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PET Imaging of Incretin Receptors and Pancreatic Islets

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Abstract

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Diabetes is a complex metabolic disease characterised by impaired regulation of blood glucose and, in some forms, loss or dysfunction of insulin-producing pancreatic beta cells. Incretin receptors, particularly the glucagon-like peptide-1 receptor (GLP-1R) and glucose-dependent insulinotropic polypeptide receptor (GIPR), have become important therapeutic targets in diabetes and obesity. At the same time, the ability to non-invasively visualise pancreatic islets remains an important need, particularly for monitoring islet replacement therapies.

This thesis explores the development and application of positron emission tomography (PET) imaging to study incretin receptors and pancreatic islets, with the overall aim of improving our understanding of diabetes biology and emerging, incretin based, therapeutic strategies. In **Paper I**, the GIPR-targeting PET tracer [^{68}Ga]Ga-DOTA-GIP1234 was developed with a fatty acid modification designed to prolong blood circulation time through albumin binding and improve receptor accessibility. The tracer demonstrated specific GIPR binding, although its prolonged circulation was accompanied by high blood and liver activity. In **Paper II**, PET imaging was used to investigate target engagement of the dual incretin agonist tirzepatide and the triple agonist SAR441255 in healthy pigs. Dose-dependent GLP-1R occupancy was demonstrated in the pancreas, pituitary and medial hypothalamic region, showing the potential of using PET to quantify incretin drug–target engagement in vivo. In **Paper III**, the small-molecule tracer [^{11}C]Methyl-V-0219 was developed for imaging GLP-1R in the brain. Although the tracer crossed the blood–brain barrier, no specific GLP-1R binding could be demonstrated in vivo, highlighting important challenges for brain GLP-1R PET imaging. In **Paper IV**, a 68Ga-labelled nanobody targeting DPP6 was developed for PET imaging of pancreatic islets. The tracer enabled sensitive detection of transplanted human islets and stem cell-derived islets in mice, supporting DPP6 as a promising target for monitoring islet replacement therapies.

In conclusion, this thesis demonstrates the versatility of PET imaging for studying incretin receptor and pancreatic islet biology. The developed approaches provide tools for investigating where and to what extent some of incretin-based drugs engage their targets, while also advancing the possibility of non-invasive monitoring of transplanted insulin-producing cells.

Keywords: PET imaging, diabetes, beta-cell mass, incretins, SC-islets, DPP6, exendin-4, GLP1, GIP

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Not all those who wander are lost...
Bilbo Baggins
(J.R.R. Tolkien)

To my family, who always believed in me
To Nelly & Mira

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Khalil, A**., Hakhverdyan, S., Cheung, P., Bossart, M., Wagner, M., Eriksson, O., & Velikyan, I. (2024). Introduction of a fatty acid chain modification to prolong circulatory half-life of a radioligand towards glucose-dependent insulinotropic polypeptide receptor. *Nuclear Medicine and Biology*, 128–129.

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IV. **Khalil, A**.*, Roca-Rivada*, A., Vasylovska, S., Balestri, L. J. I., Velikyan, I., Jawurek, S., Title, A. C., Yesildag, B., Odell, L. R., Lau, J., Eizirik, D. L., & Eriksson, O. (2026). A DPP6-targeted PET tracer for non-invasive imaging of transplanted human islets. *Diabetes, Obesity and Metabolism*.

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Abbreviations

ACN	Acetonitrile
AMF	Ammonium formate
ANOVA	Analysis of variance
BBB	Blood–brain barrier
Bmax	Maximum receptor binding capacity
BP	Binding potential
BSA	Bovine serum albumin
Cmax	Maximum plasma concentration
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
CVO	Circumventricular organ
DMF	N,N-Dimethylformamide
DO3A	1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic acid
DOTA	1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid
DPP6	Dipeptidyl peptidase-like protein 6
EC50	Half-maximal effective concentration
ELISA	Enzyme-linked immunosorbent assay
EOS	End of synthesis
FOV	Field of view
GCG	Glucagon
GCGR	Glucagon receptor
GIP	Glucose-dependent insulinotropic polypeptide
GIPR	Glucose-dependent insulinotropic polypeptide receptor
GLP-1	Glucagon-like peptide-1
GLP-1R	Glucagon-like peptide-1 receptor
GMP	Good Manufacturing Practice
GPCR	G protein-coupled receptor
HEK293	Human embryonic kidney 293 cells
HPLC	High-performance liquid chromatography
HSA	Human serum albumin
IEQ	Islet equivalents
IHC	Immunohistochemistry
iPSC	Induced pluripotent stem cell

iTLC	Instant thin-layer chromatography
Kd	Equilibrium dissociation constant
MIP	Maximum intensity projection
MRI	Magnetic resonance imaging
NCS	N-Chlorosuccinimide
NET	Neuroendocrine tumour
NHP	Non-human primate
NMR	Nuclear magnetic resonance
NOTA	1,4,7-Triazacyclononane-1,4,7-triacetic acid
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PET	Positron emission tomography
PK	Pharmacokinetics
QC	Quality control
RCP	Radiochemical purity
RCY	Radiochemical yield
ROI	Region of interest
RSA	Rat serum albumin
RT	Room temperature
SC-islets	Stem cell-derived islets
SCID	Severe combined immunodeficient
scRNA-seq	Single-cell RNA sequencing
SD	Standard deviation
sdAb	Single-domain antibody
SEM	Standard error of the mean
siRNA	Small interfering RNA
SPE	Solid-phase extraction
SPECT	Single-photon emission computed tomography
SPPS	Solid-phase peptide synthesis
SUV	Standardised uptake value
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TAC	Time–activity curve
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Tmax	Time to maximum plasma concentration
TPS	Tracer Production System
UV	Ultraviolet
VOI	Volume of interest

Introduction

Diabetes Mellitus as a Global Health Burden

Diabetes mellitus represents one of the most significant and rapidly expanding public health challenges worldwide. Currently, an estimated 589 million adults are living with the disease, corresponding to approximately one in nine adults globally. Ongoing demographic changes, including population ageing, along with changes in lifestyle and environmental factors, are expected to further increase prevalence. Projections indicate that the number of affected individuals could reach 853 million by 2050 (*1*). Beyond its high prevalence, diabetes is associated with substantial morbidity and mortality, contributing to the development of serious long-term complications and placing a considerable burden on healthcare systems (*2*).

Diabetes mellitus comprises a group of metabolic disorders characterised by chronically elevated blood glucose levels resulting from impaired insulin secretion, reduced insulin sensitivity, or both. Prolonged hyperglycemia can lead to progressive damage to multiple organ systems, including the eyes, kidneys, nervous system, cardiovascular system, and blood vessels, resulting in substantial morbidity and mortality (*3*).

The two most prevalent forms are type 1 diabetes (T1D) and type 2 diabetes (T2D). T1D is caused by autoimmune destruction of insulin-producing β-cells in the pancreatic islets, leading to an absolute deficiency of insulin (*4*). In contrast, T2D is characterised by insulin resistance combined with a progressive decline in β-cell function, ultimately resulting in inadequate insulin secretion (*5*). Although the underlying mechanisms differ, both conditions disrupt glucose homeostasis and can lead to similar long-term complications when glycaemic control is not adequately maintained (*3*).

Therefore, understanding pancreatic anatomy and islet biology is fundamental to understanding the pathophysiology of diabetes and provides the basis for developing therapeutic strategies, monitoring disease, and emerging approaches to disease prediction.

