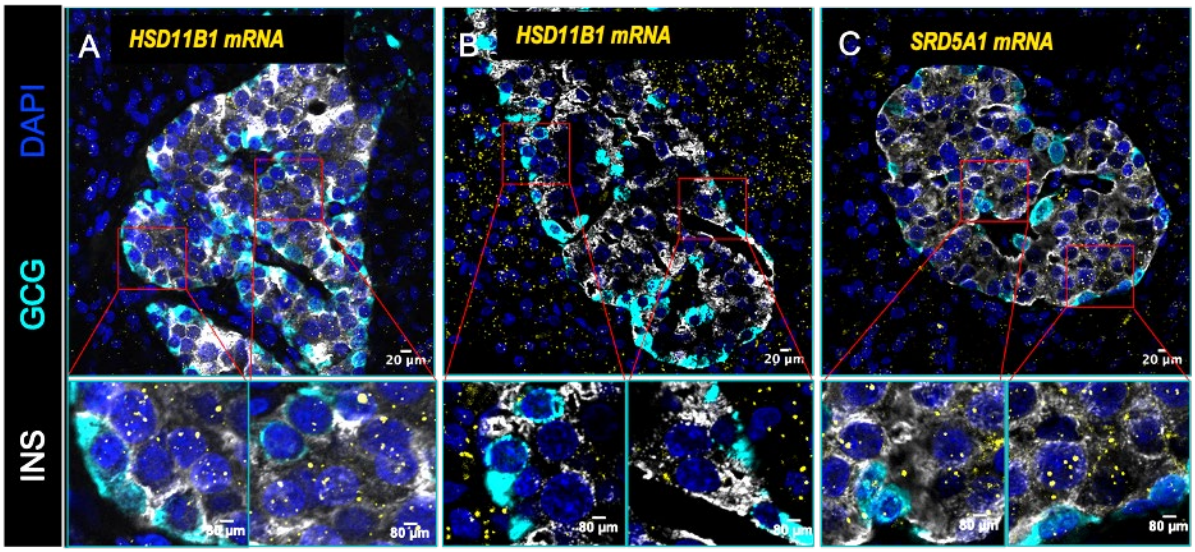

Figure 17: Expression pattern of *HSD11B1*, *HSD11B2* and SRD5A1 in lean normoglycemic islets: Representative images from *n*=1 donor, of (A) *HSD11B1*, (B) *HSD11B2*, (C) *SRD5A1* mRNA expression assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for insulin (INS, white), and glucagon (GCG, cyan), nuclei staining with DAPI (blue), on FFPE pancreatic sections from lean normoglycemic donor (BMI: <25, HbA1c: <5.7%). Scale bars, 20 µm. Red box on figures indicates section zoomed (4x), scale bar 80 µm.

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3.3.2 *HSD11B1* and *SRD5A1* are significantly decreased in islets from diabetic donors

We performed RT-PCR analysis in a cohort of 93 donors including lean normoglycemic donors (*n* = 14, BMI < 25, HbA1c < 5.7), lean and glucose intolerance (*n* = 16, BMI < 25, HbA1c ≥ 5.7), overweight and normoglycemic donors (*n* = 17, BMI ≥ 25, HbA1c < 5.7), donors with overweight and glucose-intolerance (*n* = 13, BMI ≥ 25, HbA1c ≥ 5.7), donors with obesity and normoglycemic (*n* = 11, BMI > 30, HbA1c < 5.7), donors with obesity and glucose-intolerance (*n* = 17, BMI > 30, HbA1c ≥ 5.7), and donors with overweight and T2D (*n* = 11, BMI ≥ 25, HbA1c ≥ 6.4). *HSD11B1* expression was

not significantly different when comparison was made between lean and obese donors (**Figure 18A)**, and we found no correlation between *HSD11B1* expression and BMI of donors (*n*=93) (data not shown). However, we observed a significantly lower expression in obese patients when compared to non-obese patients (p=0.0225), (**Figure 18C)**. The expression of *HSD11B2* and SRD5A1 was not significantly different irrespective of BMI classification (**Figure 18B-D, 19E-F**). Stratification of donors by HbA1c, shows a significantly lower expression for *HSD11B1* and *SRD5A1* in islets from diabetic donors compared to non-diabetic donors (p=0.0060 and p=0.0127 respectively), (**Figure 19A, C**) while no difference was observed for *11BHSD2* (**Figure 19B**). We observed a significant negative correlation of *HSD11B1* and *SRD5A1* expression with HbA1c (**Figure 19D-E**).

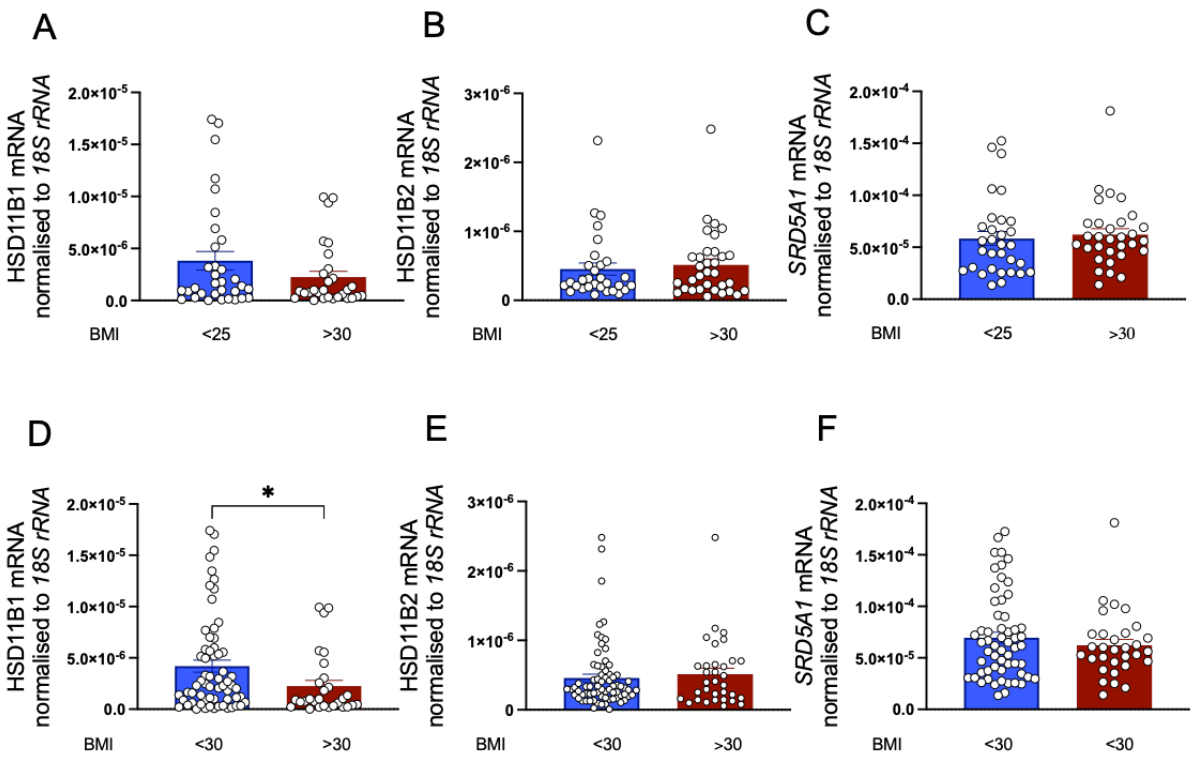

Figure 18: Comparison of the expression of *HSD11B1*, *HSD11B2* and *SRD5A1* assessed by RT-PCR in islets from lean and obese donors. **A, D**: *HSD11B1* mRNA, **B, E:** *HSD11B2* mRNA, **C, F**: *SRD5A1* mRNA levels in islets, from donors, stratified according to their BMI. A-C, lean (*n* = 30, BMI < 30) *vs.* obese (*n* = 33, BMI < 30), D-E, non-obese (*n* = 60, BMI < 30) *vs.* obese (*n* = 33, BMI < 30). Mann Whitney's unpaired t-test and non-parametric analysis was performed, *p < 0.05.

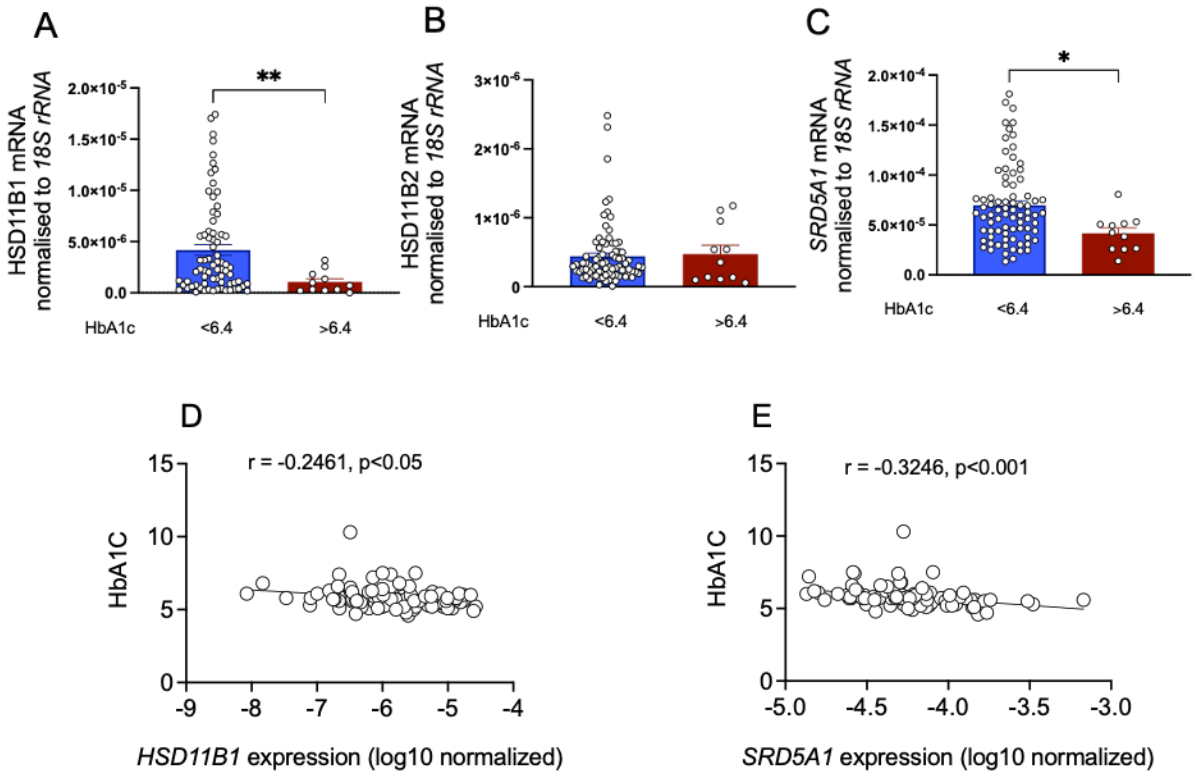

Figure 19: Comparison of the expression of *HSD11B1*, *HSD11B2* and *SRD5A1* assessed by RT-PCR in islets from non-diabetic and diabetic donors. **A**: *HSD11B1* mRNA, **B:** *HSD11B2* mRNA, **C:** *SRD5A1* mRNA levels in islets, **D**: correlation analysis of *11BHSD1* expression with HbA1c, **E:** correlation analysis of *SRD5A1* expression with HbA1c, from donors, stratified according to their HbA1c, non-diabetic (n = 82, HbA1c < 6.4) vs diabetic (n = 11, HbA1c > 6.4). Mann Whitney's unpaired t-test and non-parametric analysis was performed, **p < 0.01, *p < 0.05.

3.3.3 Visualization by RNAscope analysis revealed decreased *HSD11B1 and SRD5A1* in T2D pancreas tissue

To further confirm the decreased expression of *HSD11B1* and *SRD5A1* in islets from diabetic donors compared to non-diabetic donors, we performed RNAscope in situ hybridization combined with immunofluorescence on pancreatic section from 3 lean normoglycemic donors (BMI: <25, HbA1c: <5.7%), obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and 3 T2D donors (BMI: >30, HbA1c: ≥6.4). *HSD11B1 and SRD5A1* expression appears decreased in islets and β-cells from the obese and diabetic donors compared to the lean donors studied (**Figure 20**; **Supplementary Figure 1**). On the contrary, their expression appears increased in exocrine tissues from the diabetic donors compared to the lean or obese donors studied (**Figure 20; Supplementary Figure 1**).

HSD11B2 expression profile in endocrine islets and β-cells did not visually change in the obese or diabetic donors compared the lean donor studied while the expression in the exocrine tissue may be decreased (**Figure 21**; **Supplementary Figure 3**).

To verify that the observed lower expression of *HSD11B1* and *SRD5A1* in islets was not an artifact caused by signal oversaturation from the exocrine cells, we studied the expression pattern on isolated human islets fixed in paraffin and embedded in histogel, allowing for direct examination of gene expression without interference from surrounding exocrine tissue. We confirmed a weak expression of both *HSD11B1* (**Figure 23ADG; Supplementary Figure 7A 1-12**), and *SRD5A1* (**Figure 23CFI; Supplementary Figure 7C 1-12**) in islets and β-cells from diabetic compared to obese and lean donors while *HSD11B2* expression remain stable across all the phenotypes (**Figure 23BEH; Supplementary Figure 7B 1-12**).

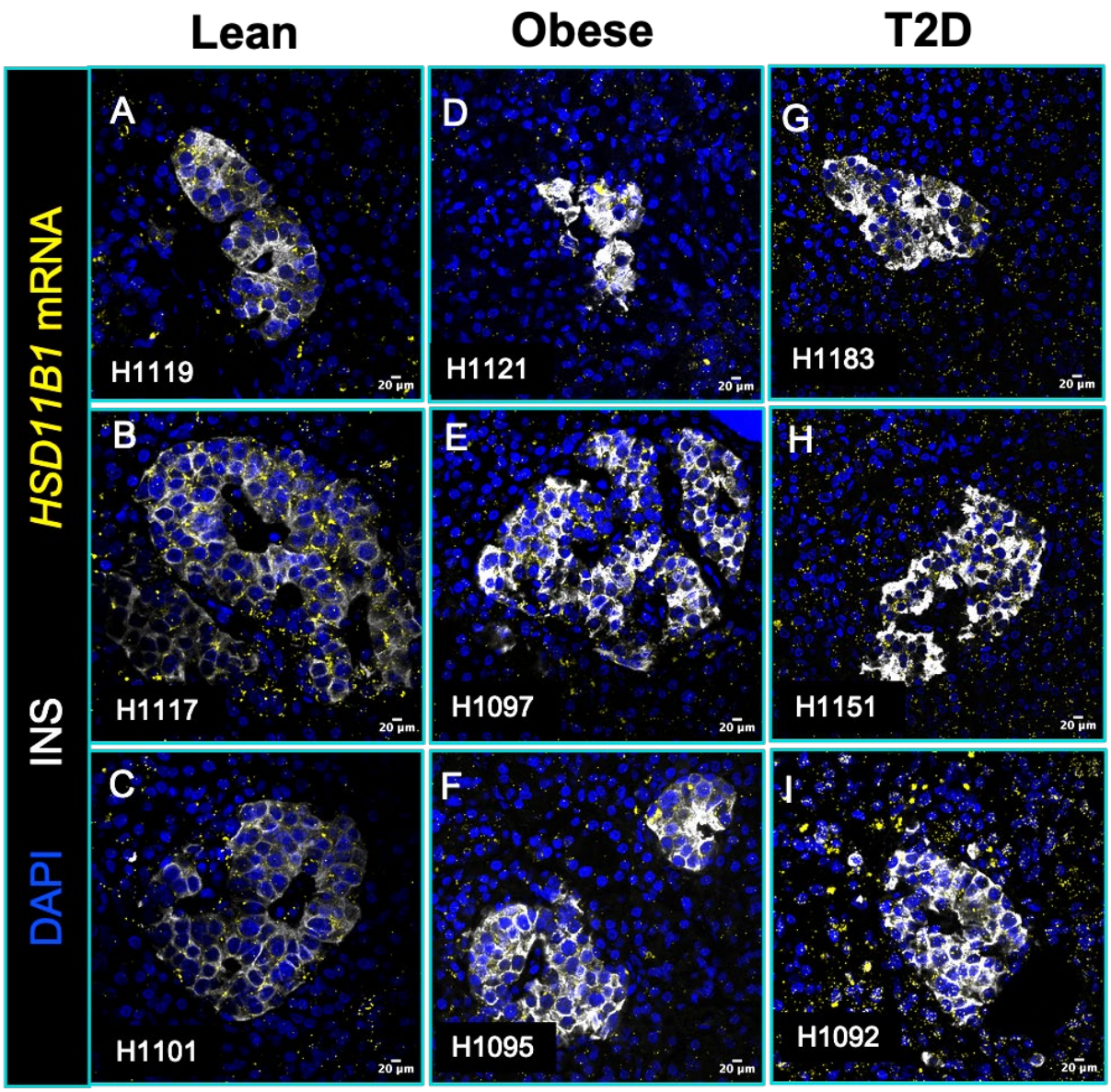

Figure 20: Expression pattern of *HSD11B1* in pancreas from lean, obese and T2D donors: Representative images of HSD11B1 mRNA expression assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for insulin (INS, white), and nuclei staining with DAPI (blue), on FFPE pancreatic sections from **(A-C)** 3 different lean normoglycemic donor (BMI: <25, HbA1c: <5.7%), **(D-F)** 3 different obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and **(G-I)** 3 different T2D donor (BMI: >30, HbA1c: >6.4%). Scale bars, 20 µm.

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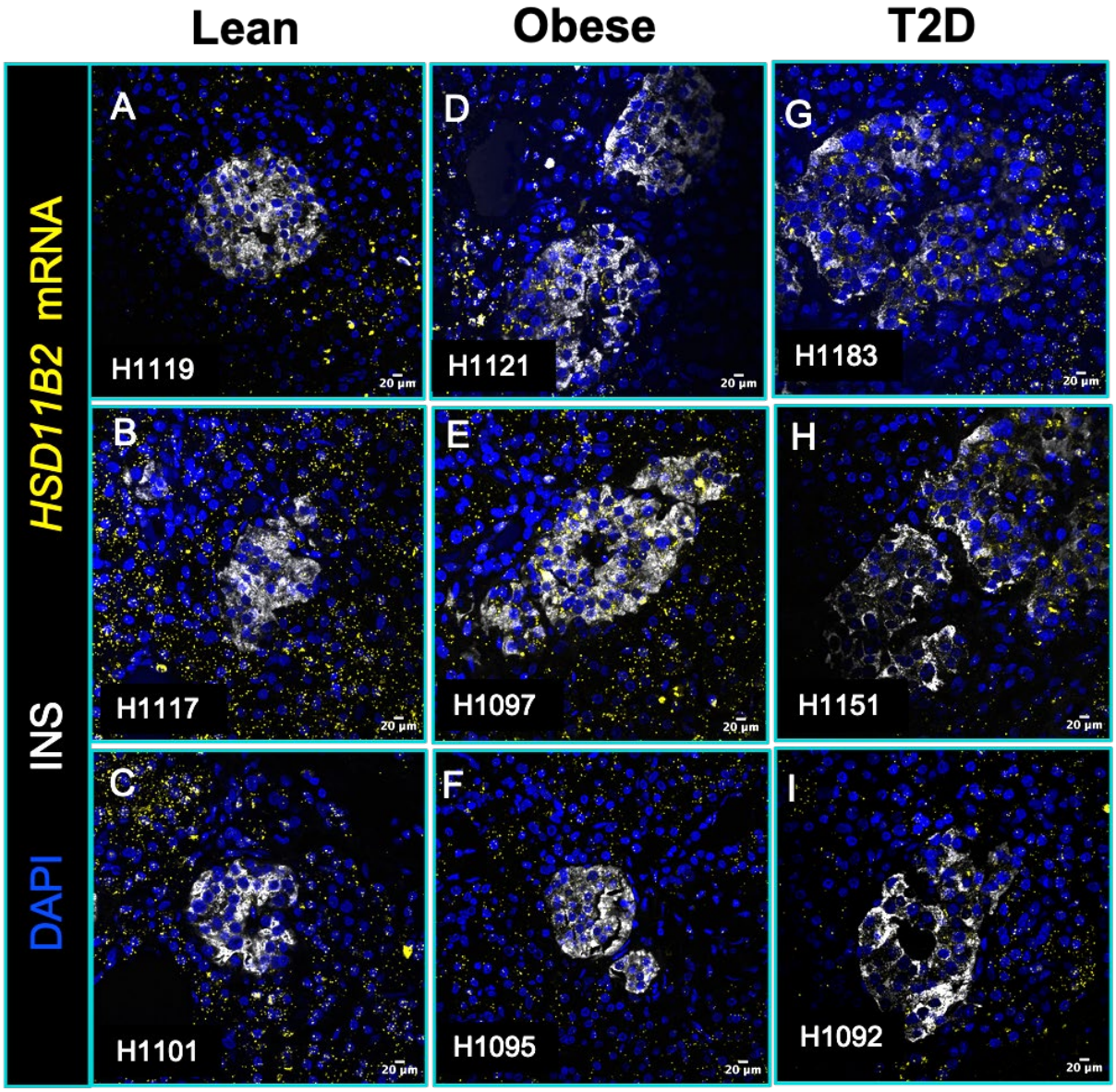

Figure 21: Expression pattern of *HSD11B2* in pancreas from lean, obese and T2D donors: Representative images of *HSD11B2* mRNA expression assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for insulin (INS, white), and nuclei staining with DAPI (blue), on FFPE pancreatic sections from (**A-C)** 3 different lean normoglycemic donor (BMI: <25, HbA1c: <5.7%), **(D-F)** 3 different obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and **(G-I)** 3 different T2D donor (BMI: >30, HbA1c: >6.4%). Scale bars, 20 µm.