Pancreas Biology

The pancreas is a mixed-function gland located in the upper abdomen, posterior to the stomach, and serves both exocrine and endocrine functions (*6*). Approximately 95–98% of the pancreatic tissue comprises the exocrine compartment, which consists of acinar and ductal cells responsible for the synthesis, secretion, and transport of digestive enzymes into the duodenum. The remaining 2–5% forms the endocrine pancreas, which plays a central role in maintaining glucose homeostasis by secreting hormones directly into the bloodstream (*7*). The endocrine pancreas is organised into clusters of cells known as the islets of Langerhans, which are dispersed throughout the pancreatic parenchyma. Human islets contain several specialised endocrine cell populations, including insulin-producing β-cells, glucagon-secreting α-cells, somatostatin-producing δ-cells, pancreatic polypeptide-producing PP cells, and ghrelin-producing ε-cells. Together, these cell types coordinate the regulation of glucose metabolism through tightly regulated hormone secretion (*8*).

Among the endocrine cell populations, β-cells are the predominant cell type, accounting for approximately 50–70% of cells in human islets, depending on the study (*9*)(*10*). In response to elevated blood glucose concentrations, β-cells secrete insulin, which promotes glucose uptake by peripheral tissues and suppresses hepatic glucose production, thereby restoring normoglycaemia. Conversely, α-cells secrete glucagon during hypoglycaemia, stimulating hepatic glucose release, while the remaining endocrine cell types modulate islet hormone secretion through paracrine signalling. This coordinated interplay among endocrine cell populations is essential for maintaining metabolic homeostasis (*11*).

Given the pivotal role of β-cells in glucose regulation, their dysfunction or loss is a defining feature of both type 1 and type 2 diabetes (*2*). Consequently, therapies aimed at preserving or enhancing β-cell function have become a major focus of diabetes research. Among these, incretin-based therapies have emerged as highly effective treatments owing to their ability to potentiate glucose-dependent insulin secretion while providing additional metabolic benefits (*12*).

Incretin Biology

The concept of incretins originated in the early 1900s when researchers hypothesised that hormones originating from the gut might influence the endocrine secretion of pancreatic islet cells and contribute to the regulation of blood glucose levels (*13*). The term *incretin* was introduced in 1932, when a Belgian physiologist, Jean La Barre, proposed the concept of "gastro-intestinal

insulinotropic action" (*14*) The original definition suggests that any gut-produced hormone that naturally stimulates or facilitates the secretion of pancreatic hormones such as insulin, glucagon, and pancreatic somatostatin is classified as an incretin (*14*). Over time, this concept has evolved into today's definition, which identifies incretins as a group of hormones comprising two main members: Glucagon-Like Peptide-1 (GLP-1) and Glucose-Dependent Insulinotropic Polypeptide (GIP). Both hormones are secreted by the gastrointestinal tract into the bloodstream in response to oral food intake and play central roles in regulating blood glucose levels (*15*). GLP-1 receptors (GLP-1R) and GIP receptors (GIPR) belong to the family of G protein-coupled receptors and are expressed across various organs and tissues, where they play significant roles in diverse physiological processes **(Figure 1)** (*15*). The insulinotropic effects of GLP-1 are mediated by GLP-1R expressed on pancreatic β-cells. Activation of these receptors stimulates insulin secretion, enhances β-cell proliferation and neogenesis, and stimulates β-cell survival (*12*). GLP-1 receptors are also widely distributed throughout the brain, particularly in regions involved in the regulation of food intake, such as the lateral, ventromedial, dorsomedial, paraventricular nucleus, and arcuate nuclei, as well as other hypothalamic regions (*16*). Furthermore, activation of GLP-1R in the heart demonstrates beneficial cardiac effects, including improved heart contractility, enhanced myocardial glucose uptake, reduced cardiomyocyte apoptosis, and protection against ischemia-reperfusion injury (**Figure 1)** (*17*).

In contrast, GIP receptors (GIPR) are primarily situated in the pancreas, adipose tissue, the gastrointestinal tract, and, to some extent, in the brain (*15*). GIP primarily promotes glucose-dependent insulin secretion and also plays an important role in regulating lipid metabolism and bone formation (**Figure 1)** (*18*).

This widespread distribution of incretin receptors across peripheral organs and the central nervous system underscores the complexity of incretin signalling, which extends beyond pancreatic insulin secretion to regulate appetite, energy balance, and cardiovascular function. Consequently, the physiological effects of incretins reflect the integrated engagement of multiple target tissues rather than isolated actions at a single site.

A defining feature of incretin hormones is their rapid enzymatic degradation. Both GLP-1 and GIP are inactivated by dipeptidyl peptidase-4 (DPP-4), resulting in short biological half-lives and tightly regulated temporal activity (*18*). Although this rapid inactivation enables precise physiological control, it also limits the duration of incretin action and contributes to differences in hormone availability across target tissues.

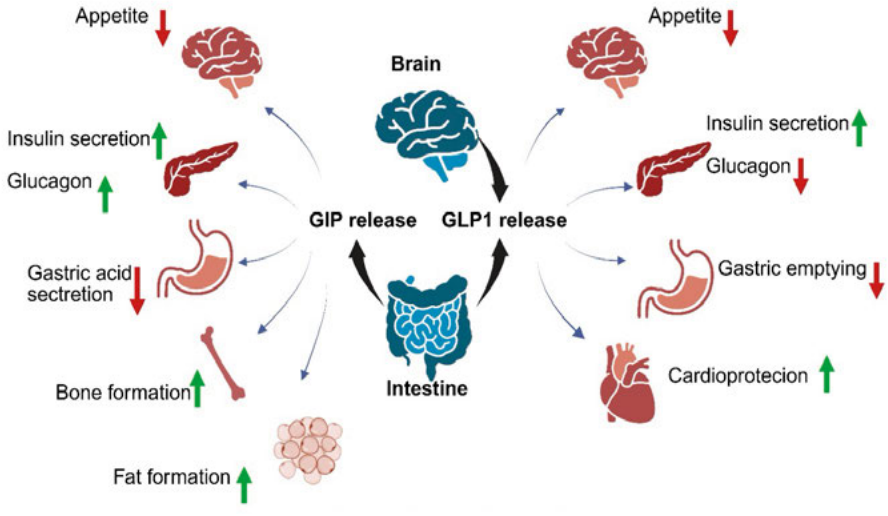

Figure 1. Overview of GLP-1 and GIP biosynthesis, receptor expression in tissues, and their action in the body. *BioRender (2026).*

Incretins and the Gut–Brain Axis

Beyond their well-established role in peripheral glucose regulation, incretins also function as key mediators of communication between the gastrointestinal tract and the central nervous system, forming an important component of the gut–brain axis (*19*). Following nutrient ingestion, incretin hormones released by intestinal enteroendocrine cells transmit metabolic signals not only to pancreatic islets but also to the brain, thereby integrating peripheral nutrient sensing with central mechanisms involved in the regulation of appetite, energy balance, and glucose homeostasis (*12*,*19*).

GLP-1, in particular, has been shown to influence central pathways through both indirect and direct mechanisms. Endogenously released GLP-1 can activate vagal afferent neurons innervating the gastrointestinal tract, transmitting signals to brainstem nuclei such as the nucleus tractus solitarius, which in turn communicate with hypothalamic regions involved in metabolic control (*19*,*20*). In addition, GLP-1–producing neurons in the brainstem project to multiple regions of the central nervous system, including the hypothalamus and mesolimbic structures, supporting a role for centrally mediated incretin signalling in the regulation of satiety and food intake (*19*,*21*).

Receptors for GLP-1 and GIP are expressed in discrete regions of the brain, including the hypothalamus, brainstem, and areas associated with reward processing (*20*,*22*). Activation of these receptors has been shown to modulate

food intake, body weight, and energy expenditure, highlighting the contribution of central incretin signalling to metabolic regulation. Importantly, the relative contributions of peripheral versus central receptor activation appear to differ between physiological incretin signalling and pharmacological interventions. Therapeutic agents, which exhibit enhanced stability and prolonged exposure, may therefore facilitate greater engagement of central receptors (*19*,*20*,*22*).

The clinical importance of the incretin gut–brain axis has become increasingly evident with the success of incretin-based therapies, which produce substantial and sustained reductions in appetite and body weight. These effects extend beyond improvements in glycaemic control, highlighting the contribution of central mechanisms to the therapeutic effects of incretin receptor agonists (*23*,*24*). However, the extent to which different incretin-based therapies directly engage central receptors, and how this influences their efficacy and adverse-effect profiles, remains incompletely understood.

Together, these observations underscore the complexity of incretin signalling along the gut–brain axis and highlight the importance of approaches that can interrogate incretin receptor engagement in both peripheral and central compartments. Improved understanding of these pathways is essential to elucidate the mechanisms underlying the metabolic effects of incretin-based therapies and for guiding the development and evaluation of next-generation therapeutic strategies.

Incretins as Therapeutic Targets

Growing evidence supporting the diverse physiological actions of incretin hormones has established the incretin system as an attractive therapeutic target for the treatment of metabolic diseases. Advances in the understanding of incretin biology have led to the development of pharmacological agents that enhance incretin receptor signalling. The therapeutic potential of the incretin system became apparent with the recognition of the limitations imposed by the rapid enzymatic degradation of endogenous incretin hormones (*15*). In response, efforts focused on identifying incretin-like compounds with prolonged biological activity. One such breakthrough came with the discovery of Exendin-4, a peptide originally isolated from the saliva of the Gila monster lizard (*Heloderma suspectum*) (*25*).

Exendin-4 shares structural similarities with endogenous GLP-1 and mimics its insulinotropic effects, while exhibiting increased resistance to DPP-4-mediated degradation and higher potency at the GLP-1R (*26*). Recognising the significant potential of Exendin-4 to stimulate insulin secretion and

manage blood glucose levels, the first GLP-1R agonist, called Exenatide, was synthesised based on its structure (*27*). Exenatide received FDA approval in 2005 for the treatment of T2D as an injectable suspension, marking a significant milestone in diabetes therapeutics within the category of incretin mimetics (*28*).

This achievement catalysed the development of additional injectable GLP-1R agonists, including liraglutide and later semaglutide, with improved pharmacokinetic properties, thereby expanding the therapeutic options available for the management of T2D. GLP-1R agonists were rapidly integrated into clinical practice and, when feasible, are commonly favoured over insulin-based therapy (*29*). In addition to improving glycaemic control, the effects of GLP-1R agonists on appetite regulation and satiety have supported their inclusion in clinical guidelines, including recommendations by the American Diabetes Association, for the pharmacological management of obesity (*30*).

In addition to selective GLP-1R agonism, increasing attention has been directed towards the development of dual incretin agonists that integrate complementary physiological pathways within the incretin system. This strategy combines the distinct yet overlapping roles of GLP-1R and GIPR signalling in glucose regulation and insulin secretion, aiming to achieve enhanced metabolic effects through coordinated receptor activation. This has led to the development of novel therapeutics such as tirzepatide, a dual agonist targeting both the GLP-1R and GIPR (*31*). Tirzepatide has shown promising results in clinical trials, demonstrating superior efficacy in reducing glycated haemoglobin (HbA1c) levels and reducing body weight compared to traditional GLP-1R agonists alone (*32*).

Building on this concept, ongoing research is exploring the potential of triple incretin agonists that additionally target the glucagon receptor (GCGR), in addition to GLP-1R and GIPR (*33*). By engaging all three receptors, these multi-incretin agonists aim to achieve broader metabolic benefits through complementary effects on insulin secretion, energy expenditure, appetite regulation, and glucose homeostasis. Among these agents, retatrutide has emerged as a promising therapeutic candidate, demonstrating substantial reductions in body weight and marked improvements in glycaemic control in clinical trials, highlighting the therapeutic potential of triple receptor agonism (*34*).

Despite the clinical success of incretin-based therapies, determining how these agents engage their targets across different tissues remains challenging (*24*). Incretin receptors are widely distributed throughout peripheral organs and the central nervous system, and therapeutic modulation of incretin signalling can elicit diverse biological effects that are not fully captured by systemic biomarkers alone (*24*). As incretin-based treatments have evolved from single-receptor agonists to dual- and triple-agonist strategies, the complexity of

their pharmacological profiles has increased, complicating the assessment of receptor engagement under physiological and therapeutic conditions (35). Consequently, there is a need for approaches that enable the non-invasive assessment of incretin receptor signalling in vivo, providing spatial and quantitative information on target engagement across tissues.

β-Cell Replacement Strategies

Although pharmacological therapies, including incretin-based agents, have significantly improved the management of diabetes, they cannot restore the loss of functional β-cell mass that characterises T1D or halt the progressive decline in β-cell function associated with advanced T2D (*4*,*5*). Consequently, increasing attention has been paid to therapeutic strategies aimed at replacing or regenerating insulin-producing β-cells to restore endogenous insulin secretion (*35*). The concept of β-cell replacement has been validated clinically through both whole-pancreas and pancreatic islet transplantation, demonstrating that restoration of endogenous insulin production can markedly improve glycaemic control and, in selected individuals, achieve prolonged insulin independence (*36*,*37*). More recently, advances in stem cell biology have enabled the generation of functional stem cell-derived islets (SC-islets), creating the possibility of an essentially unlimited source of transplantable β-cells (*38*). Importantly, β-cell replacement therapies have now entered clinical practice, with recent studies demonstrating successful transplantation of stem cell-derived islets in individuals with T1D (*39*). Despite these advances, reliable non-invasive methods to assess graft survival and function remain essential for evaluating therapeutic efficacy and supporting the broader clinical implementation of β-cell replacement therapies.

In Vivo Assessment of Incretin Signalling and β-Cell Mass

The expanding role of incretin-based therapies and the emergence of β-cell replacement strategies have created a growing need for methods to assess these interventions directly in vivo. Conventional clinical biomarkers, including blood glucose, HbA1c, C-peptide, and body weight, provide valuable information on therapeutic outcomes but offer only indirect measures of receptor activation, β-cell mass, and graft function (*40*). Consequently, techniques that enable the non-invasive and quantitative assessment of incretin receptor engagement and β-cell survival across different tissues are essential for

advancing our understanding of disease mechanisms, optimising therapeutic strategies, and evaluating treatment responses (*41*,*42*).

Positron Emission Tomography as a Non-invasive Molecular Imaging Tool

Positron emission tomography (PET) is a highly sensitive, non-invasive molecular imaging technique that enables the visualisation and quantification of biological processes in vivo (*43*). A PET scan involves a radioactive tracer, typically a compound labelled with a positron-emitting radionuclide, such as fluorine-18 (^{18}F), carbon-11 (^{11}C), gallium-68 (^{68}Ga), or oxygen-15 (^{15}O). Following administration, the tracer undergoes radioactive decay via positron emission, which interacts with a nearby electron, producing two gamma rays that travel in opposite directions **(Figure 2).** A ring of detectors encircling the patient detects the two gamma rays simultaneously, and this data is used to reconstruct images depicting the tracer's distribution throughout the body. PET is usually combined with Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) to obtain a structural image **(Figure 2)** (*43*).