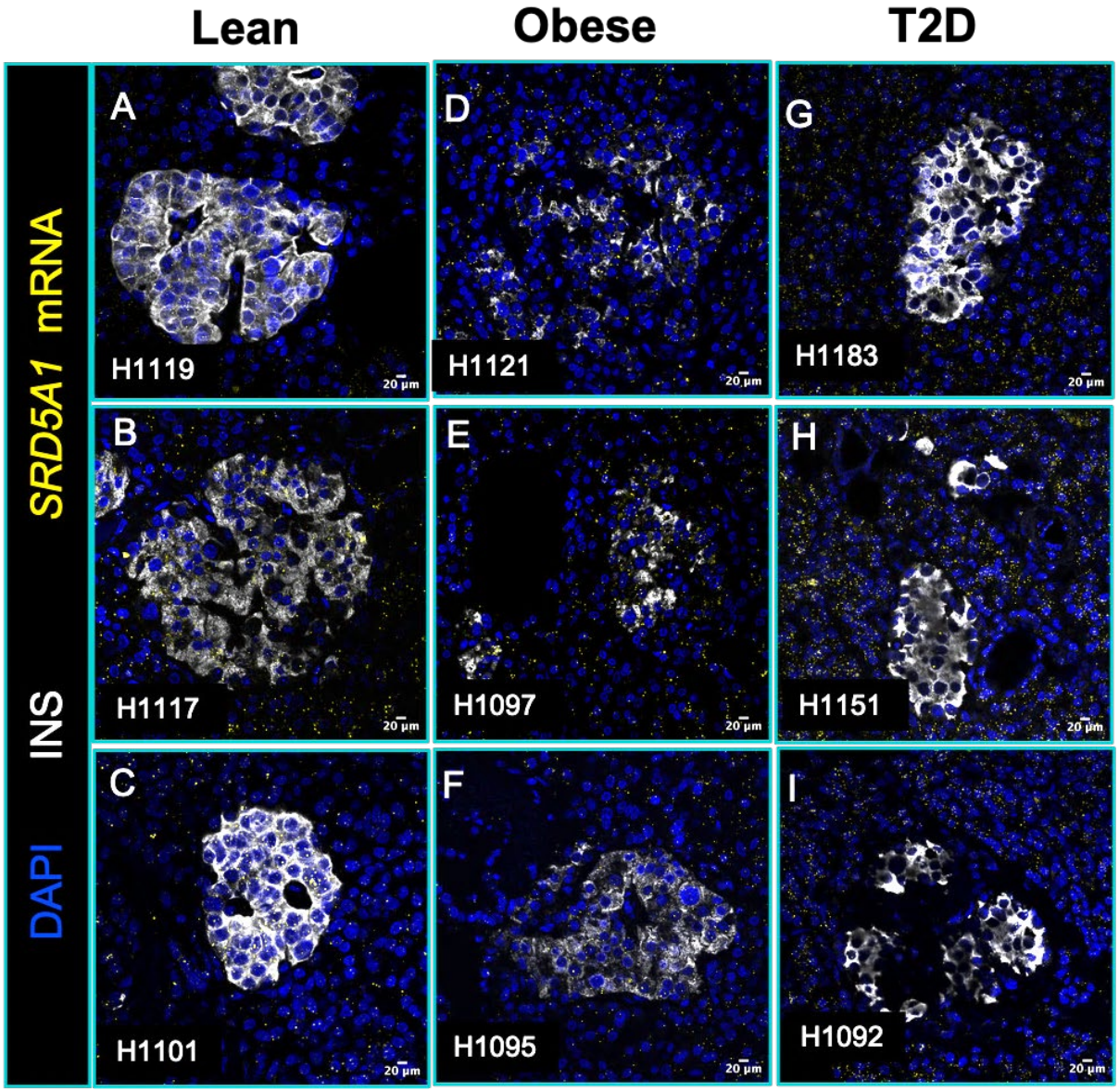

Figure 22: Expression pattern of *SRD5A1* in pancreas from lean, obese and T2D donors: Representative images of *SRD5A1* mRNA expression assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for insulin (INS, white), and nuclei staining with DAPI (blue), on FFPE pancreatic sections from (**A-C**) 3 different lean normoglycemic donor (BMI: <25, HbA1c: <5.7%), (**D-F)** 3 different obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and (**G-I)** 3 different T2D donor (BMI: >30, HbA1c: >6.4%). Scale bars, 20 µm.

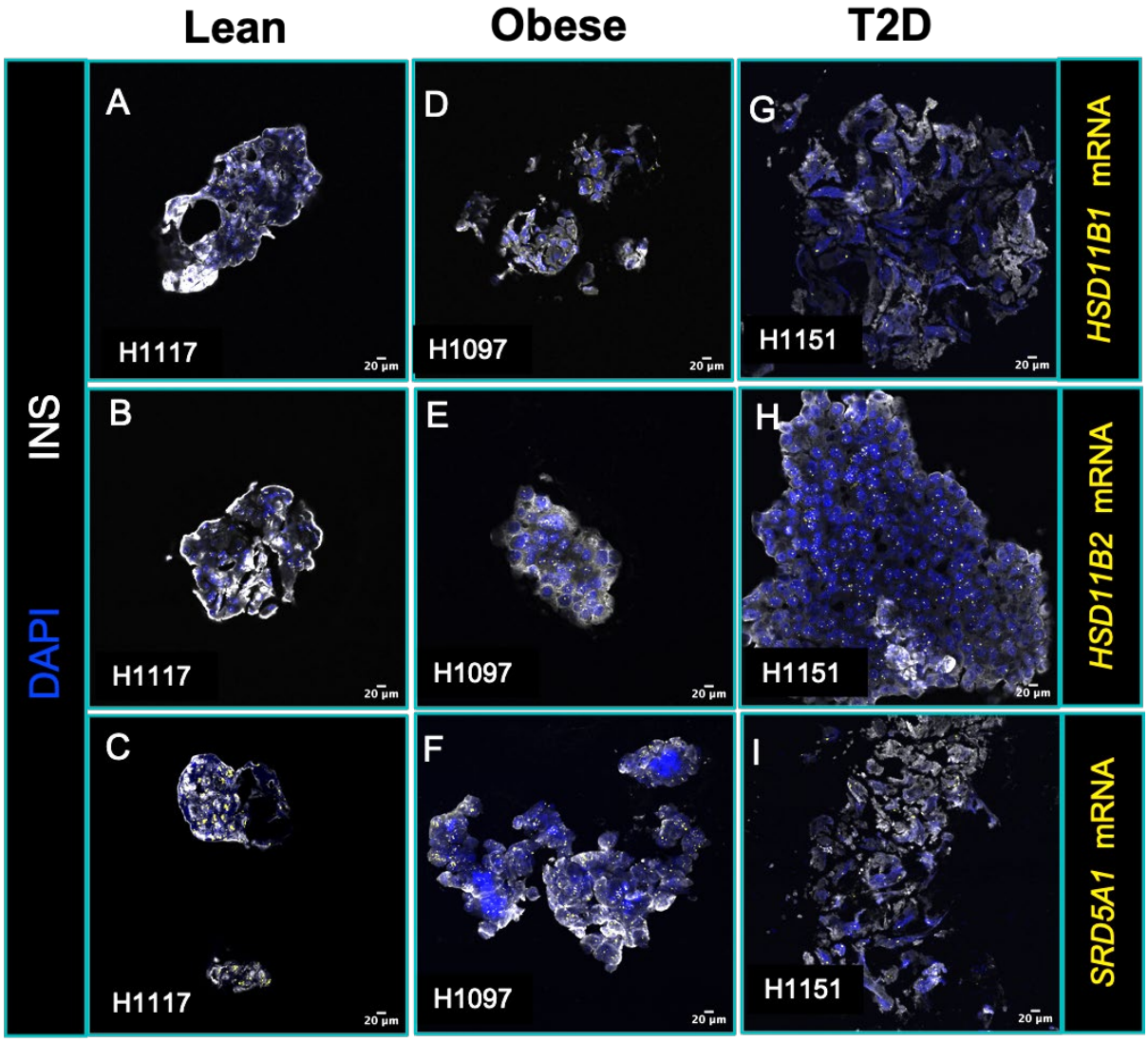

Figure 23: Expression pattern of *HSD11B1*, *HSD11B2*, *SRD5A1* in embedded islets. Representative images of **(A,D,G)** *HSD11B1*, **(B,E,H)** *HSD11B2*, **(C,F,I)** *SRD5A1* mRNA expression assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for insulin (INS) and nuclei staining with DAPI (blue), on paraffin-fixed histogel embedded islets from, (**A-C)** lean normoglycemic donors (BMI: <25, HbA1c: <5.7%), **(D-F)** obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and **(G-I)** T2D donor (BMI: >30, HbA1c: >6.4%). Scale bars, 20 µm.

3.3.4 Metabolism of cortisol in human primary islets

Pancreatic islets from 6 donors were treated with 500 nM of HC for 24h in basal glucose level. While the measured level of cortisol in islets supernatant at 24h post-treatment was lower than the initial treatment dose (**Figure 24A**), suggesting the transformation of cortisol into metabolites, the levels of 5α-THF detected however were below the limit of detection (data not shown). As expected, since *AKR1D1* is not expressed in the human islets, THF and THE levels were below the limit of detection (data not shown). The detection of cortisone in the medium (**Figure 24B**) suggests the presence of a dehydrogenase activity. Interestingly, no other metabolites including 6β-OHF could be detected using GC-MS/MS (data not shown). To assess 11β-HSD1 reductase activity and activity of SRD5A1 in reducing cortisone, we treated then human islets from 4 donors with 500 nM of cortisone for 24h (**Figure 24C**). We detected the presence of cortisol indicative the presence of 11β-HSD1 reductase activity (**Figure 24D**). However, 5α-THF levels were below the limit of detection, as well as THF and THE metabolites (data not shown).

Although cortisol is a steroid hormone and is known to diffuse passively across cell membranes, in certain tissues as the brain, the ABCB1 transporter has been recognized to function in the efflux of steroids as cortisol and synthetic GCs (Kyle et al., 2022). To confirm if some metabolites are retained within the islets, we measure cortisol and its metabolites within the human islets after treatment with either 500 nM of cortisol or cortisone for 24h. Preliminary results in 2 donors' islets confirmed the absence of THE, THF but also 5α-THF. However, we could measure 5α-DHF with cortisol at the substrate even though the concentrations were low (total concentration at 8.2 nM and 12.5 nM for each donor). We also obtained very low but quantifiable

level of 5α-DHF using cortisone as a substrate (total concentration at 13 nM and 8.2 nM for each donor, respectively).

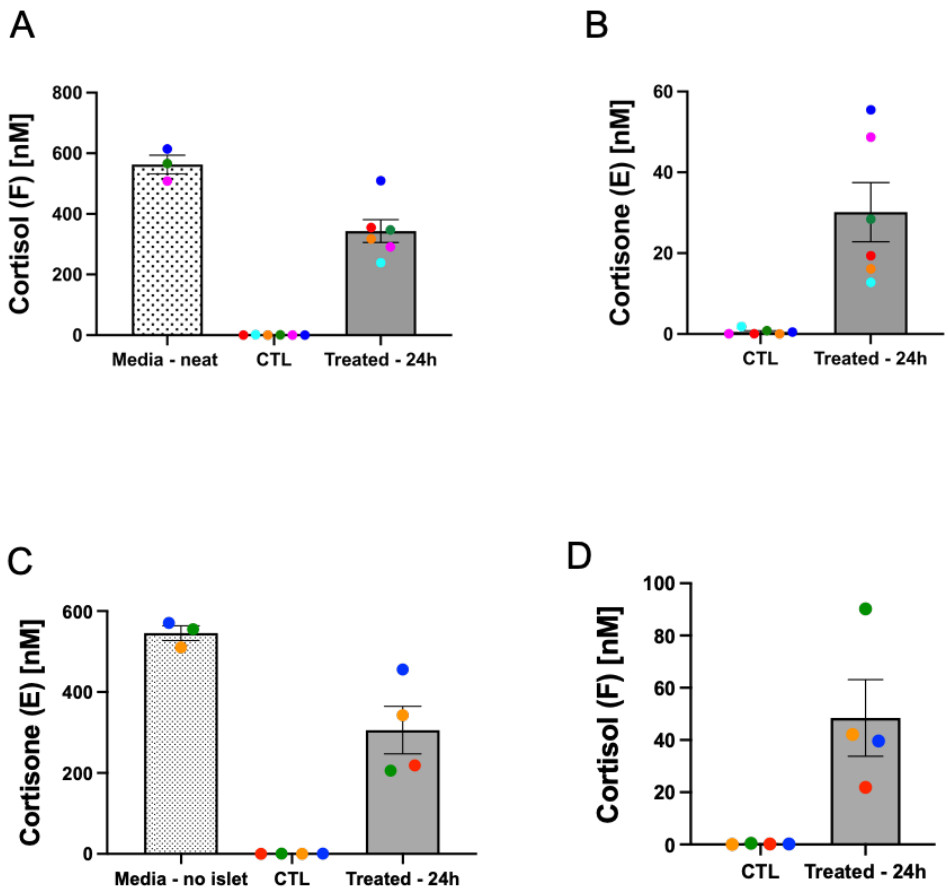

Figure 24: LC-MS/MS reveals unquantifiable activity of SRD5A1 in islets' supernatant and in islet lysate chronically treated with 500 nM of HC or cortisone for 24 hours: **A-B:** cortisol metabolites in the human islets treated with 500 nM of HC for 24 hours, **A:** Cortisol levels in media- neat (no islets), (strip bar), in control non-treated islets (CTL), (white bars) and in treated islets treated (grey bars). **B:** Cortisone generated by 11β-HSD2 activity. **C-D:** cortisol metabolites in the human islets treated with 500 nM of cortisone for 24 hours, **C:** Cortisone levels in media- neat (no islets), (strip bar), in control non-treated islets (CTL), (white bars) and in treated islets treated (grey bars**). D:** Cortisol re-generated by 11β-HSD1 activity, Experiments were carried out at least in biological duplicate and in *n* = 6 donors for 500 nM of HC treatment, and *n* = 4 donors for 500 nM of cortisone treatment (donors ID: H1192, H1128, H1184, H1142, H1152, H1154).

3.4 Discussion

Using RNAscope in situ hybridization, we have shown here that *HSD11B1* and *SRD5A1* are predominantly expressed in β-cells, with rare expression in α-cells and exocrine cells in islets from lean donors. In contrast, *HSD11B2* showed an inverse distribution pattern, with primary expression in exocrine cells and low or absent expression in β-cells and α-cells. RNAscope in situ hybridization is a highly specific technique for detecting the cellular localization of the RNA transcripts. This advanced methodology provided reliable results without requiring validation through knockout models to confirm staining specificity (Atout et al., 2022). However, as RNAscope detects mRNA expression, these findings may not directly reflect protein levels due to post-transcriptional regulation, translation efficiency, and protein stability. Such discrepancies between *HSD11B1* mRNA and 11β-HSD1 protein expression have been previously documented. Notably, in critically ill patients in intensive care units, despite an 80% decrease in *HSD11B1* mRNA expression in liver and adipose tissue, protein levels and enzyme activity remained unchanged (Boonen et al., 2013). Moreover, these results should be interpreted with caution as the number of donors studied remained relatively low (3 donors for each metabolic phenotype).

Our results are consistent with Schmid et al. who demonstrated, using immunohistochemical staining, that 11β-HSD1 protein localized in β-cells in rat INS-1 cells, primary rat islets, and primary human islets (Schmid et al., 2011). In contrast, Swali et al., employing a different antibody, found that in C57BL/6 mice and human pancreatic tissue, *HSD11B1* was predominantly localized in the islet periphery, specifically co-localizing with glucagon and pancreatic polypeptide cells, but not with insulin-producing or somatostatin-producing cells (Swali et al., 2008). Supporting

Swali's findings, Chowdhury also observed protein expression in α-cells but not in β-cells in C57BL/6 mice (Chowdhury et al., 2015). The discrepancies in these previous results may be attributed to variations in antibody specificity, sensitivity, and potential cross-reactivity, as well as differences in species, tissue preparation methods, and detection techniques used across studies. Regarding SRD5A1, our data is consistent with previous findings from immunochemistry studies (Xu et al., 2020a).

High expression of *HSD11B1* has been reported in liver and adipose tissue. RNA sequencing analyses of normal mouse tissue have revealed that *Hsd11b1* mRNA levels were extremely low in FACS-purified β and α-cells compared to other tissues (Pullen et al., 2017). Similarly, single-cell transcriptome profiling demonstrated low or absent *HSD11B1* expression levels in both human β- and α-cells (Kang et al., 2023; Segerstolpe et al., 2016). However, single-cell sequencing techniques may underestimate the expression of low-abundance transcripts due to dropout effects and technical limitations. Single-cell data suggests higher expression of *SRD5A1* compared to *HSD11B1* in β-cells (Kang et al., 2023; Segerstolpe et al., 2016). Available data suggests also the expression of *SRD5A1* is higher in β compared to α-cells (Kang et al., 2023; Segerstolpe et al., 2016).

11β-HSD1 is a bidirectional enzyme that can function as both a reductase (converting inactive 11-DHC and cortisone to active corticosterone and cortisol in mice and human respectively) and dehydrogenase (inactivating corticosterone/cortisol to 11-DHC/cortisone), although it predominantly acts as a reductase in vivo. 11β-HSD1 reductase activity has been demonstrated in vitro across various models including primary islets from ob/ob mice (Davani et al., 2000), ZDF rat islets (Duplomb et al., 2004), pancreatic explant tissue from C57BL/6 mice (Swali et al., 2008), and rat INS-

1 cells (Schmid et al., 2011). Notably, even low-dose 11-DHC treatment inhibits GSIS in ob/ob mice islets, an effect almost completely reversed by the 11β-HSD1 inhibitor carbenoxolone (Davani et al., 2000) and by GR antagonist (Ortsater et al., 2005). In contrast, low-dose 11-DHC had no effect on GSIS in C57BL/6 mice islets (Ortsater et al., 2005), possibly due to their twofold lower 11β-HSD1 protein expression compared to ob/ob mice islets. In primary human islets, we demonstrated reductase activity using 500nM cortisone treatment, although this concentration exceeds physiological levels used in previous studies, warranting further validation with 50nM corticosterone. We also observed dehydrogenase activity using 500nM cortisol, consistent with previous findings of low-level dehydrogenase activity in isolated murine islets (Davani et al., 2000; Swali et al., 2008). However, these results should be interpreted with caution given that islet preparations are never completely pure, varying between 70-95% in our preparations, and the observed dehydrogenase activity might be partially attributed to HSD11B2 expressed in contaminating exocrine cells.

We show here that SRD5A1 is the only GCs A-ring reductase expressed in islets and the higher expression of SRD5A1 compared to *HSD11B1* suggests that SRD5A1 activity may play a more significant role than 11β-HSD1 in regulating cortisol bioavailability within islets. Surprisingly, we could not detect the generation of 5α-THF metabolites in the medium after cortisol treatment. We hypothesize this could be due to limited 3α-hydroxysteroid dehydrogenase activity, despite confirming the expression of *AKR1C2*, *AKR1C3*, and *AKR1C4* in islets (data not shown). Although steroids hormones are traditionally thought to diffuse passively across membranes, recent reports suggest involvement of transporters such as ABCC1 and ABCB1 in brain and adipose tissue (Kyle et al., 2022). Therefore, we hypothesize that GCs metabolites may be retained within the islets. We finally detected inside the islets but not in the

supernatant the metabolites 5α-DHF, showing also evidence of SRD5A1 activity in reducing cortisol. This result requires confirmation with more islets preparation and using more physiological cortisol concentrations.

The endogenous SRD5A1 activity seems to be extremely low to be relevant but testosterone has been shown to be a better substrate than cortisol (Nixon et al., 2012). When comparing previous findings where 100 nmol/L testosterone treatment in primary human islets yielded dihydrotestosterone concentrations approximately 40 times lower than the substrate (Xu et al., 2020a), our observed 5α-DHF concentrations being 20 times lower than cortisol suggests that the activity is low within islets for both substrates. However, while this activity level was sufficient to impact GSIS, as dihydrotestosterone is the active hormone (Xu et al., 2020a), the conversion of cortisol to inactive 5α-reduced compounds may not be strong enough to be physiologically relevant. Further investigation using radiolabeled substrates in both isolated islets and purified β-cells would be valuable to precisely characterize SRD5A1 activity.

For the first time, we assessed islets expression of *HSD11B1* in metabolic diseases. We observed decreased expression of *HSD11B1* in both obese versus non-obese and diabetic versus non-diabetic subjects, while *SRD5A1* expression was reduced only in diabetic subjects. The reduction in *HSD11B1* expression contrasts with previous findings in ZDF diabetic rats, where increased islet *HSD11B1* expression compared to lean wild-type rats was observed independently of leptin signaling deficiency (Duplomb et al., 2004). The decreased *HSD11B1* expression in islets of diabetic individuals may serve as a protective mechanism against excessive GC activation. However, while global *11β-HSD1* knockout mice show resistance to diet-induced obesity and hyperglycemia (Kotelevtsev et al., 1997), the role of 11β-HSD1 specifically in β-cells is more nuanced. Moderate overexpression of 11β-HSD1 in β-cells has been

shown to enhance insulin secretory function and protect against high-fat diet-induced failure and inflammatory damage (Liu et al., 2014a; Turban et al., 2012a). This suggests that an optimal level of 11β-HSD1 activity in islets is crucial for maintaining proper β-cell function and insulin secretion. Finally, decreased *HSD11B1* does not preclude that 11β-HSD1 activity would be decreased. Tracer infusion studies in patients have previously demonstrated that alterations in *HSD11B1* expression in obesity or diabetes, particularly in the liver, do not necessarily correlate with changes in enzymatic activity (Anderson & Walker, 2013). Therefore, we cannot definitively conclude whether 11β-HSD1 inhibitors would directly impact β-cell function within islets in the context of GC treatment or metabolic diseases (Anderson & Walker, 2013; Morgan et al., 2014).

Regarding SRD5A1, while its tissue-specific expression has not been extensively studied in obesity, previous research found no differences in subcutaneous adipose tissue *SRD5A1* expression between patients with and without glucose intolerance or diabetes (J. W. Tomlinson et al., 2008). Our observation of decreased *SRD5A1* expression in islets from diabetic subjects, combined with previous data showing that *SRD5A1* inhibition induces glucose intolerance and diabetes (Hazlehurst et al., 2016; Upreti et al., 2014; Wei et al., 2019), suggests that this reduced expression might contribute to diabetes pathogenesis.

In conclusion, we confirmed the expression of *HSD11B1* and *SRD5A1* in islets, with predominant localization in β-cells. We provided evidence for intracellular cortisol metabolism within islets, suggesting that intracrine GCs metabolism might modulate their effect on β-cells function. While the physiological relevance of this relatively low enzymatic activity remains to be fully established, the decreased expression of both

enzymes in islets from diabetic individuals points to their potential role in diabetes pathogenesis.

CHAPTER 4

SRD5A1 overexpression in islets mitigates GCs inhibition of GSIS

SRD5A1 OVEREXPRESSION IN ISLETS MITIGATES GCS INHIBITION OF GSIS

4.1 Introduction

GCs regulate immune response but also significantly impact glucose metabolism. Excessive exposure to GCs, as observed in patients with endogenously elevated cortisol or patients treated by systemic GC therapy, can lead to glucose intolerance and/or diabetes (C. Beaupere, A. Liboz, B. Fève, et al., 2021; Feve & Scheen, 2022). If insulin resistance is one of the main mechanism of GCs-induced diabetes (Alex Rafacho et al., 2014), using human primary β-cell, we previously demonstrated that even low dose GCs, equivalent to the plasmatic peak after oral intakes of 5 to 10 mg prednisolone or 20 to 40 mg of hydrocortisone decrease GSIS (**Chapter 2**).

Existing data on SRD5A1 inhibition supports its role in regulating cortisol bioavailability and glucose homeostasis in the context of obesity or GCs therapy. *Srd5a1* inactivation in mice exposed to high fat diet predisposes them to insulin resistance (Livingstone et al., 2015; Livingstone et al., 2017). A study of over 50,000 men revealed increased diabetes risk in patients treated with dutasteride, an SRD5A1 inhibitor used for the treatment of prostate hyperplasia (Wei et al., 2019). In healthy volunteers, dutasteride for 3 months reduced insulin sensitivity (Upreti et al., 2014) and co-administration with prednisolone for 7 days led to insulin resistance compared to prednisolone alone (Othonos et al., 2020). Interestingly, we showed that *SRD5A1* expression is decreased in islets from diabetic individuals compared to non-diabetic individuals (**Chapter 3**).

As SRD5A1 is the principal enzyme metabolizing glucocorticoids in islets, and considering the deleterious effects of SRD5A1 inhibition on glucose homeostasis, understanding its specific role in β-cells is crucial. Therefore, this last part of this thesis aimed to investigate the impact of SRD5A1 overexpression on islet β-cell function.

Materials and Methods

4.2.1 Human Islet Isolation

Confer **Chapter 2**. The information on the biological and clinical characteristics of the donors used in this thesis is listed in **Supplementary Table 1.**

4.2.2 In vitro mRNA Transfection

Human islets were cultured in a 180 cm^2 flask using CMRL complete media before transfection. Islets (2500 IEQ/condition) were washed once with PBS and dissociated with acutase enzyme (PAA Laboratories, Cat No L11-007, Austria) for 2.20 minutes: 40 seconds at room temperature, 50 seconds in the water bath at 37°C, and 40 seconds at room temperature. The reaction was stopped by adding CMRL complete media. After washing once with PBS, dissociated islets were transferred to non-coated 6-well plates and resuspended in 1.752 mL of CMRL media (without additives +/- 1µM of HC or 250 nM of PRED). A separate transfection complex containing Lipofectamine Messenger MAX transfection reagent (Thermofisher Scientific, Cat No LMRNA003), and OptiMEM Reduced serum (Thermofisher Scientific, Cat No LMRNA003) was prepared using volume recommended by the manufacturer for a 6 wells plate. 0.6 µg of *SRD5A1* (see with manufacturer, VectorBuilder) or *EGFP* mRNA (Tebubio, Cat No 040L-7601-100) was then added to the transfection complex according to manufacturers' instruction. Islets in suspension was added into transfection complex mix to have per well: 2000 IEQ in 2ml total volume and 0.6 µg of mRNA. Islets were incubated in for 24hours. At the end of the experiment, islets were collected for protein and RNA extraction and for GSIS by dynamic perifusion. Islets supernatant and lysate were collected for UPLC-MC/MS analysis.

4.2.3 GCs Treatment

Human islets (300 islet equivalents) were washed twice in DPBS 1X (Gibco, Cat #14040141) and cultured in a 5.5 mM glucose media (Gibco, Life Technologies, Paris, France), supplemented with 0.625% human serum albumin (HAS), 100 U/ml penicillin-streptomycin (Gibco, Cat #15140148). The media contained either GCs (hydrocortisone [HC], prednisolone [PRED]), or 0.001% methanol/ethanol (control). Clinical oral PRED dosing ranges from 5-90 mg/day for acute treatment, while chronic therapy typically uses ≤7.5 mg/day, (Buttgereit et al., 2002), corresponding to approximately 300 to 700nM (Pickup, 1979a), (**Table 5**). To characterize cortisol metabolism in human islets, islets were treated with 1 µM of HC (Sigma-Aldrich, Cat # H0888) or 250 nM of PRED (Sigma-Aldrich, Cat # P6004), for 24hours, based on anti-inflammatory equipotent conversion factors (where 5 mg PRED equals 20 mg HC) (Buttgereit et al., 2002; Meikle & Tyler, 1977), (**Table 2**).

4.2.4 Protein Expression

Protein extraction

Human islets (1000 - 1500 IEQ) were harvested in 80 µL of lysis buffer containing 20 mmol/L Tris-Acetate, 0.27 mmol/Lsucrose, 1% Triton X-100, 1 mmol/L EDTA, 1 mM EGTA, 50 mmol/L Sodium Fluoride, and 10 mmol/L Beta glycerophosphate. Lysis buffers were supplemented with proteinase inhibitors (50 X) (Sigma-Aldrich, Cat No P8340) and phosphatase inhibitors (10X) (PhosSTOP, Roche Cat No 4906845001). Cells were sonicated for 3 sec and islets were sonicated for 4 minutes with an ultrasonic water bath (Lab companion, UCP-02). After sonication samples were left on ice for 20 min and centrifuged at 12,000 rpm for 20 minutes at 4°C to remove insoluble material.

Supernatants were transferred to clean Eppendorf tubes to measure total protein concentration.

Protein quantification

Protein concentration was determined using the Pierce BCA protein assay kit (Thermo Fisher Scientific, Ca No 23225, USA). 1 mg/mL was used to prepare the standard curve as detailed in (**Table 11**). Cells and islets were diluted 1/10 in distillate water. Samples diluted and BSA standard curve (10 µl/sample) were pipet per duplicate in a non-treated 96-well plate. The reagent B was diluted with the reagent A of the Pierce BCA kit in a 1/50 ratio, and 200 µL of mixture was added per well. The plate was incubated at 37°C for 30 minutes and read at 562 nm in the spectrophotometer (Multiskan GO, Thermo Scientific). Concentrations were determined using the standard curve.

Table 11: BSA standard curve for BCA protein measurement

Concentration (mg/mL)	Distilled water (µL)	BSA 1 mg/mL (µL)
0	30	**0**
0.1	**27**	**3**
0.2	**24**	**6**
0.4	18	**12**
0.6	**12**	**18**
0.8	**6**	**24**
1	**0**	**30**

Western Blot

Total protein (20 µg) was mixed with 4x Laemmli sample buffer (Alfa Aesar, Cat No J60015, Germany), denatured at 95°C for 5 minutes in the thermocycler (Veriti, Thermal cycler Applied Biosystems), and separated with 4% - 12% SDS-PAGE (Invitrogen, Cat No NW04122BOX, USA) for high molecular weight proteins or 20% BIS-TRIS homemade gel (**Table 12**) for low molecular weight proteins. Running was performed using the Novex Bolt Mini Gel Tank. The electrophoresis tank was prepared with the cassette clamp. Chambers were filled with 400 mL of running buffer (MES 20X: 380 mL of water + 20 mL of MES) (Thermo Scientific, Cat No J62138, Germany). After loading the samples and molecular weight (Invitrogen, Cat No LC592, USA), the migration of protein was performed for 50 minutes at 165 V. Proteins were transferred to nitrocellulose membranes (Thermo Scientific, Cat No IB23002, USA) using the iBlot2 gel transfer device (Thermo Scientific, Cat No IB21001, Israel). Proteins were transferred for 6 minutes 25 V. Then, nitrocellulose membranes were blocked in 5% BSA-TBST or milk-TBST for 1 hour at room temperature in shaking. After two washes of 5 minutes with TBST, membranes were incubated overnight in shaking with primary antibodies at 4°C (Antibodies details and dilution concentrations are detailed in (**Table 8**). After washing three times for 15 minutes each with TBST, membranes were incubated for 1 hour with anti-mouse secondary antibodies diluted 1:10000 in 5% BSA TBST or 5% milk-TBST (**Table 9**). After washing three times for 15 minutes each with TBST, membranes were developed with 1 mL of ECL Plus according to the manufacturer's instructions (GE Healthcare Life Science, Cat No RPN2236, Italy). Digital images were taken and analyzed with the Amersham 600 system. Anti-beta actin antibodies were used as a loading control (**Table 8**).

Table 12: Recipe for 4% stacking and 20 % resolving gel

Reagent	Stacking gel (4%)	Running gel (20%)
30% Acrylamide/Bis	0.66 mL	4.4 mL
0.5 M Tris-HCl pH 6.8	1.26 mL	-
1.5 M Tris-HCl pH 8.8	-	2.5 mL
10% SDS	50 µL	100 µL
dH2O	3 mL	2.9 mL
TEMED	5 µL	5 µL
10% APS	25 µL	50 µL

4.2.5 Cortisol Metabolite Measurement by HPLC-MS/MS

Cortisol and its metabolites were measured in the supernatant and lysate using HPLC-MS/MS. Islets were lysed in 250 µl of cell lytic (Sigma-Aldrich, Lot# 018M4125V) supplemented with anti-protease (1 on 50 dilution; PhosSTOP, Roche) and anti-phosphatase (1 on10 dilution; Sigma-Aldrich Product #P8340). First, we check for the possible matrix effect (Mikkaichi et al., 2004) in the islets supernatant and lysate, and was confirmed by insignificance to interfere with steroid measurement. Samples were prepared by adding 100 µl of an internal standard (IS) of known concentration of deuterated steroids (Fd4 for cortisol, ED8 for cortisone, THF-d5 for THF, THF, a-DHF and DHF, THE-D5 for THE, DHE, a-DHE) in 500 µl of cell substrate or in the whole volume of cell lysate. We produced a linear curve using an increased concentration range of standard steroids used for quantification of steroids. The culture medium associated to the IS were purified by extractions in C18 columns and filtered under nitrogen pressure using a Positive Pressure Manifold 96 Processor (PPM-96, Agilent, USA). In total, 2 extractions were performed separated by enzymatic hydrolysis with

β-gluconidase overnight. Samples were evaporated under nitrogen at 40°C after each extraction. The samples were resolubilized in assay mobile phase (55% of water, 45% of Methanol), loaded into vials and analyzed by UHPLC-MS/MS (Waters, ACQUITY UPLC H-Class PLUS, XEVO TQD, USA) in negative mode of ionization. Limit of detection were quantified for each metabolite: F; 0.55 nM, E; 0.42 nM, DHF and THF; 7.9 nM, THE; 6.03 nM, 5α-DHF and THF; 3.3 nM. As existing protocol for the measurement of dihydrocortisol (a-DHF and DHF), and dihydrocortisone (a-DHE and DHE) did not exist in the lab, we set up and optimized separation conditions for these compounds with the HPLC – MS/MS program (**Table 11**), (**Figure 11**). Experiments was performed for each donor in duplicate.

4.2.6 Glucose Stimulated Secretion Assessment (GSIS) Assessment Using Dynamic Islet Perifusion

Confer **Chapter 2**

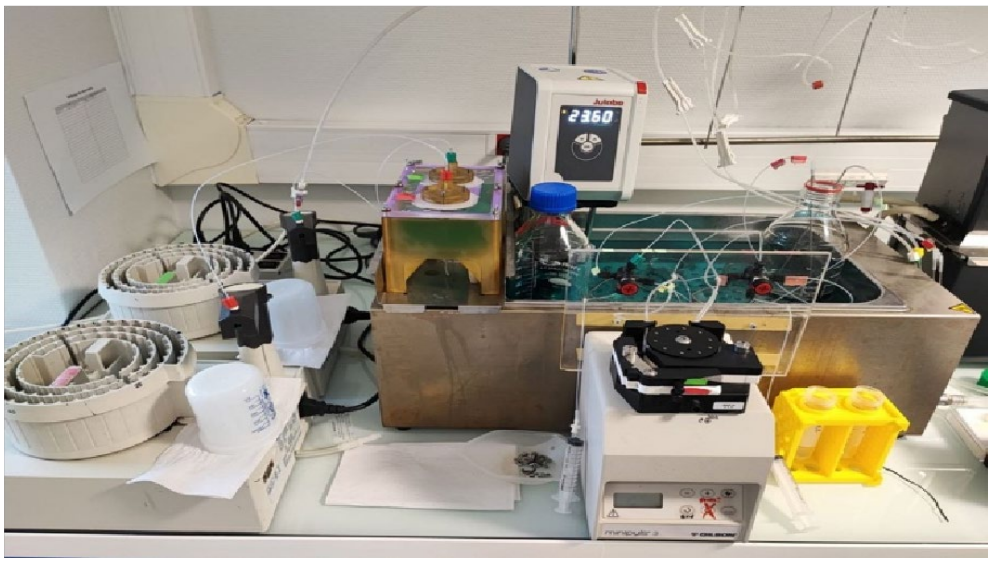

Image 1: Dynamic perifusion system

4.2.7 Statistical Analysis

Statistical analyses were performed with GraphPad Prism 10.2.0 (GraphPad Software, La Jolla, California, USA). Data are expressed as mean ± SEM. Comparison between control and GC treatment was performed using Wilcoxon paired and non-parametric t-tests. Comparison between groups was performed using Wilcoxon unpaired and non-parametric t-tests. Comparison between the different doses of PRED and GCs at equipotent dose were performed using one-way ANOVA with Sidak's multiple comparison *post hoc* tests, and two-way ANOVA and nonparametric tests with Sidak's *post hoc* tests for multiple comparisons where statistically applicable. Differences were considered significant at $p < 0.05$. Significance was expressed as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ and **** $p < 0.0001$.

Results

4.3.1 SRD5A1 overexpression increased 5 α-reduced metabolites

SRD5A1 overexpression was performed using *SRD5A1* mRNA transfection while EGFP transfection was used a control. We confirmed successful protein overexpression by western blot analysis (**Figure 25 Ai-ii**). The study was conducted using human primary islets treated for 24h by HC 1 µM. Metabolites were measured in medium and in the intracellular contents using UHPLCMS/MS analysis. Cortisol (**Figure 25B**) and cortisone (**Figure 25C**) concentration in the medium and in the intracellular content were similar between *SRD5A1* and *EGFP* transfected islets. Presence of cortisone in the media is indicative of dehydrogenase activity within the islets as previously described. We observed a significant increase in 5α-DHF, and in its secondary metabolites 5α-THF in the intracellular content of *SRD5A1* transfected islets compared to control (5α-DHF: 79.9 nM versus 37 nM, p=0.0028; 5α-THF: 20.4 nM versus 11.3 nM, p=0.0353) (**Figure 25C-D**). The level of 5α-DHE, indicative of 5α-reduction of cortisone or conversion of 5α-DHF to 5α-THF was also significantly higher in the intracellular content of SRD5A1-transfected islets compared to control. (5α-DHE: 52.1 nM versus 25.2nM, p=0.0001) (**Figure 25E**).

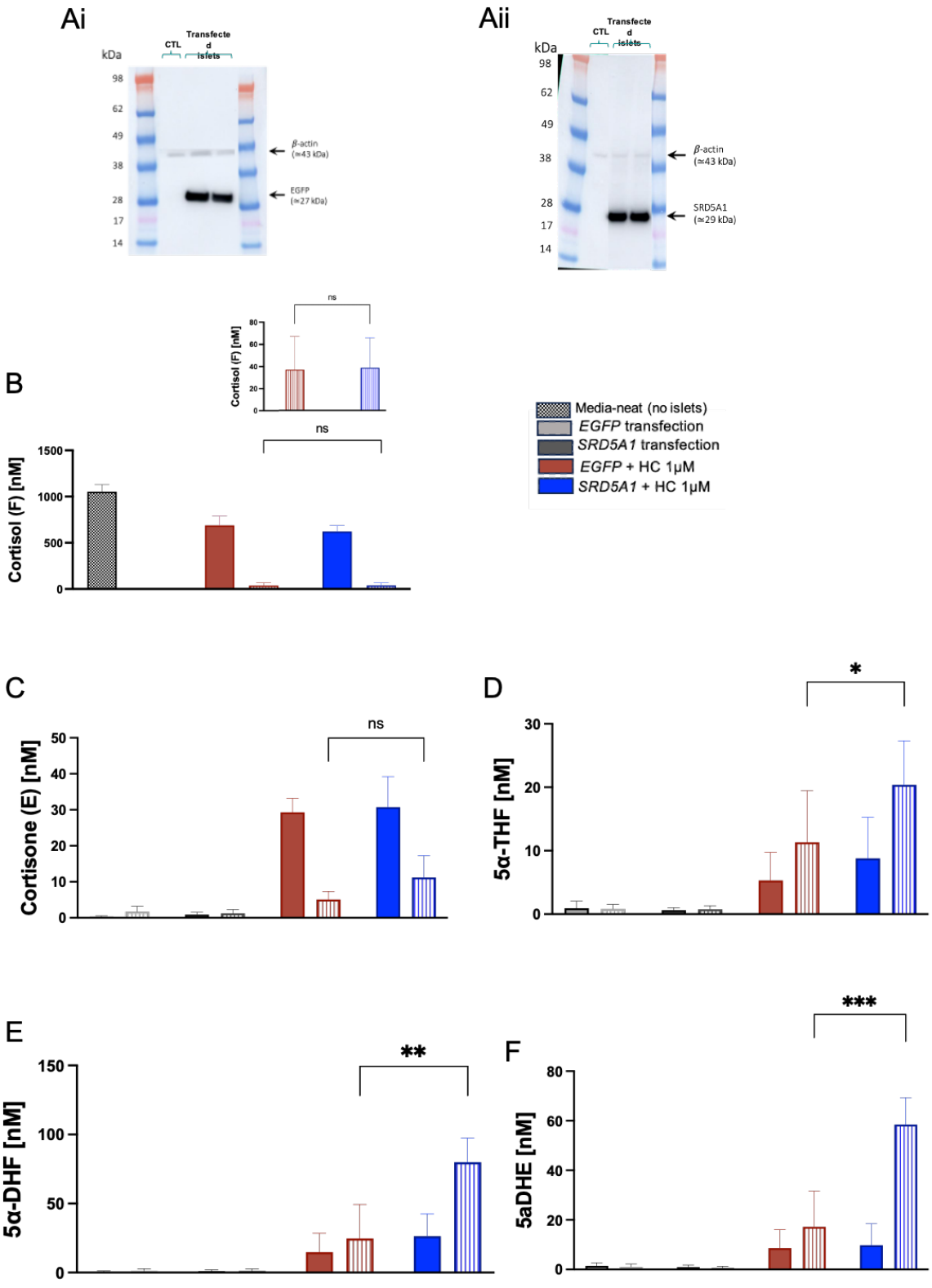

Figure 25: SRD5A1 overexpression increased cortisol degradation. Transfected islets with either *SRD5A1* or *EGFP* mRNA, were treated with 1 µM of HC for 24h. Islets supernatant (solid bars), and islet lysate (colored stripped bars), was collected for protein extraction, and

for metabolites measurement by LCMS/MS. **A:** Western Blot analysis revealing increased protein expression of (i) EGFP (band 2 and band 3), (ii) SRD5A1 (band 2 and band 3), compared to control non-transfected islets (first band in both WB revelations). Proteins were loaded at 20 µg and compared to β-actin protein expression**. B:** cortisol levels in media-neat (no islets) with which all experimental conditions were treated, (black stripped bar), levels in EGFP +HC islets (red bars) and in SRD5A1 + HC treated islets (blue bars). **C:** Cortisone levels generated by 11β-HSD2 activity. **D:** 5α-THF, **E**: 5α-DHF, **F**: 5α-DHE levels. Experiments were carried out in *n*= 4 donors. Statiscal analysis was carried out using Two-way ANOVA and Sidak's multiple *post hoc* tests. *** p<0.001, **p<0.01, *p<0.05.