The development of receptor-specific PET tracers has enabled the non-invasive investigation of incretin biology in vivo (*43*). By providing quantitative information on receptor expression, tissue distribution, and target engagement, PET complements conventional physiological and biochemical assessments and has become an important tool for investigating the mechanisms of incretin-based therapies (*41*,*44*,*45*). In particular, PET uniquely enables the evaluation of receptor occupancy after drug administration, providing direct insight into the extent and location of receptor engagement across different organs and tissues (*41*,*44*,*45*).

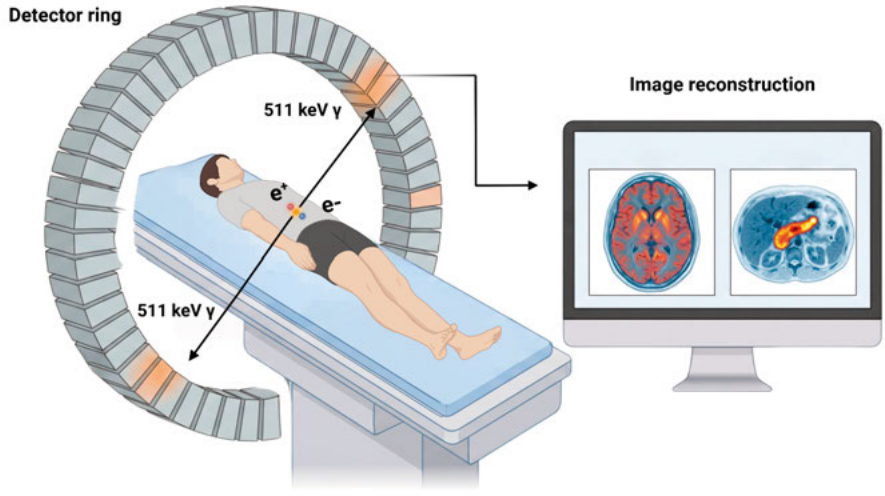

Figure 2. Principle of PET imaging. Following administration of a positron-emitting radiotracer, emitted positrons interact with electrons in surrounding tissue, resulting in annihilation and the production of two 511-keV photons emitted in two opposite directions. Coincident detection of these photons by opposing detectors defines a line of response used for image reconstruction. Reconstructed PET images provide spatial information on radiotracer distribution, illustrated here for the brain and pancreas. *BioRender (2026).*

PET Imaging of Incretin Receptors

Among the available PET tracers, radiolabelled exendin analogues targeting the GLP-1R are the most extensively developed. In particular, [^{68}Ga]Ga-DO3A-Exendin-4 has demonstrated high affinity and specificity for GLP-1R, enabling the non-invasive visualisation and quantification of receptor expression in both peripheral tissues and selected regions of the central nervous system (*41*,*46–50*). Beyond mapping receptor distribution, GLP-1R PET has been successfully applied to assess receptor occupancy following administration of GLP-1R agonists and emerging dual- and triple-incretin receptor agonists. This approach provides direct insight into tissue-specific target engagement that cannot be obtained using conventional systemic biomarkers (*41*,*44*,*45*).

In contrast, despite the increasing therapeutic importance of GIPR, particularly in the context of dual- and triple-incretin receptor agonists, PET imaging of GIPR remains at its early stages of development. Although several GIPR-targeted radiotracers have been described in recent years, their development remains preliminary, and no clinically established imaging agent is currently available (*51*,*52*). Despite the growing importance of GIPR in

emerging dual- and triple-incretin-based treatments, which play a significant role in glucose metabolism and insulin secretion, the lack of GIPR-specific PET tracers hampers direct observation and measurement of GIPR expression in living organisms. Addressing this gap would deepen our understanding of GIPR biology and its role in metabolic processes, while enabling a more accurate assessment of incretin-based therapies and target occupancy.

PET Imaging of Pancreatic Islets

Non-invasive imaging of pancreatic islets has been an active area of research for several decades, driven by the need to visualise and assess islet mass and function in vivo. Researchers have investigated several molecular targets based on their expression in islet cells or their involvement in islet physiology (*53*). These approaches have provided valuable insights into islet biology but have also revealed important limitations that constrain their broader application.

Several molecular targets have been investigated based on their preferential expression in pancreatic β-cells or their involvement in β-cell physiology. Among these, GLP-1R has been widely investigated owing to its enrichment in β-cells (*15*,*46*,*50*,*54*) and has demonstrated promising results in both preclinical and clinical studies (*45*,*47*,*49*,*50*,*55*,*56*). However, GLP-1R expression is not restricted to pancreatic islets and is also present in other tissues, which can complicate image interpretation. In addition, receptor expression may be altered under disease conditions or following pharmacological intervention, limiting its utility as a stable marker of islet mass (*15*).

Additional molecular PET targets have also been explored for PET imaging of pancreatic islets, including vesicular monoamine transporter 2 (VMAT2) (*57*), dopamine receptors (*58*), G protein-coupled receptor 44 (GPR44) (*59*), sulfonylurea receptor 1 (SUR1) (*60*), and serotonin-related pathways (*61*,*62*). While each target offers distinct advantages, none has proven to be an ideal biomarker for β-cell imaging. Reported limitations include expression in non-β-cell populations, insufficient target specificity, poor signal-to-noise ratios, interspecies differences, dependence on β-cell functional state, and limited clinical validation (*53*). Consequently, changes in tracer uptake may reflect alterations in cellular activity or receptor regulation rather than true changes in β-cell mass. These limitations highlight the need for more specific and robust imaging biomarkers.

DPP6 as a Molecular Target for Imaging Human Pancreatic Islets

Identifying molecular targets that are selectively and stably expressed in pancreatic islets is a prerequisite for developing reliable imaging strategies to visualise islet tissue in vivo. In this context, dipeptidyl peptidase 6 (DPP6) has emerged as a promising candidate target for imaging human pancreatic islets (*53*).

DPP6 is a membrane-associated protein that is highly expressed in human pancreatic β cells and, to a lesser extent, α cells, with comparatively low expression in the surrounding exocrine pancreas and other peripheral tissues (*63*). This expression pattern makes DPP6 particularly attractive as an islet-selective marker, as it enables the discrimination of islet tissue from adjacent pancreatic structures. Importantly, DPP6 expression has been shown to be preserved in human islets, supporting its potential as a stable target for longitudinal imaging of human pancreatic islets (*63*,*64*).

Targeting DPP6 is particularly attractive for monitoring islet transplantation. Preservation of DPP6 expression following transplantation enables direct visualisation of transplanted human islet grafts in vivo, facilitating the longitudinal assessment of graft survival independently of changes in insulin secretory function (*63*,*64*).

This characteristic is especially relevant for monitoring graft engraftment, survival, and loss over time, as changes in islet mass may not be immediately reflected by systemic metabolic markers.

The Role of PET Imaging in Diabetes Research

Positron emission tomography has emerged as a powerful tool for investigating diabetes at the molecular level. By enabling the non-invasive visualisation and quantification of biological processes in vivo, PET provides unique insights into receptor expression, drug target engagement, and β-cell biology that cannot be obtained using conventional clinical biomarkers alone(*65*). These capabilities make PET an important platform for investigating disease mechanisms, evaluating therapeutic interventions, and supporting the development of novel treatments.