4.3.2 SRD5A1 overexpression in human islets decreased HC impact on GSIS

In *EGFP*-transfected islets, HC (1 µM, 24h) decreased the first phase, second phase, and global AUC of GSIS (first phase, p=0.0004; second phase, p=0.0152; AUC, p=0.0285). The stimulation index remained unchanged between HC-treated and untreated EGFP islets (**Figure 26A-D**). In SRD5A1-transfected islets, HC treatment (1 µM, 24h) did not alter GSIS parameters (**Figure 26D-E**). Insulin content and stimulation index were comparable across all experimental conditions. The HC-induced reduction in GSIS AUC was more pronounced in *EGFP* versus *SRD5A1*-transfected islets and for all parameters measured (global AUC, first and second phases of insulin secretion), with the protective effect of SRD5A1 reaching statistical significance (% variation in global AUC, p=0.007; % variation in AUC of first phase, p<0.0001; % variation in AUC of second phase, p= 0.0189), (**Figure 26F-G**).

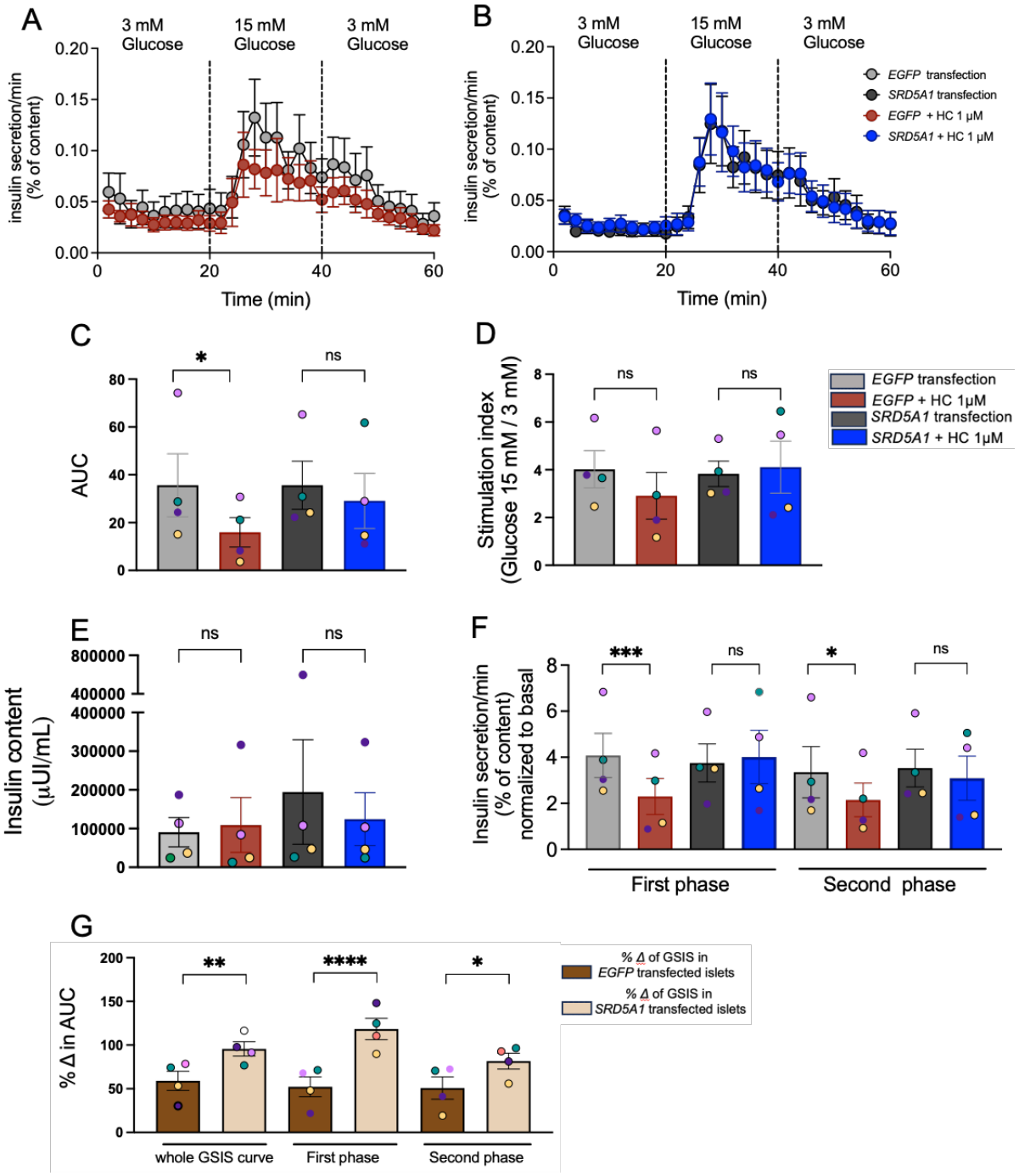

Figure 26: SRD5A1 overexpression in human islets decreases HC impact on GSIS. Glucose-stimulated insulin secretion assessed by dynamic perifusion in human islet transfected with EGFP mRNA at 0.6µg alone (light grey bar) or co-treated with 1 µM of HC for 24h (red bar), and in human islets transfected with SRD5A1 mRNA at 0.6µg alone (dark grey bar) or co-treated with 1 µM of HC (blue bar) for 24h (n=4 donors). **A:** GSIS profile in islets from one representative

donor transfected with *EGFP* mRNA +/- HC treatment and perfused with 3 mmol/L, and 15 mmol/L glucose concentrations. Insulin secretion was normalized to % of insulin content. **B:** GSIS evaluation in the same donor islets as (B), but transfected with *SRD5A1* mRNA +/- HC treatment **C:** Global GSIS AUC. **D:** Stimulation index calculated as 20 minutes 15 mM glucose stimulation / initial low 3 mM glucose stimulation. **E:** Insulin content in human islets at the end of dynamic perifusion experiments. **F:** First and second phases of insulin secretion normalized to baseline. The first and second phases of insulin secretion were calculated as the first 10 minutes and the last 10 minutes of 15 mmol/L glucose stimulation, respectively and divided by the mean of insulin during the first 3 mM period. **G:** % variation in AUC (whole AUC, first phase and second phase of insulin secretion) in islets treated with HC compared to non-treated islets. Comparison was performed using one-way ANOVA and Friedman non-parametric multiple comparison *post hoc* test for panel C-E. Two-way ANOVA and Sidak's multiple comparison *post hoc* test was carried out for panel F-G. ns: nonsignificant; ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05

4.3.3 SRD5A1 overexpression in human islets tend to decrease PRED impact on GSIS

After 24h of transfection and co-treatment, we observed a mild decrease in insulin secretion in islets transfected with *EGFP* and treated with PRED (250 nM for 24h) (**Figure 27A**) while PRED did not impact GSIS in the *SRD5A1*-transfected islets from these donors (**Figure 27B**). However, when analyzing all the islets preparations, PRED did not significantly affect the first phase, second phase, global AUC of GSIS or the stimulation index in *EGFP*-transfected islets compared to untreated controls. Similarly, in *SRD5A1*-transfected islets, PRED treatment did not alter GSIS parameters (**Figure 27B-D**). Nontreated *SRD5A1* transfected islets had a significantly higher insulin content compared to other experimental conditions (**Figure 27E**). Finally, the PRED-induced changes in GSIS AUC were not significantly different between EGFP and SRD5A1-transfected islets across all parameters measured (global, first and second phases) (**Figure 27F-G**). The absence of PRED effect on

GSIS in EGFP-transfected islets precluded the possibility of detecting any protective effect of SRD5A1 against PRED action, in contrast to what was observed with HC treatment. Interestingly, since transfection procedure altered baseline GSIS compared to non-transfected islets (Supplementary Figure 12), this methodological constraint might have interfered with PRED action. The pre-existing alteration of the secretory response due to transfection could have limited our ability to detect the previously reported effects of PRED on GSIS observed in non-transfected islets.

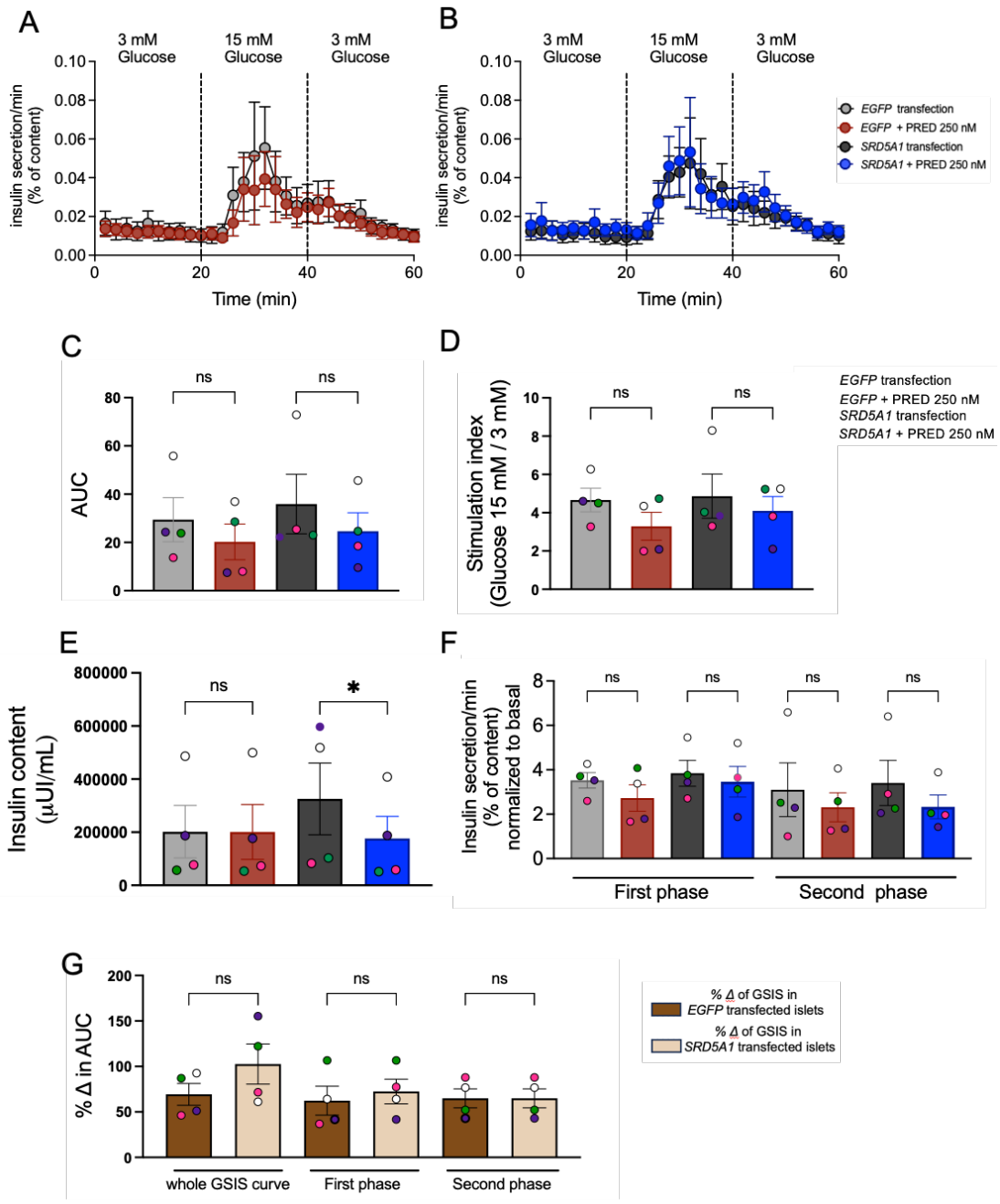

Figure 27: SRD5A1 overexpression show tendency to inhibit the impact of PRED on human islets. Glucose-stimulated insulin secretion assessed by dynamic perifusion in human islet transfected with EGFP mRNA at 0.6µg alone (light grey bar) or co-treated simultaneously with PRED (red bar), and in human islets transfected with SRD5A1 mRNA at 0.6µg alone (dark grey bar) or co-treated simultaneously with PRED (blue bar) for 24h. **A:** GSIS evaluation in islets transfected with *EGFP* mRNA +/- PRED treatment and perfused with 3 mmol/L, and 15 mmol/L glucose concentrations, and insulin secretion was normalized to % of insulin content (n = 4). **B:** GSIS evaluation in the same donor islets as (A), and transfected with *SRD5A1* mRNA +/- PRED

treatment and perfused with 3 mmol/L, and 15 mmol/L glucose concentrations, and insulin secretion was normalized to % of insulin content (*n* = 4). **C:** Comparison between all four conditions' AUC. **D:** Insulin content in human islets at the end of dynamic perifusion experiments. **E:** Comparison between stimulation index in all experimental conditions. **F:** comparison between the first and second phases of insulin secretion. The first and second phases of insulin secretion were calculated as the first 10 minutes and the last 10 minutes of 15 mmol/L glucose stimulation, respectively. **G:** % variation in AUC (whole AUC of the GSIS curve, AUC of the first phase of insulin secretion, and AUC of the second phase of insulin secretion. Comparison was performed using one-way ANOVA and Friedman non-parametric multiple comparison *post hoc* test for panel **C-E.** Two-way ANOVA and Sidak's multiple comparison *post hoc* test was carried out for panel **F-G**. ns: nonsignificant; *p<0.05.

4.4 Discussion

In this study, we demonstrated that SRD5A1 protein overexpression attenuates the negative effects of HC on GSIS, while the potential protective effect of SRD5A1 against PRED action could not be assessed due to the lack of clear PRED effect on GSIS in transfected islets. The lack of statistical power may have also limited our ability to detect subtle effects of PRED on GSIS. Notably, our previous work (**Chapter 3**) suggested a more pronounced impact of HC compared to PRED on GSIS, although this difference did not reach statistical significance. The present results further support the observation that HC may exert stronger inhibitory effects on GSIS compared to PRED.

The protective effect of SRD5A1 against HC-induced GSIS impairment is likely mediated through enhanced cortisol degradation, as evidenced by increased intracellular levels of 5α-reduced cortisol metabolites. Planned perform RNA sequencing analysis will provide crucial insights into the modulation of GR-target genes, as well as pathways related to apoptosis and inflammation. This will further support that the observed effects are due to modulation of GC action on the GR, rather than effects on other pathways. Studies of inflammation pathways are particularly relevant given that inflammation contributes to β-cell dysfunction in diabetes (Eguchi & Nagai, 2017). β-cells produce both cytokines and chemokines (Burke et al., 2014b; Collier et al., 2021; Lund et al., 2008; Maedler et al., 2002; Piemonti et al., 2002) and their receptors (Böni-Schnetzler et al., 2018), which are important for their function. GCs exposure can improve β-cell function, likely by suppressing inflammatory signaling (Hult, Ortsäter, et al., 2009; Lund et al., 2008). Moreover, local GC regeneration within β-cells has been shown to protect against inflammatory β-cell

destruction in transgenic mice overexpressing 11β-HSD1 exposed to high-fat diet (Turban et al., 2012b) or streptozotocin (Liu et al., 2014b). Interestingly, the 5α-metabolites of GCs produced by SRD5A1 maintain anti-inflammatory characteristics while lacking significant metabolic properties (Yang et al., 2011), potentially offering a more favorable balance between the beneficial and detrimental effects of GCs on β-cell function.

Our findings support the concept of intracrine regulation of GC availability within β-cells. The detection of SRD5A1 metabolites in the intracellular content, but not in the supernatant, suggests that GC bioavailability is modulated directly inside the cells. This local regulation mechanism may be particularly relevant in metabolic conditions such as obesity, where tissue-specific GC exposure may differ from systemic levels (Anderson et al., 2021; Dube et al., 2014). This observation aligns with previous studies demonstrating the protective effects of local GC metabolism in various tissues, including adipose tissue and liver. For instance, tissue specific 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) knockout mice have been shown to be protected from the adverse metabolic effects of GC excess, emphasizing the critical role of tissue-specific GC activation in the development of metabolic disorders (Chapman et al., 2013).

Several experimental limitations should be considered. First, the transfection of both *SRD5A1* and control *EGFP* mRNA affected β-cell function, as evidenced by the altered GSIS profiles and stimulation index compared to non-transfected cells. Consequently, the impact of modulating cortisol bioavailability may not perfectly reflect the situation in intact islets. While we employed “low therapeutic” GC doses typical of clinical therapy, these do not represent local exposure at physiological state or in conditions

such as obesity. Future studies with lower cortisol concentrations (50nM) could provide insights into physiological regulation, but the effects on GSIS could be too subtle to detect.

Conversly, higher GCs doses may have produced more pronounced effects on GSIS, potentially yielding clearer results, but high dose might also saturate the enzymatic capacity of *SRD5A1*. The very high overexpression achieved in our system may not necessarily translate to a proportional increase in enzyme activity. This could be due to several factors, including potential limitations in essential cofactors such as NADPH, or issues with protein folding and conformation at high expression levels. Additionally, cellular machinery for post-translational modifications and proper subcellular localization may become saturated, further impacting the functional activity of the overexpressed enzyme. Interestingly, during the optimization of *SRD5A1* transfection, we observed using higher amount of mRNA (1.5 ug) that islets transfected with *SRD5A1* and subsequently treated with GCs exhibited a dramatic decrease in insulin content. In contrast, islets transfected with *SRD5A1* but not treated with GCs, as well as control islets transfected with *EGFP* (both treated and untreated with GCs), maintained similar insulin content levels (data not shown), this might be explained by the induction of endoplasmic reticulum (ER) stress or metabolic substrate shift, potentially diverting cellular resources away from insulin production. This strong overexpression also represents a major limitation of the study, as it creates highly artificial conditions that may not be replicable in a therapeutic context. Indeed, potential SRD5A1 activator would likely achieve more modest increases in activity. Furthermore, this extreme overexpression does not accurately model the physiological restoration of normal SRD5A1 levels as observed in lean individuals compared to the reduced expression seen in diabetic subjects.

Despite these limitations, our study demonstrates that intracrine cortisol metabolism modulates GCs action within β-cells. We provide proof of concept that cortisol degradation by SRD5A1 within cells enhances β-cell function. This highlights intracellular cortisol metabolism as a potential therapeutic target to support β-cell functionality under GC exposure.

CHAPTER 5

General Discussion

GENERAL DISCUSSION

This PhD research established the foundational work required to investigate SRD5A1's role in β-cell function. We demonstrated that moderate GCs exposure impairs GSIS. Subsequently, we confirmed the expression of both *HSD11B1* and *SRD5A1* in β-cells. Our results indicated that SRD5A1 overexpression enhanced cortisol degradation, thereby attenuating GC-mediated inhibition of GSIS.

5.1 Strength of the work

In the first part, we used human islets and perifusion methods for the first time to demonstrate that, *in vitro,* PRED, even at low doses typically administered in chronic oral GC therapy, directly impact insulin release in response to glucose stimulation. We compared PRED, DEX, and HC at anti-inflammatory equipotent doses and showed that DEX has a more pronounced impact on insulin release than PRED and HC. Furthermore, we present original data showing that BMI, age, or sex do not strongly influence the direct impact of PRED.

In the second part, we confirmed the expression of *HSD11B1* and *SRD5A1* mRNA within the β-cells of normoglycemic human islets. We demonstrated for the first time in intact primary human islets cultured under dynamic experimental settings, the evidence of intracrine metabolism of cortisol. We also provide original data showing that *HSD11B1* expression is decreased in islets in obese or diabetic subjects, contrary to what had been previously hypothesized. Moreover, our results position SRD5A1 as a potentially regulator of cortisol bioavailability within islets compared to 11β-HSD1, despite documented limitations in studying its activity in vitro. Indeed, characterization

of its protein structure has been challenging due to an instability of the protein during its expression and the purification (Han et al., 2021). Despite its good expression in the liver, we (data not shown), and other were unable to study its activity in intact primary human hepatocytes culture, primary rat hepatocytes or in immortalized cells line (Han et al., 2021). A previous team tested as well in other tissue as in rat adipocytes, prostrate cells treated with corticosterone, HepG2 cells, 2S-FAZA cells, fresh liver homogenate, frozen liver homogenate, microsomes, cytosol, nuclear fraction, mitochondria, H4IIE cells, adrenal, and kidney.

In the last part, we provide proof of concept that the modulation of cortisol bioavailability by SRD5A1 mitigates the inhibitory effect of GCs on GSIS. We demonstrated this using the synthetic form of the endogenous GC, HC, while the effect was less clear with PRED. This complements previous work showing that SRD5A1 inhibition in humans treated with PRED and dutasteride worsens the impact of GCs on glucose homeostasis (Othonos et al., 2020).

5.2 Limitation of the work

i. β-cell function is not restricted to insulin secretion

Our research presents some limitations regarding mechanistic insights, particularly in the first part. Our study primarily focused on GSIS, which, while crucial, does not encompass all aspects of β-cell function. Additional parameters such as β-cell viability, proliferation, and other secretory functions were not investigated in this work, limiting our understanding of the full impact of GCs and SRD5A1 on β-cell function. Regarding the decrease in GSIS observed with low therapeutic dose of GCs, we did not provide mechanistic insight as it was not the objective of the project. We hypothesize that using

lower doses would not engage different mechanisms or molecular pathways than those already well-characterized. Transcriptomic studies from the part 3 of the work (in transfected islets), we still provide some mechanistic data.

Our experimental design using 24-hour GC exposure may not fully represent the effects of chronic GC therapy. This timeframe was selected to examine initial PRED effects at therapeutic doses. Future studies investigating chronic effects of prolonged GC exposure on β-cell function would require extended culture periods and potential *in vitro* model adaptations.

The use of an immortalized cell line, such as Human EndoC-βH1, would allow for the impact of longer durations of GC treatment. With less restrictive access, it permits obtaining more material to study additional aspects of β-cell function, such as viability. Finally, transcriptomic analysis also planned for the third part will allow the study of the impact of GCs and SRD5A1 overexpression on the expression of genes involved in apoptosis and inflammation.

v.

ii. GCs also impact α-cells function

It is generally accepted that the primary function of the pancreatic α-cells under homeostatic condition is to produce and secrete the hormone glucagon. Glucagon plays a key role in maintaining glucose homeostasis primarily by stimulating hepatic glucose production and glycogenolysis, counteracting the action of insulin. Its secretion increases in response to hypoglycemia and decreases under hyperglycemic conditions. (Quesada et al., 2008). While glucagon was traditionally thought to function primarily in opposition to insulin, recent research has revealed its importance in GSIS within intact islets (Leibiger et al., 2012).

The effect of GCs on the α-cells has been conflicting. The specific effect of GCs on glucagon release remains understudied owing largely to several complexities inherent in studying this relationship with results varying across species and with experimentation methods. In a study on patients with Cushing's syndrome and non-obese and obese humans treated by 2 mg/day of DEX for 3 days, glucagon secretion was increased compared to non-treated patients, with a more pronounced increase in Cushing's syndrome patients (Wise et al., 1973). Similarly, pre-treatment in mice with PRED increased glucagon release from mice islets (Marco et al., 1976). Contrary to these in vivo data, rat islets cultured with 10 nM of DEX showed approximately 50% decrease in glucagon receptor (Abrahamsen & Nishimura, 1995). In mice islets cultured in PRED media (50 -100 μM), PRED failed to increase glucagon levels (Abrahamsen & Nishimura, 1995). Swali et al, 2008 showed that DEX dose-dependently decrease glucagon secretion in human and mice islets, and glucagon levels were restored when co-treated with GR antagonist, mifepristone (Swali et al., 2008). While we recognize the importance of understanding GCs effects on glucagon release, given these contradictory data, we anticipated a high risk of obtaining inconclusive results that would be challenging to interpret. In addition, the low expression of 11β-HSD1 and SR5DA1 in α-cells suggests that variations in cortisol metabolism are unlikely to significantly affect these cells' function, though a paracrine effect cannot be excluded. Further studies should be conducted to determine whether local variations in GC bioavailability affect α-cell function and whether these changes impact β-cell function.

iii. The heterogeneity of human biology

Although the sample size of human islet donors might be considered limited, this restriction is inherent to human islet accessibility. Our study incorporated donors with diverse BMI, age, and sex, enabling assessment of response heterogeneity. In the first part, we also explore if these factors may influence the impact of PRED 250nM on GSIS. We did not observe any difference in the impact in islets when comparing by the age, the sex or the BMI of the donors. This analysis may lack the sensitivity to detect significant differences. Larger cohort studies would be needed to further elucidate these relationships, though they may be challenging to obtain in practice.

The heterogeneity in GC impact among donors complicates the study, particularly in part 3. In donors where GCs have minimal impact on GSIS, it will also be more challenging to demonstrate the effects of decreased cortisol bioavailability induced by SRD5A1 overexpression. On the other side, while primary mouse islets or immortalized cell lines may yield more consistent results, they may not fully capture the complexity of human biology.

Sex dimorphism is an important point to take in consideration. The dihydrotestosterone, locally generated from testosterone by SRD5A1 enhances as well GSIS (Xu et al., 2020b). However, metabolic disorders induced by *Srd5a1*-/- knockout or SRD5A1 inhibitors in Zucker rats were more pronounced in females or persisted after castration, suggesting that phenotypic changes may be primarily influenced by altered glucocorticoid metabolism rather than androgens (Livingstone et al., 2015; Livingstone et al., 2017). In the RNAscope in situ hybridization, given the low number of patients studied, we could not assess heterogeneity in gene expression patterns by sex. However, we did not observe any differences in the expression of

SRD5A1, HSD11B1, or HSD11B2 between male and female samples when analysing the large cohort of 93 patients. In the final part of this work, we did not examine the impact of sex on the effects of SRD5A1. Given the high level of overexpression, we do not expect sex to influence the response. Interestingly, in the previous study by Xu et al., testosterone-treated islets from females were also impacted by dutasteride treatment, as observed in islets from males (Xu et al., 2020a).

iv. Clinical relevance of the experimental GCs concentration

The correlation between *in vitro* islet doses and *in vivo* equivalent doses presents complexity. Additional factors affecting GCs bioavailability must be considered, particularly CBG and albumin (Perogamvros et al., 2012). While our perfusion medium does not contain CBG, it contains a very low percentage of BSA (0.1%), which is essential to prevent the islets from sticking to the tubing. We also measured HC concentrations in the treatment medium (neat media-no islets) via LC-MS/MS after 24 hours of treatment (**Figure 21, 25; Supplementary figure 9, 11**) and compared to the concentration measured in islets supernatant and intra-islets content. Our findings indicate a dose-dependent metabolism of cortisol by the islets in our experimental system. Specifically, when islets were exposed to 1 µM cortisol, approximately 300 nM was supposed to be metabolized, while treatment with 500 nM cortisol resulted in the metabolism of around 200 nM. This suggests a non-linear relationship between the administered cortisol concentration and the amount metabolized, potentially reflecting the saturation of cortisol transport inside the cells or the saturation of the metabolism. This seems to be the case for the dehydrogenase activity which consistently produced cortisone within the ranges of 20 – 40 nM irrespective of the concentration of cortisol used (**Figure 21; Supplementary figure 19,11**). Unfortunately, we were unable to

perform similar measurements for PRED and DEX due to the unavailability of specific assays in our institution at this time.

Interestingly, in a study of 10 non-diabetic obese patients undergoing bariatric surgery, cortisol was measured at 20.3 ± 2.3 µg/dL (~550 nM) in the artery and 17.6 ± 2.3 µg/dL (~485 nM) in the portal vein, while cortisone levels were 1.45 ± 0.12 µg/dL (~38 nM) in the artery and 3.55 ± 0.44 µg/dL (~96 nM) in the portal vein (Basu, Diabetes, 2009). The splanchnic bed uptake of approximately 3 µg/dL (~83 nM) cortisol suggests significant pre-hepatic cortisol metabolism, though patients were under stress and receiving D4 cortisol infusion which may have affected enzyme activity. Notably, our experimental concentration of 1 µM is twice the physiological level observed in this study.

Notably, we wanted to study the effect of a mild cortisol overexposure, and our experimental concentration of 1 µM is twice the physiological level observed in this study but, when considered the concentration of the cortisol “used” by the cells, it may be only one third more in our experimental model. In vitro, the cortisol uptake was twice more than what has been observed in vivo but the cortisone release was twice lower. It seems also that a physiological dose of HC would be around 250 nM. Although a full dose-response analysis for GCs effects on GSIS was beyond this study and scope, future investigations in this area would clarify the effective dose thresholds for GCs’ inhibitory influence on β-cell function.

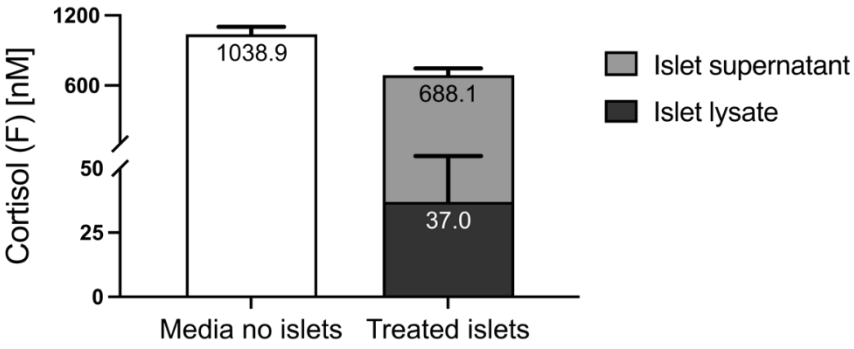

Figure 25: Quantification of hydrocortisone (HC) by LC-MS/MS in human islets treated with estimated 1 µM HC for 24 hours. HC concentration in neat media without islets, which was used to treat the islets under experimental conditions (1038.9 nM of HC, white bar); HC concentration in the islet supernatant (688.1 nM, light grey bar); and in the islet lysate (37 nM, dark grey bar) after 24 hours of incubation with the 1038 nM of HC in the neat media-no islets. Experiment carried out in *n* = 5 donors. Data expressed as mean ± SEM.

5.3 Relevance of cortisol metabolism dysregulation in metabolic disease

Previous studies have extensively documented the expression patterns of 11β-HSD1 in metabolic diseases. In obesity, 11β-HSD1 expression is increased in SAT and VAT but decreased in the liver. However, data regarding these enzymes' expression in diabetes remain scarce. Our findings demonstrate that both SRD5A1 and 11β-HSD1 expression are decreased in the islets of diabetic subjects. While correlation studies exist, direct mechanistic links between these altered expression patterns and the development of metabolic diseases are lacking. Notably, evidence for GR activation or the therapeutic potential of GR antagonists in metabolic complications remains limited. Furthermore, studies using tracer methodologies to assess cortisol or cortisone production, and uptake have not revealed significant differences between lean and obese patients (Anderson et al., 2021; Stimson et al., 2009). The strongest evidence for the involvement of these enzymes in metabolic complications comes from animal models with genetic or pharmacological modulation of HSD11B1 or SRD5A1

(Chapman et al., 2013; Dowman et al., 2013; Livingstone et al., 2015; Livingstone et al., 2017). Clinical trials using 11β-HSD1 or SRD5A1 inhibitors have also supported these findings (Gregory et al., 2020; Hazlehurst et al., 2016; Stomby et al., 2014; Upreti et al., 2014). However, these interventional approaches are likely to produce stronger changes in enzyme activity compared to the subtle expression modifications observed in pathological conditions. While minor changes in enzymatic activity could still influence intracrine or paracrine regulation, the fundamental question remains whether subtle alterations in GC exposure significantly impact GR signaling and its downstream effects. There is a critical need for more sensitive methodologies to measure small changes in cortisol concentrations and identify relevant GC biomarkers to address these questions.

5.4 Perspectives

i. SRD5A1: A therapeutic target for metabolic disease

Multiple lines of evidence suggest that SRD5A1 activator could be a promising therapeutic direction for metabolic complications associated with GCs.

SRD5A1 is highly expressed in liver (Thigpen et al., 1993) and is crucial in GCs metabolism within the liver, as *SRD5A1*-deficient mice exhibit an approximately eightfold decrease in corticosterone clearance rate. In humans, 5α-reduced cortisol metabolites constitute a significant fraction, roughly one-third to one-half, of urinary cortisol breakdown products (Andrew et al., 1998). The increase of SRD5A1 activity could be beneficial for metabolic-associated fatty liver disease (MAFLD). This is supported by findings that reduced SRD5A1 activity is associated with progression from simple steatosis to NASH (Ahmed et al., 2012) while *SRD5A1* knockout mice

exhibit increased hepatic steatosis on both normal chow and high-fat diets, with female models showing more severe effects (Dowman et al., 2013; Livingstone et al., 2015; Livingstone et al., 2017). Studies showing dual SRD5A inhibition with dutasteride increased intrahepatic lipid accumulation in healthy volunteers further support this protective role (Hazlehurst et al., 2016).

Regarding glucose homeostasis, SRD5A1 expression is also expressed in adipose tissue (Barat et al., 2007; Wake et al., 2007), and skeletal muscles (Aizawa et al., 2010), as well as in the human pancreas even if the expression is significantly lower than in the liver. Given that GCs primarily impact glucose homeostasis through the induction of insulin resistance, modulation of cortisol bioavailability by SRD5A1 appears crucial in regulating insulin sensitivity. Evidence supporting SRD5A1 agonism as a therapeutic approach for insulin resistance comes from both clinical and preclinical studies. Clinical studies show that dual SRD5A inhibition with dutasteride decreases insulin sensitivity in healthy volunteers (Upreti et al., 2014). This is supported by animal studies where *Srd5a1* knockout mice on high fat-high sucrose diet develop insulin resistance compared to wild-type mice (Livingstone et al., 2015; Livingstone et al., 2017). Female *Srd5a1* knockout mice show an even more severe metabolic phenotype mouse (Livingstone et al., 2017). Importantly, treatment with a liver-selective GR antagonist improves insulin sensitivity in *Srd5a1* knockout mice, suggestive of SRD5A1's metabolic effects (Mak et al., 2019).

Finally, testosterone plays a crucial role in regulating multiple metabolic pathways, including carbohydrate, fat, and protein metabolism, with its deficiency being associated with increased central adiposity, reduced insulin sensitivity, impaired glucose tolerance, and adverse lipid profiles (Zitzmann, 2009). Therefore, increasing

active testosterone levels through enhanced SRD5A1 activity could provide metabolic benefits by improving insulin sensitivity, body composition, and lipid metabolism. This beneficial effect has already been demonstrated for GSIS, where increased SRD5A1 activity enhances GSIS in human islets through testosterone-to-dihydrotestosterone conversion (Xu et al., 2020a).

ii. From anti-inflammatory balance to metabolic effects

While chronic inflammation plays a crucial role in metabolic diseases, suggesting benefits from enhanced glucocorticoid action, a careful balance in GC metabolism is essential. Interestingly, the 5α-reduced metabolites of corticosterone (5α-THB) demonstrate anti-inflammatory properties without the adverse metabolic effects typically associated with GCs (Livingstone et al., 2024; Yang et al., 2011). This suggests that SRD5A1 agonism might maintain some anti-inflammatory benefits through its metabolites, although the anti-inflammatory equipotency of human metabolites 5α-THF and 5α-DHF remains to be established. The importance of balanced GC action is further supported by studies in β-cells, where optimal elevation of 11β-HSD1 shows unexpectedly beneficial metabolic outcomes, while either higher or lower levels suppress glucose-stimulated insulin secretion, demonstrating an inverted U-shaped dose-response (Turban et al., 2012a). Moreover, in liver fibrosis, decreased cortisol metabolism might actually be beneficial through maintained anti-inflammatory action (Ahmed et al., 2012), highlighting the complexity of targeting GC metabolism therapeutically. Therefore, while SRD5A1 agonism could help prevent metabolic complications, careful optimization would be needed to maintain sufficient anti-inflammatory effects.

Finally, SRD5A1 is also expressed in the brain, eyes, adrenal glands, and in the kidney (Azzouni et al., 2012; Russell & Wilson, 1994; Thigpen et al., 1993; Weinstein et al.,

1991; Xu et al., 2020a). The action mediated in certain of these organs have been studied. In the eyes, 5α-dihydrocortisol is synthesized and present in the aqueous humor of the eyes and may play a role in the regulation of aqueous humor formation (Weinstein et al., 1991). 5α-reductase converts progesterone to 5α-dihydroprogesterone (5α-DHP), a precursor for neurosterioids that modulate GABA receptors and have anxiolytic and sedative effects (Stoffel-Wagner, 2003). Increased production of neurosterioids in the brain, through enhanced 5α-reductase activity, could potentially help in conditions like depression and anxiety by elevating levels of 5α-DHP and its downstream metabolites.

iii. SRD5A1 Agonism: Androgen-related safety concerns

SRD5A1 is notably expressed in the human male reproductive tract, in ovary and in skin (Thigpen et al., 1993). While increasing active testosterone may improve metabolic conditions, potential adverse effects must be considered due to the conversion of testosterone to DHT by SRD5A1, especially if the potential activator lack specificity for the isoform SRDA1.

DHT is a critical hormone in prostate growth, development, and male sexual differentiation (Azzouni et al., 2012). SRD5A inhibitors are used to treat prostate hyperplasia. An SRD5A1 activator could also lead to the development or to worsen pre-existing prostate hyperplasia. It may also potentially promote prostate cancer development by enhancing androgen receptor (AR) signaling, which drives prostate cell proliferation, growth, and survival (Azzouni et al., 2012). This concern is particularly relevant in advanced and castration-resistant prostate cancer, where SRD5A1 contributes to elevated DHT concentrations through alternative synthesis pathways involving 5α-androstanedione (Azzouni et al., 2012; Chang et al., 2011).

In the skin, SRD5A1 contributes to sebum production and hair growth but it has not been involved in idiopathic hirsutism (Taheri et al., 2015). The SRD5A inhibitors have been also proposed for scalp hair loss treatment. Polycystic Ovary Syndrome (PCOS), the leading cause of hirsutism in women, is characterized by hyperandrogenism, abnormal anovulation, and polycystic ovary morphology, with elevated androgens driving the symptoms (Sadeghi et al., 2022; Wu et al., 2017). SRD5A1 polymorphism is associated with prevalence of PCOS among lean women (Goodarzi et al., 2006; Graupp et al., 2011). Therefore, an activator of SRD5A1 protein could potentially exacerbate PCOS, as SRD5A1 is expressed in the ovary, and hirsutism by intensifying AR signaling in hair follicles and ovaries.

COMMUNICATIONS AND PUBLICATION

COMMUNICATIONS

Tijani K. O., Moreno-Lopez M., Acosta-Montalvo A., et al. “Impact of glucocorticoids on islets function role of SRD5A1 as a modulator of GCs bioavailability”. **60th EASD**, Madrid, September, **2024.**

Tijani K. O., Moreno-Lopez M., Acosta-Montalvo A., et al. “Impact of glucocorticoids on islets function role of SRD5A1 as a modulator of GCs bioavailability”. **9th Scientific Symposium**, Institute Pasteur de Lille, June **2024**.

Tijani K. O., Moreno-Lopez M., Acosta-Montalvo A., et al. “Impact of glucocorticoids on islets function role of SRD5A1 as a modulator of GCs bioavailability”. **6th European Genomic Institute for Diabetes** (EGID) Thematic School, Lille, September **2024**.

Tijani K. O., Saponaro C., Moreno-Lopez M. et al. “Impact of Low Therapeutic Concentration of Prednisolone on Pancreatic β-Cell Function: Insights into Equipotent Dose Effects of Glucocorticoids” **European Islet Study Group** (EISG), Helsinki, June **2024.**

Tijani K. O., Moreno-Lopez M., Acosta-Montalvo A., et al. “Impact of glucocorticoids on islets function role of SRD5A1 as a modulator of GCs bioavailability”. **7th EGID Symposium**, Lille, November **2023**.

PUBLICATIONS

Omolara Khadijat Tijani, Maria Moreno Lopez, Isaline Louvet, Ana Acosta-Montalvo, Anaïs Coddeville, Valery Gmry, Julie Kerr Conte, Francois Pattou, Marie-Christine Vantyghen, Chiara Saponaro, Caroline Bonner, and Stéphanie Espiard. **Impact of Therapeutic Doses of Prednisolone and Other Glucocorticoids on Insulin Secretion from Human Islets.** Accepted in Annales d'Endocrinologie, for December 2024 publication.

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SUPPLEMENTARY TABLE

Supplementary Table 1: Donor Phenotype & Human Islet Preparation Characteristics.