In the context of incretin-based therapies, PET imaging offers the opportunity to directly assess receptor distribution and occupancy, thereby improving our understanding of the pharmacological mechanisms underlying single-, dual-, and triple-receptor agonists (*44*,*45*,*51*). At the same time, advances in β-cell-specific imaging biomarkers have created new opportunities to quantify endogenous β-cell mass and monitor transplanted islet grafts, supporting the

development and clinical translation of β-cell replacement therapies (*53*,*63*,*64*).

Continued advances in radiotracer development, imaging technology, and quantitative image analysis are expected to further expand the role of PET in diabetes research. Together, these developments position PET as a valuable translational tool for advancing our understanding of diabetes pathophysiology, guiding therapeutic development, and enabling more precise assessment of treatment response.

Aims of the Thesis

The overall aim of this thesis was to develop and apply PET imaging approaches to advance the understanding of incretin receptor and pancreatic islet biology through preclinical and translational imaging studies. Specifically, this work aims to investigate receptor biology and target engagement of emerging incretin-based therapies, while developing novel PET tracers for imaging GIPR and pancreatic islets.

The specific objectives of the individual papers were:

Paper I. To develop and evaluate the modified GIPR-targeting PET tracer [^{68}Ga]Ga-DOTA-GIP1234, incorporating a fatty acid moiety to prolong its circulatory half-life and improve receptor accessibility. The tracer was characterised in terms of receptor binding, pharmacokinetics, and biodistribution using relevant in vitro and in vivo models.

Paper II. To investigate the dose-dependent target engagement and receptor occupancy of the dual incretin agonist tirzepatide and the triple incretin agonist SAR441255 at GLP-1R in the pancreas and central nervous system using [^{68}Ga]Ga-DO3A-Exendin-4 PET. In addition, GIPR target engagement by SAR441255 in the pancreas was evaluated using [^{68}Ga]S02-GIP-T4 PET. These studies were performed in healthy pigs as a translational large-animal model.

Paper III. To establish and optimise the radiochemical synthesis of [^{11}C]Methyl-V-0219 and to evaluate its potential as a brain-penetrant PET tracer for imaging GLP-1R through in vitro and in vivo characterisation.

Paper IV. To develop and evaluate a ^{68}Ga-labelled 4hD29 nanobody targeting DPP6 for PET imaging of pancreatic islets. The tracer was evaluated in mouse models transplanted with human islets or stem cell-derived islets to assess its sensitivity for detecting grafts containing low numbers of transplanted islets.

Methodology

Radiolabelling

Radiolabelling of [^{68}Ga]Ga-DOTA-GIP1234 and [^{68}Ga]Ga-NOTA-4hD29 for the healthy pig biodistribution studies, as well as [^{68}Ga]Ga-DO3A-Exendin-4, [^{68}Ga]Ga-S02-GIP-T4 and [^{11}C]Methyl-V-0219, was performed at Akademiska Sjukhuset. Radiolabelling of [^{68}Ga]Ga-DOTA-GIP1234 and [^{68}Ga]Ga-NOTA-4hD29 for in vitro cell studies, ex vivo biodistribution studies and rodent PET imaging was performed by the author at the Preclinical PET Platform (PPP), Uppsala University.

Tracer Stability

The stability of evaluated [^{68}Ga]Ga-DOTA-GIP1234 and [^{68}Ga]Ga-NOTA-4hD29 tracers was assessed at predefined time points in a formulation buffer and plasma. In vitro stability was evaluated in rat or human plasma, while in vivo stability was assessed in rat plasma following tracer administration. These analyses were performed to confirm radiochemical stability of the tracers over the PET imaging period.

In Vitro Autoradiography

Relevant tissue or cell pellet samples were cryosectioned at 20 µm using a microtome. Sections were incubated with the radiotracer under baseline or blocking conditions to assess specific binding and, where applicable, receptor selectivity. After incubation, the sections were washed, dried and exposed to phosphor imaging plates for at least four half-lives of the radionuclide. The plates were then scanned using a phosphor imaging scanner, and the resulting autoradiograms were analysed using ImageJ software.

The technique relies on capturing localised radioactive decay from a radiolabelled ligand that selectively binds to its target within thin, structurally preserved biological sections. This allows for the precise visualisation and quantification of both the spatial distribution and relative density of tracer accumulation across the section.

Stem-cell Derived Islets

Human embryonic stem cells (hESCs; H1 cell line, WiCell®) were differentiated into stem cell-derived islet-like clusters (SC-islets) using an established seven-stage differentiation protocol (*66*) . The differentiation was performed by Dr Joey Lau's research group at Uppsala University.

In Situ Albumin Binding Assay Using Native PAGE

To evaluate the binding interactions of DOTA-GIP1234, [^{68}Ga]Ga-DOTA-GIP1234 and DOTA-GIP-C803 with bovine serum albumin (BSA) or rat serum albumin (RSA), in situ native polyacrylamide gel electrophoresis (PAGE) was performed. This technique separates molecules according to their intrinsic net charge, size and conformation while preserving non-covalent interactions. Because the acrylamide matrix acts as a molecular sieve, the highly mobile free peptide can be distinguished from the larger, more slowly migrating albumin. A shift in migration pattern or reduction in the free-peptide signal therefore indicates peptide–albumin interaction. The experiments were performed at Akademiska Sjukhuset.

Ethics Approval and Consent to Participate

All the performed procedures were in accordance with the ARRIVE guidelines for animal research. The Animal Ethics Committee of the Swedish Animal Welfare Agency ethically reviewed and authorised the animal experiments, which were carried out in accordance with European Directive 2010/63/EEC and the institutional guidelines at Uppsala University (UFV 2007/724).

All human biological sample donors in Sweden provide informed consent that their donated tissues may be entered into a BioBank and used in medical research, pending review and approval by the Swedish Ethical Review Authority of individual studies. The use of human tissues and islets from Uppsala Biobank (registration #827) was approved by the Swedish Ethical Review Authority (2015/401(pancreas, spleen), 2019/02790 (liver), 2024-00969) (heart, lung) and were anonymised, collected, and treated according to local institutional and Swedish national rules and regulations. The use of human blood from the local biobank was approved by the Swedish Ethical Review Authority (2024-05050). The differentiation and use of iPSCs into islet-like cells was approved by the Ethics Committee of the Erasmus Hospital, Université Libre de Bruxelles, reference P2019/498.

Animal Handling

Healthy Yorkshire × Swedish Landrace × Hampshire pigs, weighing approximately 25–35 kg, were transported from a commercial farm to the imaging facility on the day of the experiment. Anaesthesia was induced intravenously using fentanyl, ketamine and midazolam. The animals were intubated and mechanically ventilated, and arterial and venous catheters were placed for blood sampling, tracer administration and maintenance of anaesthesia.

Male Sprague–Dawley rats, weighing approximately 300–350 g, were housed in pairs under controlled temperature and humidity, with a 12 h light/dark cycle and free access to food and water. Procedures were performed under general anaesthesia with isoflurane. Depending on the study, rats were used for dynamic PET imaging, biodistribution, blocking or competition experiments.

Immunodeficient NMRI nu/nu mice were used for PET imaging of transplanted human islets and stem cell-derived islets. Islet grafts were implanted intramuscularly, and imaging procedures were performed under isoflurane anaesthesia. The mice were maintained under standard animal facility conditions with free access to food and water.

PET/CT Imaging of Pigs

The pigs were positioned supine in a clinical PET/CT scanner (Discovery MI, GE Healthcare), with either the brain or pancreas centred in the field of view depending on the study. A CT scan was acquired for attenuation correction and anatomical localisation, followed by dynamic PET imaging initiated at the time of intravenous tracer administration. Whole-body PET/CT imaging was performed when required. For selected abdominal studies, contrast-enhanced CT was acquired after PET to improve delineation of the pancreas and surrounding soft tissues.