Donor ID	Age (years)	Sex	BMI (kg/m^2)	HbA_{1c} (%)	Cause of death	Cold ischemia time (h)	Purity (%)	Viability (%)	Culture time (h)	GSIS assay at t=0 (islets functionality)	Experiments conducted
H354	64	M	39.2	10.3	Suicide	5.33	80	87.6	21	Static: 1.37	mRNA expression
H431	61	F	33.3	5.6	Suicide	5.33	80	87.6	21	Static: 1.37	mRNA expression
H459	58	M	32.9	7.2	Suicide	5.33	80	87.6	21	Static: 1.37	mRNA expression
H504	57	F	18.9		Suicide	5.33	80	87.6	21	Static: 1.37	mRNA expression
H531	49	M	25.7	5.7	Traumatic accident	9.38	90	98.5	19.5	Static: 2.88	mRNA expression
H571	51	F	23.5	6.2	Stroke	6.53	85	80	18	Static: 1.83	mRNA expression
H578	56	F	40.1	9.3	Stroke	11.20	80	97.4	22	NA	mRNA expression
H595	47	F	21.9	5.7	Meningioma	7.30	90	98.2	36	Static: 0.91	mRNA expression
H596	48	M	30.2	5.6	Traumatic accident	5.35	70	95.8	12	NA	mRNA expression
H616	64	F	25.7	6	Stroke	5.36	80	98	20	Static: 2.76	mRNA expression
H645	25	M	24.1	5.9	Traumatic accident	4.44	80	97.8	18	N/A	mRNA expression
H672	65	F	20.2	5.8	Stroke	3	95	86.8	12	Static: 1.64	mRNA expression

Donor ID	Age (years)	Sex	BMI (kg/m^2)	HbA_{1c} (%)	Cause of death	Cold ischaemia time (h)	Purity (%)	Viability (%)	Culture time (h)	GSIS assay at t=0 (islets functionality)	Experiments conducted
H704	77	M	32.3	6	Meningeal Hemorrhage	3.15	80	97	18	NA	mRNA expression
H727	65	F	20.2	5.8	Stroke	3	95	86.8	12	Static: 1.64	mRNA expression
H739	56	M	33.7	5	Stroke	5	70	98.4	18	NA	mRNA expression
H763	59	F	24	6.6	Traumatic non-AVP	5.40	90	95.5	16	NA	mRNA expression
H765	58	F	27.6	5.6	Cerebral. Hemorrhage	5.2	90	97.5	16	Static: 0.84	mRNA expression
H823	40	F	25.6	4.7	Cerebral. Hemorrhage.	7.18	95	96.2	18	Static: 2.33	mRNA expression
H826	57	M	27.4	5.6	Cerebral. Hemorrhage.	3.06	80	98.5	10	Static: 1.05	mRNA expression
H834	58	F	20.8	5.6	Stroke	6.55	90	97.6	18	Static: 1.00	mRNA expression
H836	44	F	17.1	6	Choking	6.04	80	95.5	18	Static: 0.58	mRNA expression
H841	58	F	31.3	5.7	Stroke	5.16	80	92.9	10	Static: 1.78	mRNA expression
H842	51	M	30.7	5.2	Aneurysm	6.12	90	95.7	18	Static: 0.91	mRNA expression
H848	58	M	33.8	6.6	Head trauma	9.88	70	91.9	18	Static: 2.18	mRNA expression
H853	56	M	24.3	5.1	Stroke	3.35	85	88.5	18	NA	mRNA expression
H859	55	F	25.7	5.4	Stroke	11.15	90	96.1	18	Static: 1.08	mRNA expression
H869	68	F	25.4	5.4	Stroke	3.3	80	93.5	18	Static: 0.94	mRNA expression
H873	24	F	19.4	5.9	Traumatic accident	3.58	80	98	18	Static: 1.63	mRNA expression
H891	45	M	32.9	8	Stroke	4	70	96.6	20	Static: 1.76	mRNA expression RNAscope for *SRD5A1*, HSD11B1, HSD11B2
H895	65	M	34.3	5.2	Choking	9.05	70	91.8	20	Static: 2.54	mRNA expression

Donor ID	Age (years)	Sex	BMI (kg/m^2)	HbA_{1c} (%)	Cause of death	Cold ischaemia time (h)	Purity (%)	Viability (%)	Culture time (h)	GSIS assay at t=0 (islets functionality)	Experiments conducted
H896	52	M	24.5	5.9	Aneurysm	2.52	80	87.7	38	Static: 1.28	mRNA expression
H906	51	M	32.3	5.5	Stroke	6.16	70	91	20	NA	mRNA expression
H904	51	M	28.6	4.8	Stroke	5.22	80	88.9	15	Static: 6.07	mRNA expression
H911	37	M	24.8	5	Choking	3.22	80	91.8	19	Static: 1.22	mRNA expression
H912	65	F	27	6.1	Traumatic accident	7.36	85	96.5	36	Static: 1.54	mRNA expression
H913	56	F	34.5	7.9	Stroke	5.07	70	98.4	18	Static: 1.15	mRNA expression RNAscope for *SRD5A1*
H914	31	M	27.8	5.2	Traumatic accident	8.27	90	97.1	21	Static: 2.08	mRNA expression
H916	38	F	34.7	4.9	Stroke	3.24	70	95.2	42	Static: 1.15	mRNA expression
H917	57	M	26.1	5.7	Aneurysm	6.53	80	99	18	Static: 1.13	mRNA expression
H919	47	M	33.8	5.2	Head trauma	8.12	70	95.6	24	Static: 2.05	mRNA expression
H924	59	M	26.5	6.8	Aneurysm	5.92	90	95.4	15,5	Static: 1.69	mRNA expression
H926	40	F	32.8	5	Choking	4.08	80	92.3	18	Static: 1.57	mRNA expression
H927	36	F	30.9	5.1	Stroke	4.22	70	93.1	56	Static: 1.17	mRNA expression
H928	57	F	23.6	6	Aneurysm	3.41	80	91.1	34	Static: 0.75	mRNA expression
H940	53	M	32.8	6.2	Head trauma	6.2	80	93.7	18	Static: 3.67	mRNA expression
H943	48	F	22	5.7	Suicide	4.09	80	99	18	Static: 2.46	mRNA expression
H944	46	M	23.1	5.7	Suicide	6.01	90	99.3	20	Static: 1.02	mRNA expression
H962	64	F	21.4	6	Cereb. Hemorrh.	9.33	90	95	21	Static: 0.62	mRNA expression

Donor ID	Age (years)	Sex	BMI (kg/m^2)	HbA_{1c} (%)	Cause of death	Cold ischaemia time (h)	Purity (%)	Viability (%)	Culture time (h)	GSIS assay at t=0 (islets functionality)	Experiments conducted
H974	22	M	21.6	5.5	Stroke	4.5	80	98.3	42	Static: 1.38	mRNA expression
H975	77	F	28.7	5.5	Stroke	8.76	80	95.7	20	Dynamic: 5.03	mRNA expression
H983	44	M	33	5.3	Stroke	5.03	70	90.8	39	Dynamic: 14.62	mRNA expression
H986	52	M	22.9	5.9	Stroke	5.58	80	96.3	18	Dynamic: 7.06	mRNA expression
H989	68	F	24.1	5.2	Cerebral. Hemorrhage.	5.56	90	93.3	18	Dynamic: 5.14	mRNA expression RNAscope for *SRD5A1*
H991	52	M	32.3	5.6	Stroke	9.42	80	95	18	Dynamic: 7.73	mRNA expression
H993	63	M	25.9	5.3	Stroke	7.14	80	98.7	18	Dynamic: 3.80	mRNA expression
H996	62	F	26.2	4.2	Stroke	5.27	90	94.2	18	Dynamic: 10.02	mRNA expression
H1006	60	M	30.9	5.7	Traumatic accident	7	80	95.2	40	Dynamic: 1.89	mRNA expression
H1012	55	M	23.5	5	Stroke	2.13	90	96.9	72	Dynamic: 3.16	mRNA expression
H1013	37	F	31.2	5.9	Post brain surgery	7.11	95	95.1	48	Dynamic: 2.68	mRNA expression
H1015	50	M	21.7	5.5	Traumatic accident	3	80	98.6	72	Dynamic: 3.16	mRNA expression
H1020	26	M	28.1	5.5	Traumatic accident	14.28	90	86.8	67	Dynamic: 20.46	mRNA expression
H1021	81	M	31.1	6.6	Stroke	8.06	75	94.5	19	Dynamic: 1.80	mRNA expression
H1028	57	M	18.4	6.1	Stroke	4.12	90	89.6	16	Dynamic: 1.92	mRNA expression
H1030	59	F	30.8	6	Stroke	12.08	90	94.5	20	Dynamic: 11.62	mRNA expression
H1033	39	M	24.3	5.6	Choking	3.14	80	96.2	18	Dynamic: 3.26	mRNA expression

Donor ID	Age (years)	Sex	BMI (kg/m^2)	HbA_{1c} (%)	Cause of death	Cold ischaemia time (h)	Purity (%)	Viability (%)	Culture time (h)	GSIS assay at t=0 (islets functionality)	Experiments conducted
H1034	68	M	37.1	6.4	Traumatic non-AVP	7.09	70	95.7	22	Dynamic: 5.63	mRNA expression
H1038	51	M	26	5.7	Heart attack	3.01	80	93.2	39	Dynamic: 27.04	mRNA expression
H1039	54	F	24.2	6.3	Cerebral. Hemorrhage.	6.22	80	94.7	18	Dynamic: 3.04	mRNA expression
H1042	51	F	26.5	5.3	Stroke	9.21	90	94.5	24	Dynamic: 1.98	mRNA expression, 24h treatment with HC and cortisone
H1043	52	F	19.8	5.3	Traumatic accident	4.12	90	94.5	48	Dynamic: 6.46	mRNA expression
H1045	60	M	31.2	5.7	Stroke	4.47	80	93.1	18	Dynamic: 5.38	mRNA expression
H1046	50	M	19.6	4.6	Stroke	5.3	80	94.8	12	Dynamic: 10.2	mRNA expression
H1055	57	M	26.1	5.7	Traumatic accident	4.11	90	98.5	18	Dynamic: 3.47	mRNA expression
H1059	76	F	29.4	6.7	Stroke	2.48	90	95	11	Dynamic: 2.44	mRNA expression
H1061	32	M	24.7	5.4	Traumatic accident	8.72	90	90	14	Dynamic: 1.95	mRNA expression
H1063	59	F	31.9	5.7	Stroke	6.24	90	92.5	12	Dynamic: 5.71	mRNA expression
H1067	43	M	24.8	5.2	Stroke	5.04	80	97.4	15	N/A	mRNA expression
H1069	42	F	20.2	5.4	Stroke	9.03	90	97.2	20	Dynamic: 2.83	mRNA expression
H1071	37	M	23.4	6.1	Traumatic accident	4.58	80	99.1	14	Dynamic: 2.09	mRNA expression
H1072	59	F	30.5	5.8	Stroke	7.58	70	94.8	N/A	Dynamic: 3.64	mRNA expression
H1075	37	H	31.3	5.8	Stroke	6.55	70	94.7	18	Dynamic: 4.82	mRNA expression
H1077	55	F	22.9	5.1	Stroke	5.56	80	97.7	20	Dynamic: 3.04	mRNA expression
H1083	86	F	29.3	5.7	Stroke	6.11	80	94.9	18	Dynamic: 5.47	mRNA expression

Donor ID	Age (years)	Sex	BMI (kg/m^2)	HbA_{1c} (%)	Cause of death	Cold ischaemia time (h)	Purity (%)	Viability (%)	Culture time (h)	GSIS assay at t=0 (islets functionality)	Experiments conducted
H1086	35	F	30.9	5.2	Stroke	9.29	75	92.7	45	Dynamic: 1.62	mRNA expression
H1092	61	M	34.9	6.5	Stroke	6.11	90	98.9	16	Dynamic: 3.83	mRNA expression, RNAscope analysis for *SRD5A1*, HSD11B1, HSD11B2
H1094	62	M	31.1	5.1	Stroke	6.25	80	97	17	Dynamic: 4.88	mRNA expression,
H1095	56	M	30.5	5.5	Choking	7.22	80	98.1	23	Dynamic: 2.77	mRNA expression, RNAscope analysis for *SRD5A1*, HSD11B1, HSD11B2
H1097	53	F	29.1	5.3	Traumatic accident	3.38	90	94.5	10	Dynamic: 1.76	mRNA expression, RNAscope analysis for *SRD5A1*, HSD11B1, HSD11B2
H1099	54	F	24.3	5.8	Stroke	7.02	90	96.2	60	Dynamic: 4.74	mRNA exprssion
H1101	47	M	25.7	4.3	Stroke	3.35	80	96.1	20	Dynamic: 4.55	mRNA expression, RNAscope analysis for *SRD5A1*, HSD11B1, HSD11B2
H1103	62	F	20.7	5.8	Cerebral. Hematoma	4.19	90	99.3	17	Dynamic: 3.15	mRNA expression
H1106	33	M	24.5	5.1	Choking	3.62	90	97	19	Dynamic: 3.16	mRNA expression
H1109	65	F	27.4	6.1	Stroke	6.18	90	99	18	Dynamic: 0.91	mRNA expression
H1114	50	M	34	5.5	Suicide	5.50	95	99.2	21	Dynamic: 2.93	mRNA expression
H1117	50	H	24.7	5.4	AVC	5.51	80	95.9	14	Dyanimc: 2.07	mRNA expression, RNAscope analysis for *SRD5A1*, HSD11B1, HSD11B2
H1119	61	F	23.3	5.6	Choking	8.25	80	97.5	15	N/A	mRNA expression, RNAscope analysis for *SRD5A1*, HSD11B1, HSD11B2
H1120	52	M	29.5	5.7	Traumatic non-AVP	3.59	90	96.7	18	Dyanimc: 6.69	mRNA expression,
H1121	65	M	32	5.5	Stroke	9.24	90	94.9	14	Dyanimc: 1.41	mRNA expression, RNAscope analysis for *SRD5A1*, HSD11B1, HSD11B2
H1122	54	F	22	5.6	Stroke	4.54	60	98.2	14	Dyanimc: 1.28	mRNA expression, RNAscope analysis for *SRD5A1*, HSD11B1, HSD11B2
H1128	46	M	22.54	5.6	“Fausse Route”	3.08	70	96.9	10	Dyanimc: 2.09	mRNA expression, and 24h treatment with HC, and cortisone

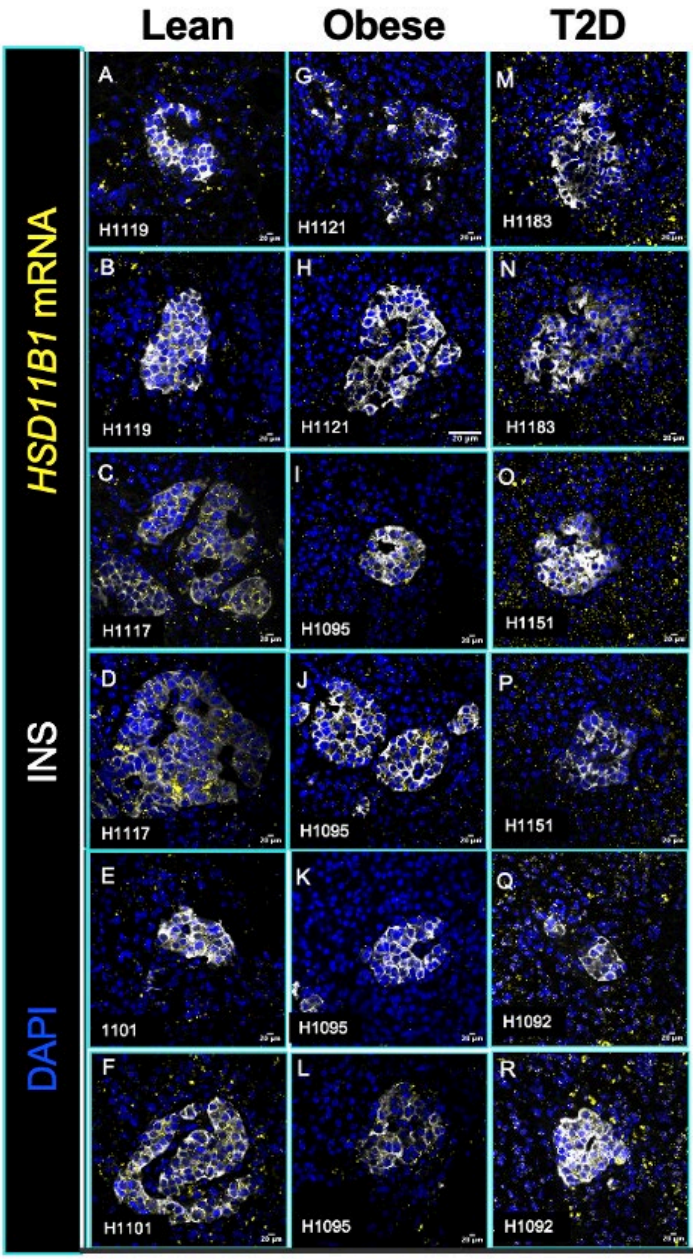
Donor ID	Age (years)	Sex	BMI (kg/m^2)	HbA_{1c} (%)	Cause of death	Cold ischaemia time (h)	Purity (%)	Viability (%)	Culture time (h)	GSIS assay at t=0 (islets functionality)	Experiments conducted
H1138	39	H	29.4	5.6	AVC	3.56	70	96.6	18	Dynamic: 1.24	mRNA expression
H1142	58	M	26.7	5.8	Stroke	8.33	75	91.4	61	Dynamic: 2.29	mRNA expression, and 1h-24h treatment with HC
H1151											
H1152	66	H	25.6	5.8	AVC	9.37	90	92.9	12	Dynamic: 1.45	mRNA expression, and 24h treatment with HC and cortisone treatment
H1154	62	M	24.7	5.2	Stroke	312	75	92.9	8	Dynamic: 1.04	mRNA expression, and 24h treatment with HC and cortisone treatment
H1156	57	F	33.4	5.9	Stroke	11	80	91.3	16	Dynamic: 8.54	mRNA expression, and cortisol metabolites measurement by LCMS/MS
H1159	39	M	26.3	5.2	Traumatic accident	8.36	80	94.6	6	Dynamic: 2.23	mRNA expression Chronic treatment and perifusion-FGF21 25 nM
H1167	69	F	28.9	5.6	Stroke	12.16	80	92.3	46	Dynamic: 2.25	mRNA expression
H1170	45	M	26.8	5.4	Suicide	4.03	80	93.8	18	Dynamic: 1.83	mRNA expression
H1177	65	F	27.2	5.8	AVC	6.44	80	93.93	20	Dynamic: 1.30	mRNA expression
H1178	59	F	29.2	5.8	Stroke	6.44	80	93.9	20	Dynamic: 3.93	mRNA expression
H1183	61	H	31.3	7.4	AVC	9.02	NA	91.3	NA	Dynamic: 2.01	mRNA expression, RNAscope analysis for *SRD5A1*, HSD11B1, HSD11B2
H1184	46	M	28.1	5.8	Stroke	3.42	80	88.5	19	Dynamic: 5.14	mRNA expression, and 8-24h of HC and cortisone treatment

Donor ID	Age (years)	Sex	BMI (kg/m^2)	HbA_{1c} (%)	Cause of death	Cold ischaemia time (h)	Purity (%)	Viability (%)	Culture time (h)	GSIS assay at t=0 (islets functionality)	Experiments conducted
H1192	28	M	28.1	5.7	Stroke	3.17	90	93.1	16	Dynamic: 1.05	mRNA expression, 1-8-24h of HC and cortisone treatment
H1194	62	F	36.7	6.5	Stroke	6.92	40	92.4	10	Dynamic: 2.00	mRNA expression,
H1195	54	M	21.3	5.9	Choking	4.33	70	90.8	8	Dynamic: 3.04	mRNA expression,
H1202	47	F	30.1	5.9	Stroke	6.33	90	88	16	Dynamic: 1.20	mRNA expression, dynamic perifusion after 24h of 250 nM PRED treatment,
H1203	56	F	46.3	6.1	Stroke	7.4	80	97.4	21	Dynamic: 1.56	mRNA expression
H1206	66	M	23.3	5.7	Stroke	9.15	80	97.8	22	Dynamic: 6.45	mRNA expression
H1208	49	H	20.9	5.5	AVC	7.47	90	96.7	33	N/A	mRNA expression, dynamic perifusion after 24h of 250 nM PRED treatment,
H1210	70	H	26.6	5.6	AVC	9.00	70	96	18	Dynamic: 3.15	mRNA expression, dynamic perifusion after 24h of 250 nM PRED treatment,
H1211	29	M	42.4	5.8	Stroke	NA	60	91.8	66	Dynamic: 6.23	mRNA expression, dynamic perifusion after 24h of 250nM PRED treatment,
H1215	65	M	27.3	5.5	Stroke	7.37	70	89.5	67	Dynamic: 3.35	mRNA expression
H1223	60	F	28.4	5.6	Stroke	5.37	90	92.6	38	Dynamic: 3.67	mRNA expression
H1225	39	F	21.5	5.2	Stroke	7.05	90	98	232	Dynamic: 3.4	mRNA expression
H1228	54	F	NA	NA	Anoxia	1	70	93.4	21	Dynamic: 5.14	mRNA expression and cortisol metabolites measurement by LCMS/MS
H1236	49	M	29.2	5.7	Stroke	7.17	90	98.9	20	Dynamic: 2.22	mRNA expression, dynamic perifusion after 24h of 250 nM PRED treatment,
H1241	58	M	24,.5	5.6							mRNA expression, dynamic perifusion after 24h of 250 nM PRED treatment

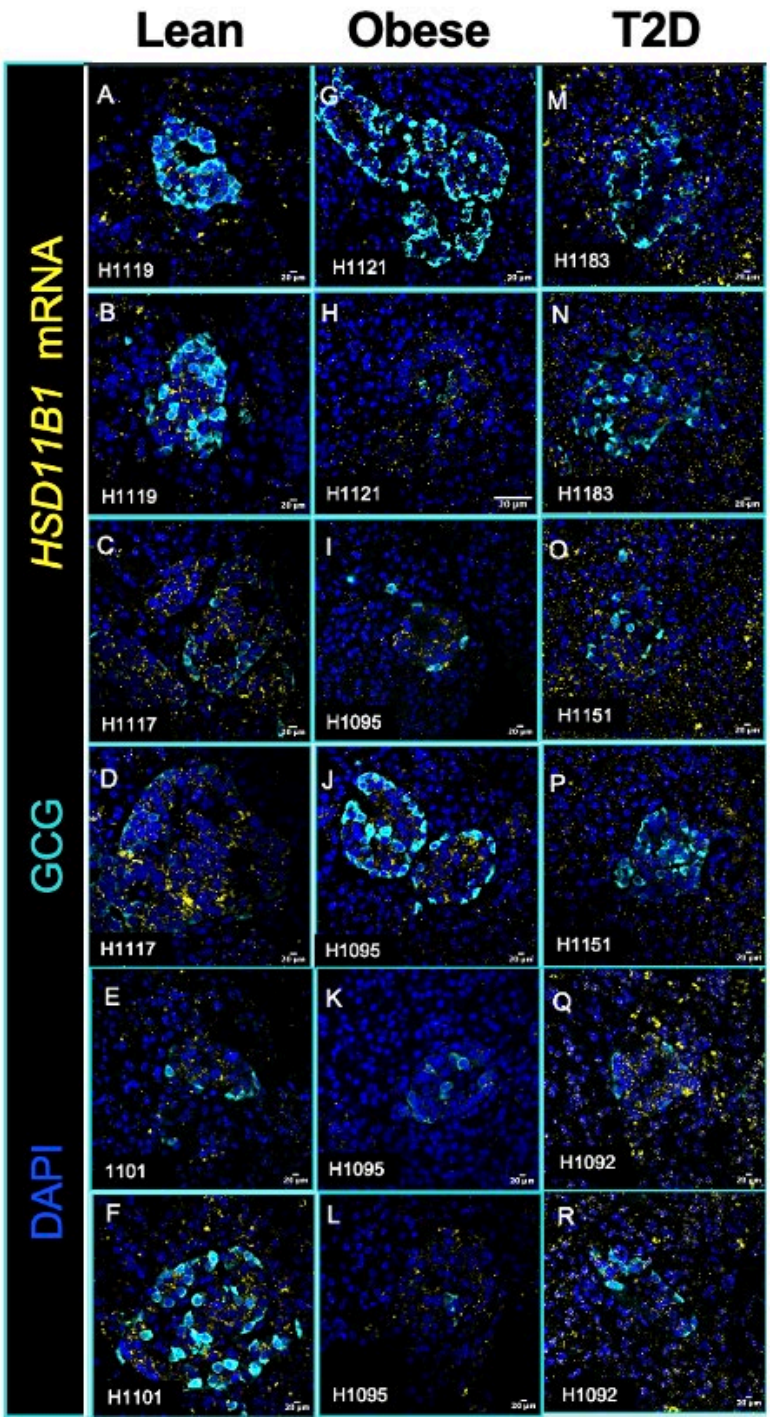
Donor ID	Age (years)	Sex	BMI (kg/m^2)	HbA_{1c} (%)	Cause of death	Cold ischaemia time (h)	Purity (%)	Viability (%)	Culture time (h)	GSIS assay at t=0 (islets functionality)	Experiments conducted
H1242	55	M	37.1	5.3	Stroke	4	NA	NA	NA	NA	mRNA expression
H1247	50	M	39.5	5.1	Stroke	8.20	60	90.9	18	Dynamic: 3.22	mRNA expression
H1251	55	F	29.1	5.7	Stroke	6	90	88.8	12	Dynamic: 2.16	mRNA expression, dynamic perifusion after 24h of 250 nM PRED treatment,
H1252	55	F	33.9	5	Stroke	9.02	50	91.9	12	Dynamic: 2.23	mRNA expression and 24h of HC and cortisone treatment (LCMS/MS)
H1254	63	M	24.5	7.5							mRNA expression, and 24h of HC and cortisone treatment (LCMS/MS)
H1255	62	M	25.9	5.7	Stroke	7.08	85	86.6	42	Dynamic: 3.09	mRNA expression, and dynamic perifusion after 24h of 250 nM PRED, 1 µM HC, and 38 nM DEX treatment
H1256	65	M	32.1	5	Stroke	6.26	80	88.4	16	Dynamic: 1.82	mRNA expression, and dynamic perifusion after 24h of 250 nM PRED, 1 µM HC, and 38 nM DEX treatment,
H1257	58	M	24.5	5.6	Stroke	4.33	80	90.3	31	Dynamic: 2.04	mRNA expression, and WB after *SRD5A1* transfection
H1263	58	F	24.2	NA	Anoxia	4.55	80	92.5	9	NA	mRNA expression, and dynamic perifusion after 24h of 250 nM PRED, 1 µM HC, and 38 nM DEX treatment,
H1265	50	F	34	10	AVC	14.00	90	93.9	3	N/A	mRNA expression, and dynamic perifusion after 24h of 250 nM PRED, 1 µM HC, and 38 nM DEX treatment,
H1273	58	M	25	NA	Anoxia	12.16	90	94.6	15	Dynamic: 1.95	mRNA expression
H1274	53	F	29.8	5	Stroke	7.06	70	94.1	10	Dynamic: 1.73	mRNA expression, , and dynamic perifusion after 24h of 250 nM PRED, 1 µM HC, and 38 nM DEX treatment,
H1284	18	M	21.3	4.7	Traumatic AVP	6.56	70	98	9.5	Dynamic: 14.87	Chronic HC 1 µM + *SRD5A1* transfection, dynamic perifusion, and LCMS/MS for cortisol metabolites
H1289	69	F	26.4	5.3	Stroke	5	70	94.3	17	Dynamic: 1.31	Chronic HC 1 µM + *SRD5A1* transfection, dynamic perifusion, and LCMS/MS for cortisol metabolites
H1292	50	F	34	10	AVC	14.00	30	93.9	3	N/A	mRNA expression

Donor ID	Age (years)	Sex	BMI (kg/m^2)	HbA_{1c} (%)	Cause of death	Cold ischaemia time (h)	Purity (%)	Viability (%)	Culture time (h)	GSIS assay at t=0 (islets functionality)	Experiments conducted
H1296	56	M	20	6.3	Stroke	4.30	65	95.7	61	Dynamic: 1.16	Chronic HC 1 µM + *SRD5A1* transfection, dynamic perifusion, and LCMS/MS for cortisol metabolites
H1302	19	M	26.6	5.2	Traumatic AVP	8	75	85.4	7	NA	Chronic HC 1 µM + *SRD5A1* transfection, dynamic perifusion, and LCMS/MS for cortisol metabolites
H1303	98	M	27.8	5.9	Stroke	8.55	75	93	14	Dynamic: 2.32	Chronic HC 1 µM + *SRD5A1* transfection, dynamic perifusion, and LCMS/MS for cortisol metabolites
H1309	64	M	24.6	5.7	Stroke	7.33	90	93.8	8	Dynamic: 2.31	Chronic PRED 250nM + *SRD5A1* transfection, and dynamic perifusion
H1310	58	M	29.1	5.9	Stroke	5.18	80	95.9	58	NA	Chronic PRED 250nM + *SRD5A1* transfection, and dynamic perifusion
H1313	64	M	29.5	5.7	Stroke	6.20	80	93.8	11	NA	Chronic PRED 250 nM / HC 1 µM + *SRD5A1* transfection, dynamic perifusion, and LCMS/MS for cortisol metabolites
H1314	44	M	25	6.4	Anoxia	4.15	75	94	41	NA	Chronic PRED 250nM + *SRD5A1* transfection, and dynamic perifusion

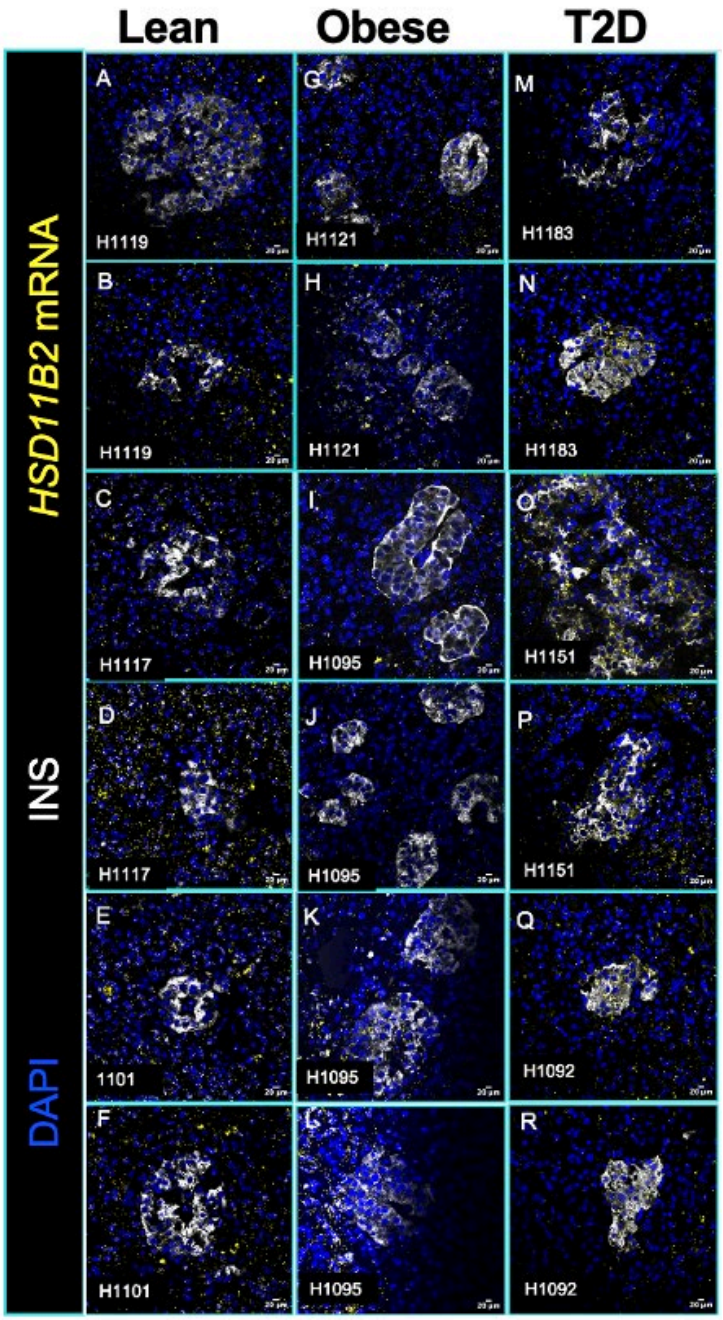
SUPPLEMENTARY FIGURES:



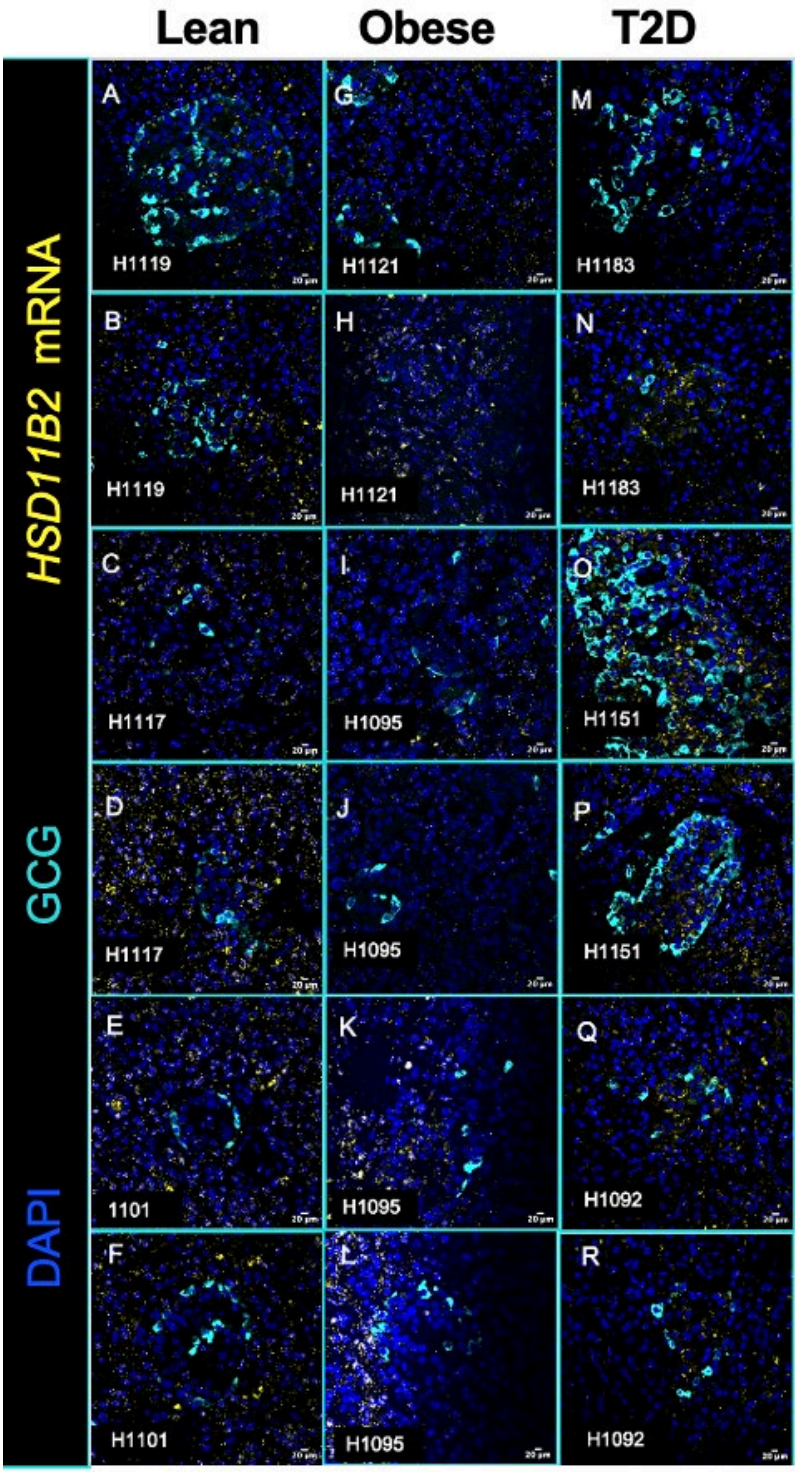
Supplementary Figure 1: *HSD11B1* mRNA is prominent in the endocrine islets of the lean normoglycemic donors and is induced in the exocrine of the T2D pancreatic islets: Representative images of *HSD11B1* mRNA expression in *n* = 3 donors (2 representative images per donor), assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for insulin (INS), (white), and nuclei staining with DAPI (blue), on FFPE pancreatic sections from (**A-F)** lean normoglycemic donors (BMI: <25, HbA1c: <5.7%), **(G-L)** obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and (**M-R)** T2D donor (BMI: >30, HbA1c: ≥6.4%). Scale bars, 20 μm.



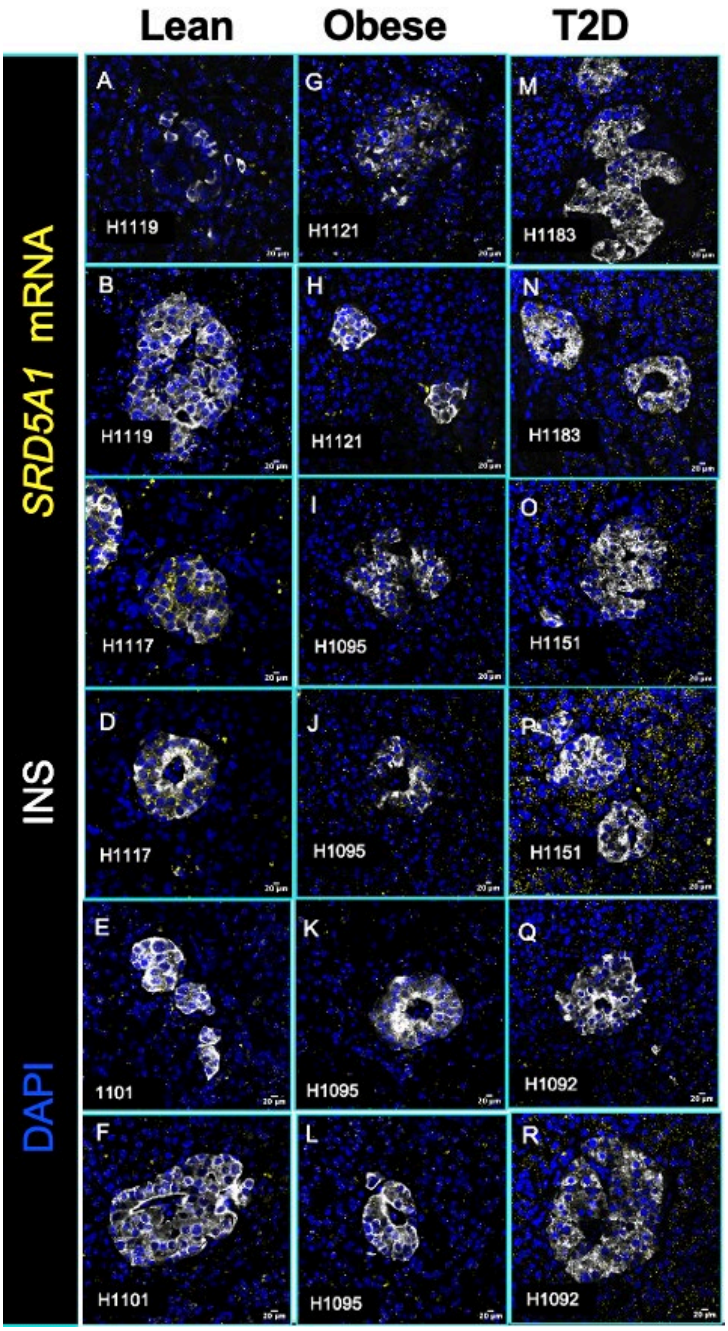
Supplementary Figure 2: *HSD11B1* expression pattern in α-cells.: Representative images of *HSD11B1* mRNA expression in 3 donors (2 representative images per donor), assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for glucagon (GCG), (cyan), and nuclei staining with DAPI (blue), on FFPE pancreatic sections from **(A-F)** lean normoglycemic donors (BMI: <25, HbA1c: <5.7%), **(G-L)** obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and **(M-R)** T2D donor (BMI: >30, HbA1c: ≥6.4%). Scale bars, 20 µm.



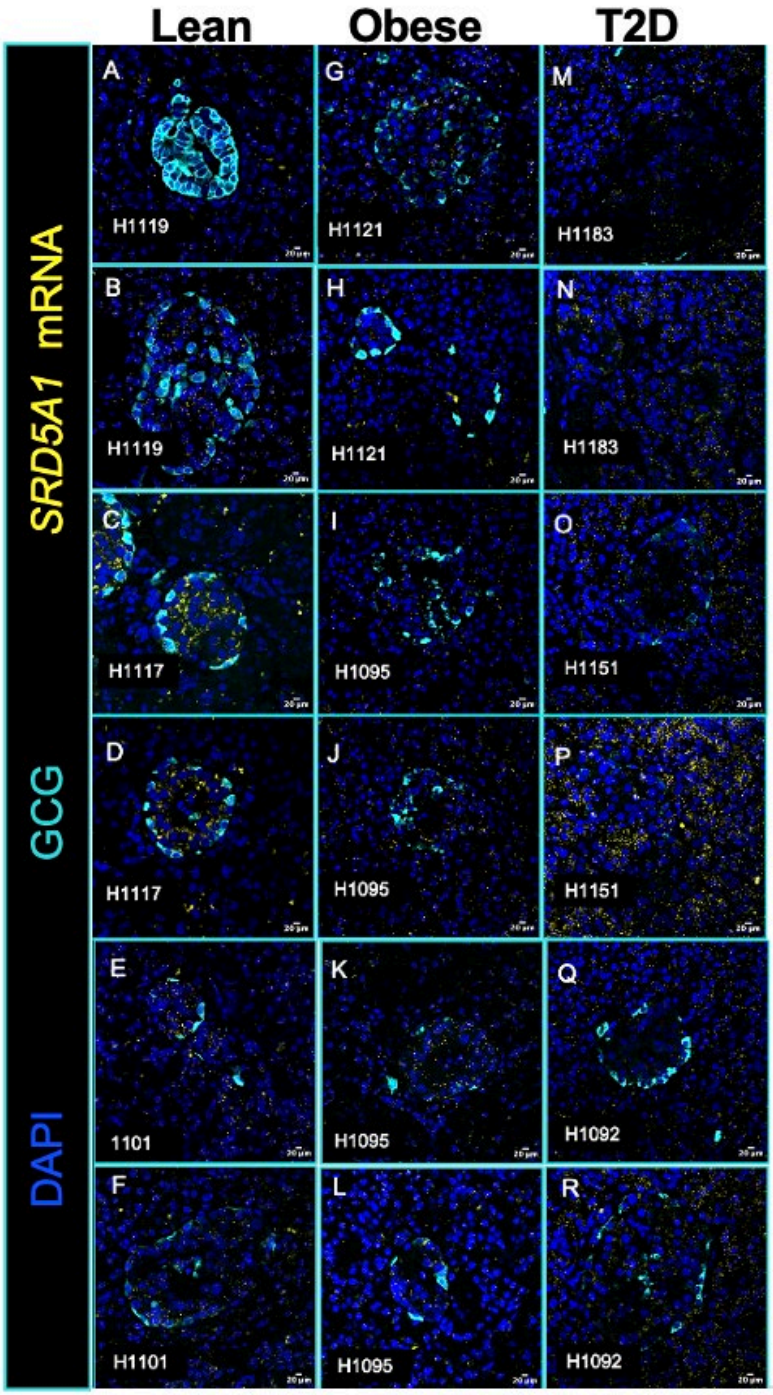
Supplementary Figure 3: *HSD11B2* mRNA endocrine pancreas expression is comparable across metabolic phenotype but with diminishing exocrine expression: Representative images of *HSD11B2* mRNA expression in *n* = 3 donors (2 representative images per donor), assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for insulin (INS), (white), and nuclei staining with DAPI (blue), on FFPE pancreatic sections from (**A-F)** lean normoglycemic donors (BMI: <25, HbA1c: <5.7%), **(G-L)** obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and **(M-R)** T2D donor (BMI: >30, HbA1c: ≥6.4%). Scale bars, 20 µm.