PET Imaging of Rats and Mice

Rodent PET imaging was performed using a nanoPET/MRI system (Mediso Medical Imaging Systems, Budapest, Hungary). Animals were anaesthetised with isoflurane and positioned prone in the scanner, with either the brain, abdominal region or graft-containing muscle centred in the field of view depending on the experiment. Dynamic PET acquisition was initiated at the time of intravenous tracer administration and was followed, where applicable, by whole-body PET imaging. MRI or CT was used for anatomical

localisation, co-registration and attenuation correction, depending on the experimental protocol.

PET Image Analysis

Reconstructed PET images were analysed using PMOD software (PMOD Technologies LLC, Zurich, Switzerland). Regions of interest (ROI) were defined manually on co-registered PET/CT or PET/MRI images to delineate relevant organs, tissues and grafts. For pig brain analysis, PET images were co-registered with the PMOD pig brain MRI atlas for regional segmentation. Tracer uptake was expressed as standardised uptake value (SUV), calculated from the measured tissue activity concentration and normalised to the decay-corrected injected activity and animal body weight

Ex Vivo Biodistribution

Ex vivo biodistribution was performed in rodents to quantify tracer uptake in selected organs and tissues. At predefined time points after tracer administration, animals were euthanised, and tissues of interest were collected, weighed and measured for radioactivity using a well counter. Radioactivity was corrected for decay and normalised to injected activity and tissue weight, and expressed as SUV.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism v.11. Comparisons between two groups were performed using paired or unpaired t-tests, depending on the experimental design. Comparisons involving multiple groups were performed using one-way ANOVA with appropriate multiple-comparison testing. A p-value < 0.05 was considered statistically significant.

The present investigation

Paper I - Introduction of a fatty acid chain modification to prolong circulatory half-life of a radioligand towards glucose-dependent insulinotropic polypeptide receptor

Background and Aim

Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone secreted by intestinal K cells in response to nutrient intake. Together with glucagon-like peptide-1 (GLP-1), GIP stimulates glucose-dependent insulin secretion and contributes to the regulation of glucose homeostasis (*15*). Although the therapeutic potential of GIP was initially considered limited because of its impaired insulinotropic activity in type 2 diabetes (T2D), evidence that GIP responsiveness can be restored has renewed interest in GIP-based therapies (*67*,*68*). This has led to the development of dual and triple-incretin receptor agonists, which have demonstrated benefits for glycaemic control and body weight (*32*,*34*,*68*,*69*).

The growing clinical relevance of GIP has spurred the development of positron emission tomography (PET) tracers for the non-invasive imaging of the GIP receptor (GIPR) (*44*,*51*). However, existing GIPR radioligands have limitations, including suboptimal receptor affinity, rapid clearance, or high hepatic uptake, which reduce their suitability for imaging the healthy pancreas, where GIPRs are expressed (*44*,*51*). Although the previously developed GIPR PET tracer, [^{68}Ga]Ga-S02-GIP-T4, demonstrated high receptor affinity, its relatively high hepatic uptake reduced image contrast. To address this limitation, we subsequently developed [^{68}Ga]Ga-DOTA-C803-GIP, which exhibited lower hepatic retention. However, this tracer had relatively low affinity for GIPR and was rapidly cleared from the blood, limiting tissue exposure and receptor binding (*44*,*51*).

Based on this rationale, we hypothesised that incorporating an albumin-binding moiety into [^{68}Ga]Ga-DOTA-C803-GIP would prolong its circulatory half-life **(Figure 3)**, thereby increasing tissue exposure and improving GIPR

binding. This, in turn, was expected to enhance image contrast, particularly in the pancreas. Accordingly, the aim of this study was to evaluate the receptor binding properties and biodistribution of [^{68}Ga]Ga-DOTA-GIP1234 following the introduction of an albumin-binding moiety.

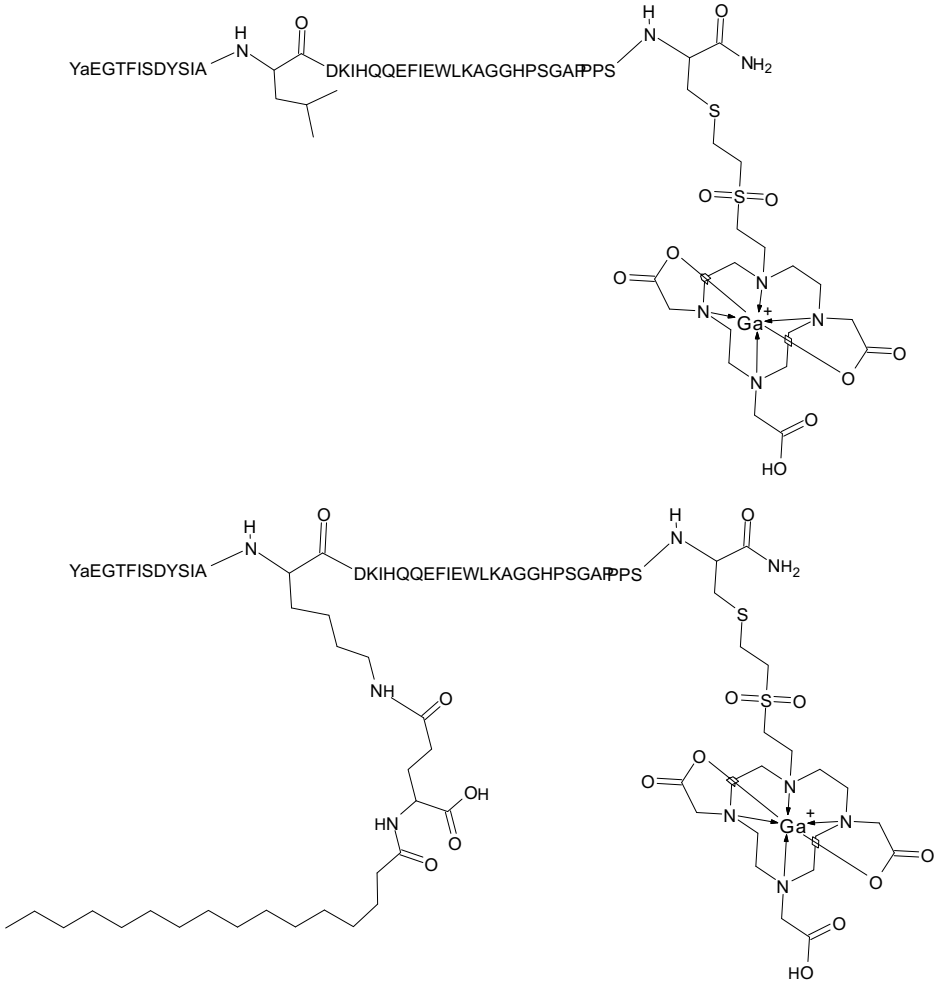

Figure 3. Amino acid sequences and key structural features of the GIPR-targeting tracers. Upper panel: [^{68}Ga]Ga-DOTA-C803-GIP containing Leu14. Lower panel: [^{68}Ga]Ga-DOTA-GIP1234, in which a palmitic acid chain is attached to Lys14 through a glutamic acid linker. In both constructs, the DOTA chelator is conjugated at the C-terminal Ser40 via a cysteine amide residue and a diethylsulfone linker.