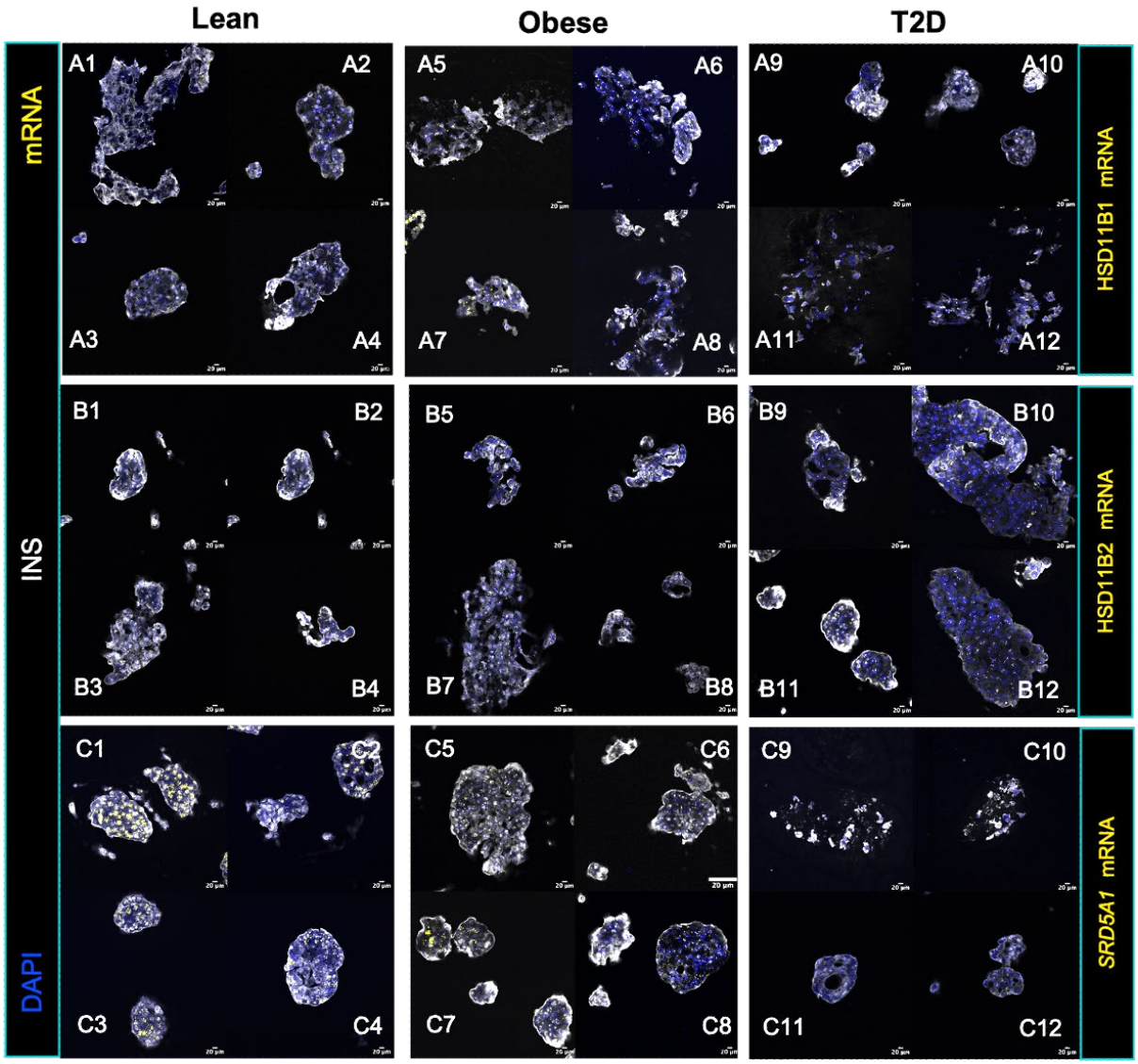
Supplementary Figure 4: *HSD11B2* expression pattern in α-cells.: Representative images of *HSD11B2* mRNA expression in *n* = 3 donors (2 representative images per donor), assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for glucagon (GCG), (cyan), and nuclei staining with DAPI (blue), on FFPE pancreatic sections from **(A-F)** lean normoglycemic donors (BMI: <25, HbA1c: <5.7%), **(G-L)** obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and **(M-R)** T2D donor (BMI: >30, HbA1c: ≥6.4%). Scale bars, 20 µm.



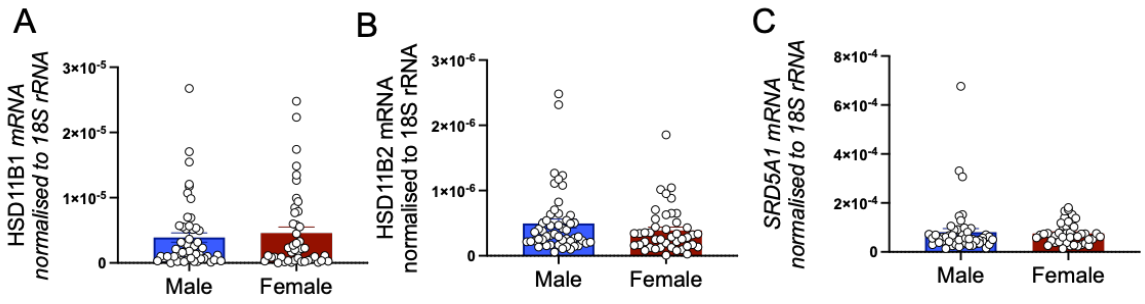
Supplementary Figure 5: *SRD5A1* mRNA is prominent in the endocrine islets of the lean normoglycemic donors and is induced in the exocrine of the T2D pancreatic islets: Representative images of *SRD5A1* mRNA expression in *n* = 3 donors (2 representative images per donor), assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for insulin (INS), (white), and nuclei staining with DAPI (blue), on FFPE pancreatic sections from **(A-F)** lean normoglycemic donors (BMI: <25, HbA1c: <5.7%), **(G-L)** obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and **(M-R)** T2D donor (BMI: >30, HbA1c: ≥6.4%). Scale bars, 20 μm.



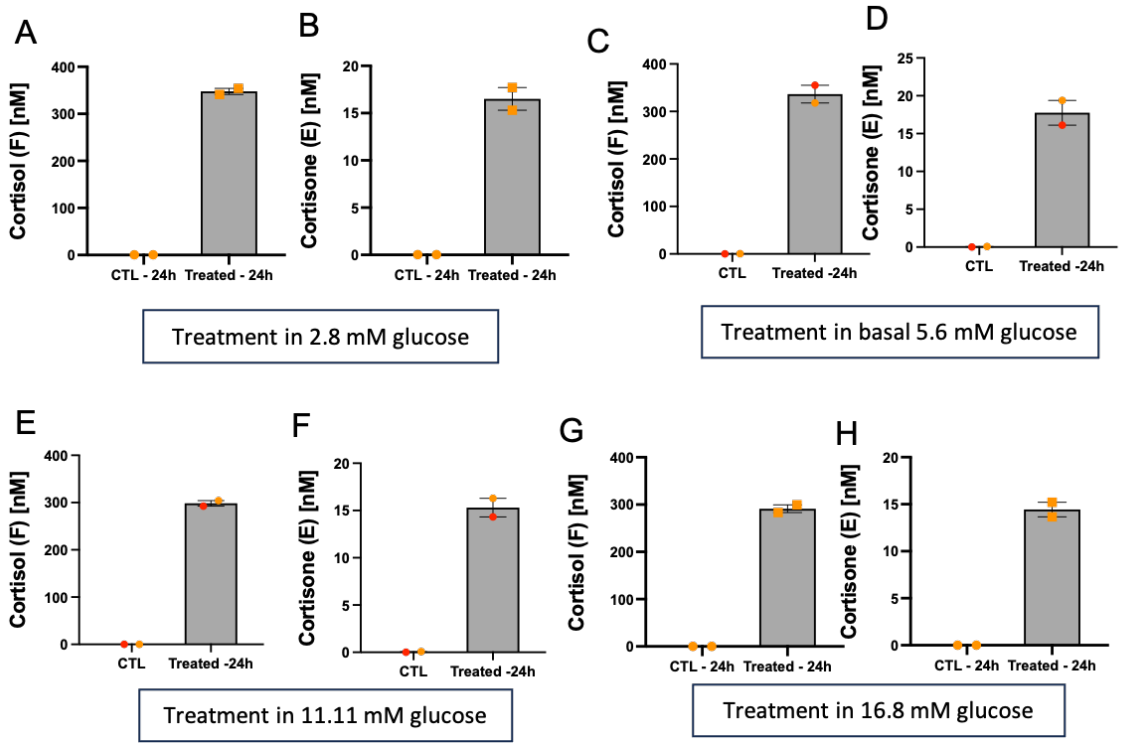
Supplementary Figure 6: *SRD5A1* expression pattern in α-cells. Representative images of HSD11B2 mRNA expression in *n* = 3 donors (2 representative images per donor), assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for glucagon (GCG), (cyan), and nuclei staining with DAPI (blue), on FFPE pancreatic sections from **(A-F)** lean normoglycemic donors (BMI: <25, HbA1c: <5.7%), **(G-L)** obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and **(M-R)** T2D donor (BMI: >30, HbA1c: ≥6.4%). Scale bars, 20 µm.



Supplementary Figure 7: Mild expression of *HSD11B1* and *SRD5A1* in the diabetic endocrine cells, with sustained expression of HSD11B2 mRNA across metabolic phenotype: Representative images of genes mRNA expression in additional *n* = 2 donors (2 representative images per donor), assessed by *in situ* hybridization (yellow dots), and immunofluorescence staining for insulin (INS), and nuclei staining with DAPI (blue), on FFPE pancreatic sections from **(A-F)** lean normoglycemic donors (BMI: <25, HbA1c: <5.7%), **(G-L)** obese normoglycemic donors (BMI: >30, HbA1c: <5.7%), and **(M-R)** T2D donor (BMI: >30, HbA1c: ≥6.4%). Scale bars, 20 µm.

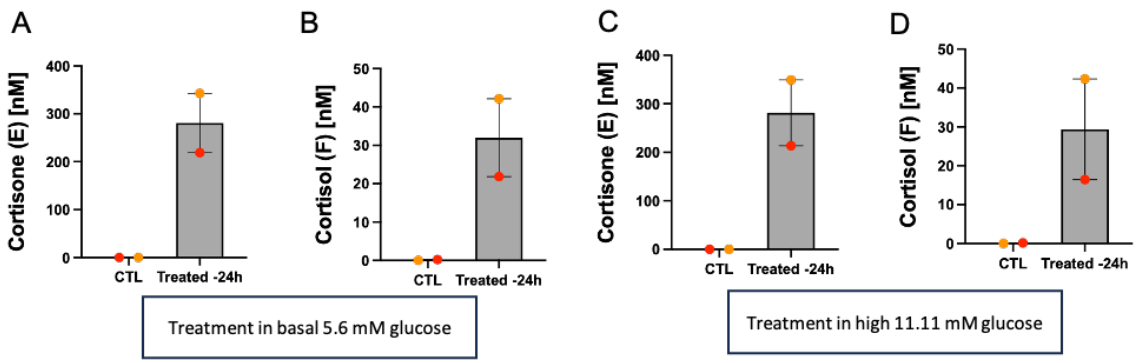


Supplementary Figure 8: Impact of sex dimorphism on *HSD11B1*, *HSD11B2* and *SRD5A1* expression profile in human metabolic cohort: **(A)** HSD11B1 mRNA, **(B)** HSD11B2 mRNA, and (**C)** *SRD5A1* mRNA levels in islets from *n* = 93 donors, stratified according to their sex from lean normoglycemic donors (*n* = 15, BMI < 25, HbA1c < 5.7), normoglycemic donors with obesity (*n* = 25, BMI ≥ 25, HbA1c < 5.7), donors with obesity and glucose-intolerance (*n* = 24, BMI > 25, HbA1c ≥ 5.7), and donors with obesity and T2D (*n* = 12, BMI ≥ 25, HbA1c ≥ 6.4). Unpaired and non-parametric t-test was performed.

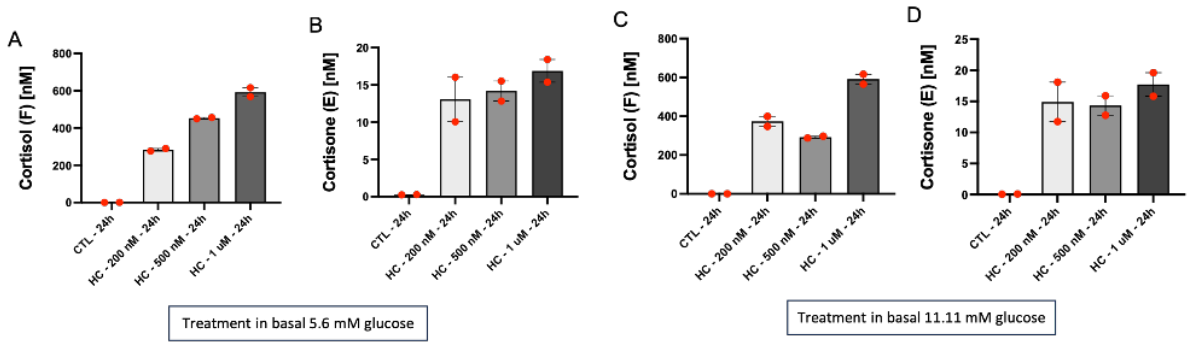


Supplementary Figure 9: Varying glucose concentration did not affect levels of HC uptake by the islets or on the cortisol metabolites generated.

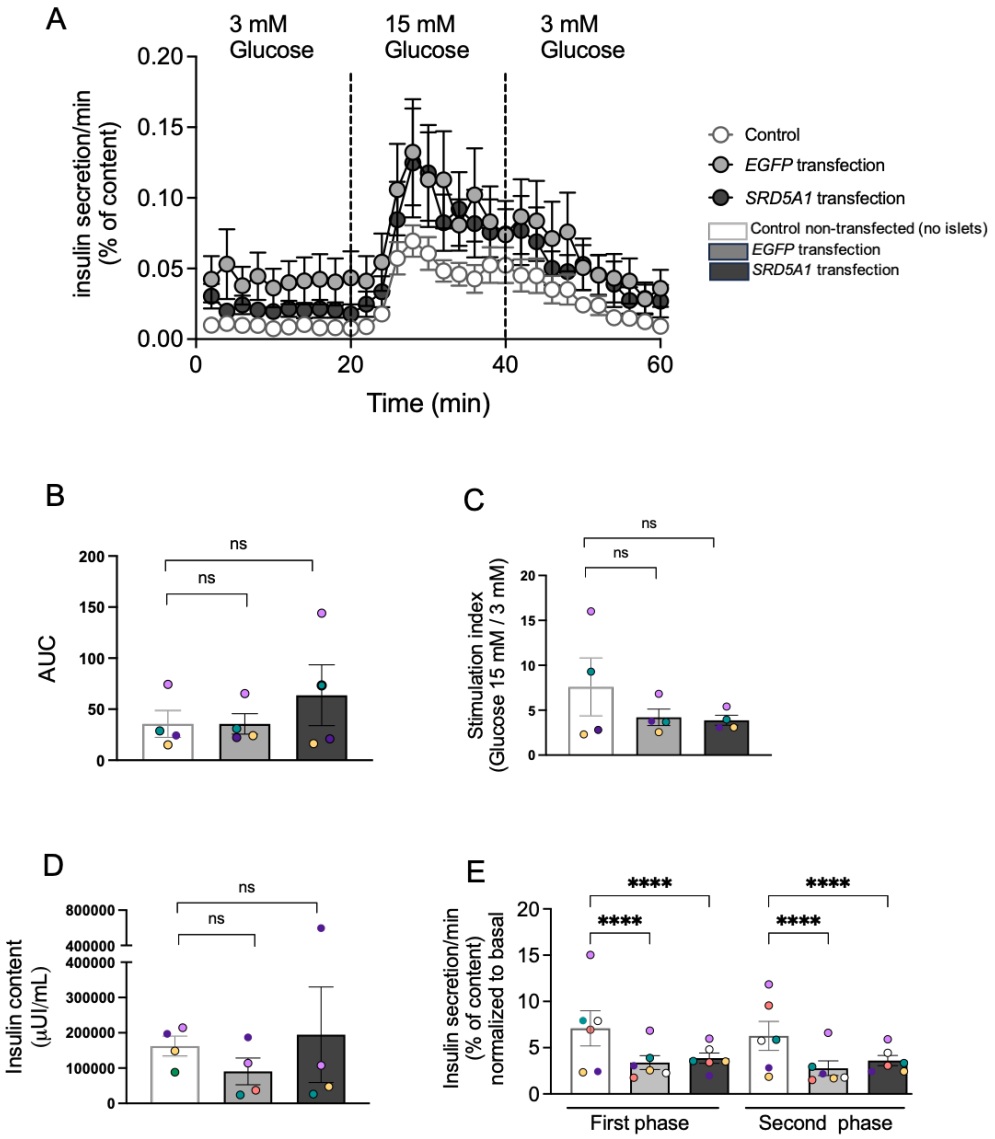
A-H: Islets treated with 500 nM of HC and cultured in low, basal, high and superhigh glucose for 24H. **A**: Cortisol levels in control non-treated islets (CTL), (white bars) and in treated islets treated (grey bars); **B**: Cortisone generated by 11β-HSD2 activity, cultured, in low 2.8 mM glucose. **C:** Cortisol levels in control non-treated islets (CTL), (white bars) and in treated islets treated (grey bars); **D:** Cortisone generated by 11β-HSD2 activity, cultured, in 5.6 basal mM glucose. **E:** Cortisol levels in control non-treated islets (CTL), (white bars) and in treated islets treated (grey bars**); F:** Cortisone generated by 11β-HSD2 activity, cultured, in high 11.11 mM glucose. **G**: Cortisol levels in control non-treated islets (CTL), (white bars) and in treated islets treated (grey bars); **H**: Cortisone generated by 11β-HSD2 activity, cultured, in high 11.11 mM glucose. Experiments were carried out in *n*= 2 (donors ID: H1152, H1154), and at least in biological duplicate.



Supplementary Figure 10: Varying glucose concentration did not affect levels of cortisone uptake by the islets or on the cortisol metabolites generated. **A-B:** Islets treated for 24h with 500 nM of cortisol and cultured in 5.6 mM basal glucose, **A:** Cortisone levels in control non-treated islets (CTL), (white bars) and in treated islets treated (grey bars); **B:** Cortisol generated by 11β-HSD1 activity, cultured. **C-D**: Islets treated for 24h with 500 nM of cortisone and cultured in 5.6 mM basal glucose, **C:** Cortisone levels in control non-treated islets (CTL), (white bars) and in treated islets treated (grey bars); **D:** Cortisol generated by 11β-HSD1 activity, cultured. Experiments were carried out in *n*= 2 (donors ID: H1152, H1154), and at least in biological duplicate



Supplementary Figure 11: Varying glucose concentration did not affect levels of cortisol uptake by the islets or measured cortisol metabolites in islet supernatant. Islet were treated with 200 nM, 500 nM and 1 µM of HC for 24h. **A-B:** Islets cultured in 5.6 mM basal glucose, **A:** Cortisol levels, and B: Cortisone generated by 11β-HSD2 activity in control (white bar) and treated islets (grey bars)**. C-D:** Islets cultured in 11.11 mM high glucose, **C:** Cortisol levels, and **B:** Cortisone generated by 11β-HSD2 activity in control (white bar) and treated islets (grey bars). Experiments were carried out in *n*= 2 (donors ID: H1152, H1154), and at least in biological duplicate.



Supplementary Figure 12: GSIS profile of non-transfected and transfected islets used for the HC treatment. Glucose-stimulated insulin secretion assessed by dynamic perifusion in non-transfected human islet (white bars), or islets transfected with EGFP (light grey bar) or SRD5A1 mRNA at 0.6µg (dark grey bar) for 24h (*n*=4). **A:** GSIS evaluation. **B:** AUC. **C:** Stimulation index. **D**: Insulin content in human islets at the end of dynamic perifusion experiments. **E:** comparison between the first and second phases of insulin secretion. The first and second phases of insulin secretion were calculated as the first 10 minutes and the last 10 minutes of 15 mmol/L glucose stimulation, respectively. Comparison was performed using one-way ANOVA and Friedman non-parametric multiple comparison *post hoc* test for panel. ****p<0.0001