Results

[^{68}Ga]Ga-DOTA-GIP1234 was successfully radiollabeled with a high radiochemical yield (88 ± 3.7%, n = 5) and radiochemical purity (97.8 ± 0.8%). The tracer remained stable in both the formulation buffer and rat plasma for at least 60 min after radiolabeling, indicating sufficient stability for subsequent biological evaluation.

In vitro autoradiography showed selective binding of [^{68}Ga]Ga-DOTA-GIP1234 to human GIPR-expressing HEK293 cells, with negligible binding to GLP-1R- and GCGR-expressing cells **(Figure 4A)**. The tracer exhibited a dissociation constant (K_d) of 40 ± 12.5 nM. Pre-incubation with tirzepatide reduced tracer binding by approximately 90%, confirming the receptor-specific binding to GIPR and indicating that the tracer and tirzepatide compete for the same binding site **(Figure 4B)**.

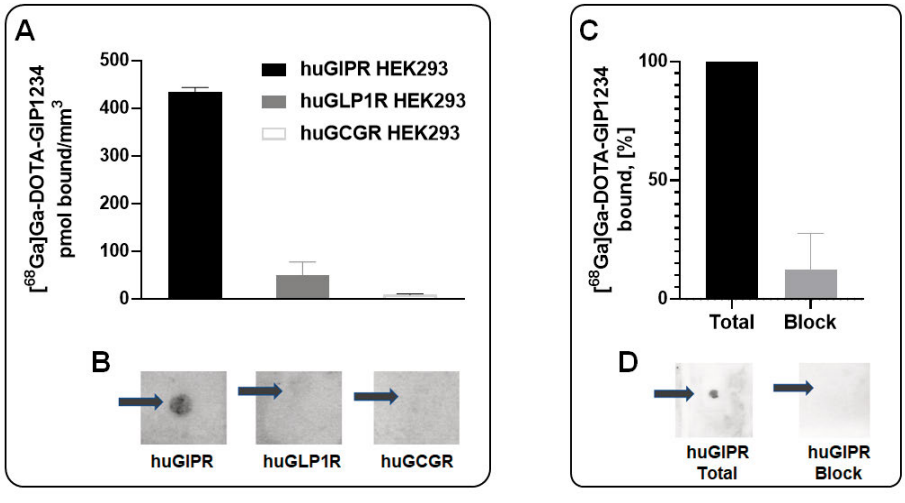

Figure 4. (A) Total tracer binding to frozen sections of huGIPR-, huGLP1R-, and huGCGR-expressing HEK293 cell pellets, expressed as pmol/mm^3. (B) Representative autoradiograms showing pronounced binding to huGIPR-expressing sections and minimal signal in huGLP1R- and huGCGR-expressing sections. (C) Quantification of [^{68}Ga]Ga-DOTA-GIP1234 binding to huGIPR-expressing cell pellets before and after pre-incubation with 2 µM tirzepatide. (D) Representative autoradiograms illustrating the reduction in tracer binding to huGIPR-expressing sections following tirzepatide pre-blocking.

Dynamic PET imaging in rats showed rapid tracer distribution within the blood pool, followed by uptake in highly perfused organs, particularly the liver, lungs, and spleen. Compared with the parent compound [^{68}Ga]Ga-DOTA-C803-GIP, incorporation of the albumin-binding fatty acid markedly altered the biodistribution profile, resulting in increased blood retention,

reduced renal uptake, and increased hepatic accumulation over time **(Figure 5A,B)**. Ex vivo biodistribution confirmed approximately two-fold higher blood activity at 60 min post-injection, along with increased uptake in several highly perfused tissues such as the spleen, lungs, and liver, indicating prolonged systemic exposure **(Figure 5C)**. No measurable tracer uptake was detected in the brain.

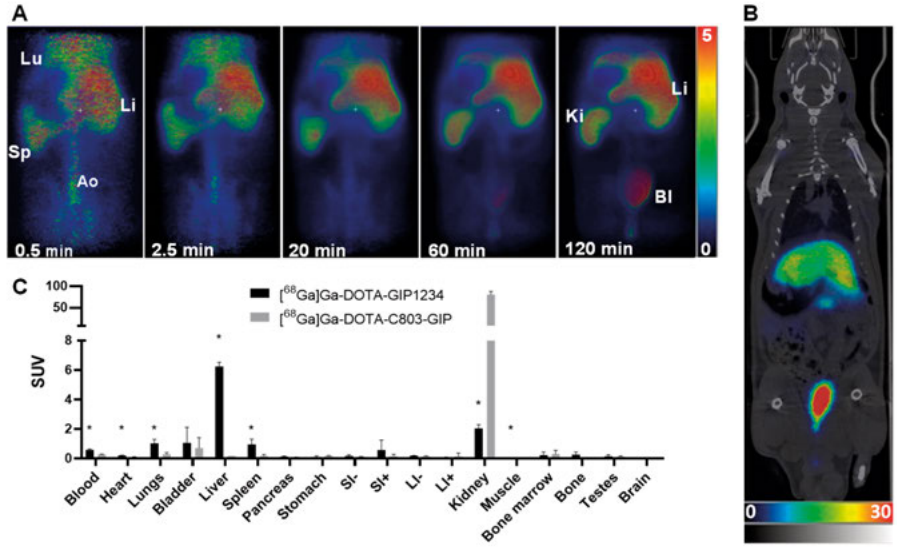

Figure 5. (A) Maximum intensity projection PET images of the abdominal region acquired from 0 to 120 min after tracer administration and normalised to SUV 5. (B) Representative whole-body coronal PET/CT image at 60 min post-injection, normalised to SUV 30. (C) Ex vivo organ distribution of [^{68}Ga]Ga-DOTA-GIP1234 in rats (n = 5) at 60 min post-injection, compared with previously reported data for [^{68}Ga]Ga-DOTA-C803-GIP. Asterisks indicate statistically significant differences ($p < 0.05$). Lu, lung; Ao, aorta; Sp, spleen; Ki, kidney; Li, liver; Bl, urinary bladder.

PET/CT imaging in healthy pigs showed a tracer distribution profile similar to that observed in rats. The albumin-binding modification increased blood activity by almost fivefold compared with [^{68}Ga]Ga-DOTA-C803-GIP and shifted tracer clearance from predominantly renal towards hepatic excretion **(Figure 6A-C)**. Despite the prolonged circulation time and increased tissue exposure, pancreatic uptake remained low throughout the imaging period **(Figure 6B)**. Co-injection of excess unlabeled DOTA-GIP1234 increased tracer retention in the blood and liver but did not alter pancreatic uptake, suggesting limited receptor-specific accumulation in vivo **(Figure 6C-E)**.

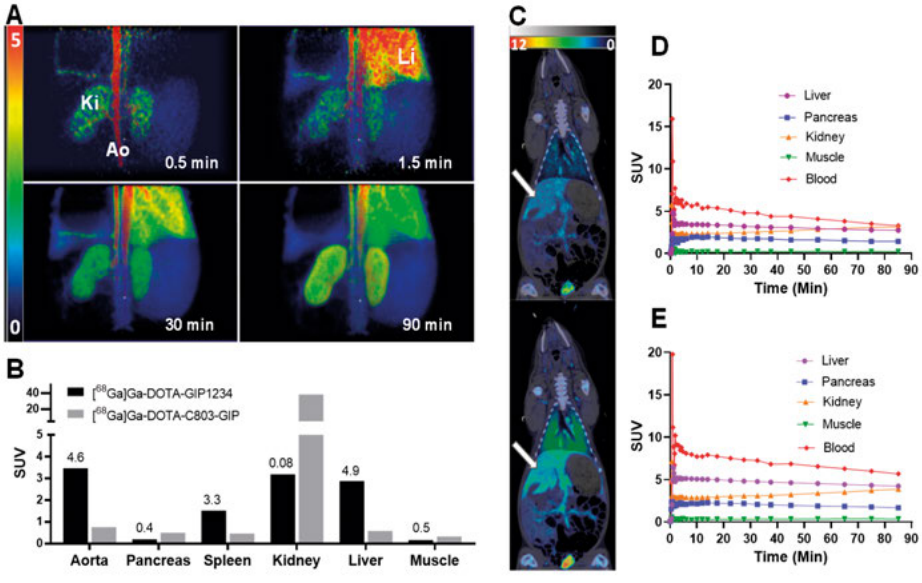

Figure 6. (A) Maximum intensity projection images, normalised to SUV 5, acquired at 0.5, 1.5, 30 and 90 min after tracer administration. (B) In vivo tissue uptake at 60 min post-injection, compared with previously reported data for [^{68}Ga]Ga-DOTA-C803-GIP. Values above the bars indicate the fold difference in uptake between the two tracers. (C) Representative coronal PET/CT images acquired 60 min after administration of [^{68}Ga]Ga-DOTA-GIP1234 alone (top) or together with 100 µg/kg unlabelled DOTA-GIP1234 (blocking scan, bottom). Arrows indicate the liver. (D) Time–activity curves for selected tissues following administration of [^{68}Ga]Ga-DOTA-GIP1234 alone. (E) Corresponding time–activity curves following co-injection of 100 µg/kg unlabelled DOTA-GIP1234. Ao, aorta; Ki, kidney; Li, liver.

The interaction of DOTA-GIP1234 with albumin was assessed using native polyacrylamide gel electrophoresis (native PAGE). Increasing BSA concentrations progressively reduced the amount of free DOTA-GIP1234, demonstrating concentration-dependent albumin binding **(Figure 7A,B)**. At a BSA-to-peptide concentration ratio of 0.51, the concentration of free DOTA-GIP1234 was reduced by 69%. In contrast, DOTA-GIP-C803, which lacks the palmitic acid moiety, showed no concentration-dependent interaction with BSA **(Figure 7)**, confirming that albumin binding was mediated by the introduced fatty acid chain.

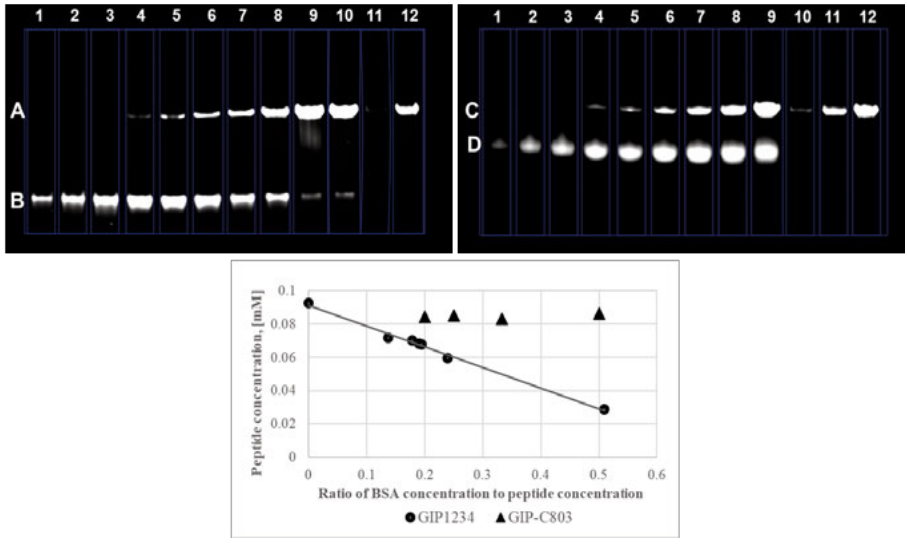

Figure 7. PAGE analysis of the interaction between BSA and DOTA-GIP1234 (left) or DOTA-GIP-C803 (right). (A) Increasing BSA concentrations were applied in lanes 4–9 (13, 15, 19, 23, 31 and 46 µM), with replicate measurements in lanes 9–10 and detection-limit assessment in lanes 11–12. (B) DOTA-GIP1234 was maintained at 93 µM during incubation with BSA (lanes 4–10); concentrations of 56, 74 and 87 µM were used for signal calibration (lanes 1–3), with replicate measurements in lanes 9–10. (C) Increasing BSA concentrations were applied in lanes 4–9 (13–46 µM), while 13, 26 and 37 µM BSA were used for calibration (lanes 10–12). (D) DOTA-GIP-C803 was incubated with BSA at 60 µM (lanes 4–5) or 94 µM (lanes 6–9), with 21, 42 and 52 µM used for signal calibration (lanes 1–3). The lower panel shows the concentration of unbound peptide as a function of the BSA-to-peptide concentration ratio for DOTA-GIP1234 (●) and DOTA-GIP-C803 (▲).

Albumin binding of the radiolabeled tracer was further assessed by native PAGE combined with autoradiography (**Figure 8**). Following incubation of [^{68}Ga]Ga-DOTA-GIP1234 with BSA, no detectable radioactivity corresponding to free tracer was observed, while the radioactive signal was associated with the BSA fraction. These results confirmed that [^{68}Ga]Ga-DOTA-GIP1234 retained its albumin-binding properties following radiolabeling.

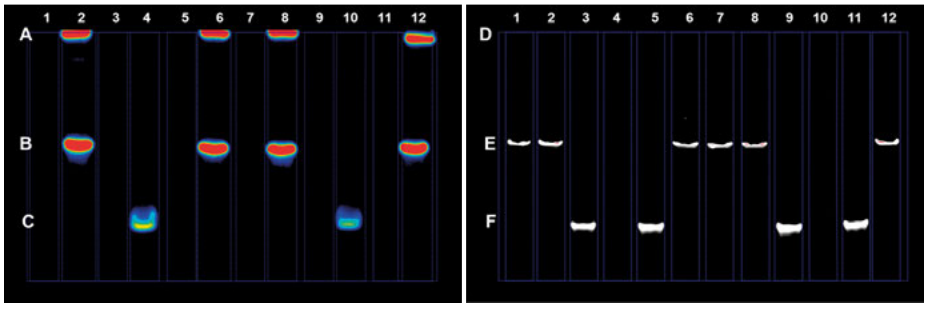

Figure 8. Gel autoradiography (left) and corresponding PAGE analysis (right) showing the interaction between BSA and [^{68}Ga]Ga-DOTA-GIP1234. BSA (31 µM) and [^{68}Ga]Ga-DOTA-GIP1234 (15 µM) were incubated together in lanes 2, 6, 8 and 12. Reference samples containing BSA alone (31 µM; lanes 1 and 7), DOTA-GIP1234 (104 µM; lanes 3, 5, 9 and 11), or [^{68}Ga]Ga-DOTA-GIP1234 (10 µM; lanes 4 and 10) were included. Rows A–F indicate the loading wells (A), BSA-associated [^{68}Ga]Ga-DOTA-GIP1234 (B), free [^{68}Ga]Ga-DOTA-GIP1234 reference (C), wells (D), BSA and BSA-bound radiotracer (E), and DOTA-GIP1234 reference (F).

Discussion and Conclusions

The introduction of a palmitic acid moiety into DOTA-GIP1234 was intended to increase blood residence time through reversible binding to serum albumin and thereby improve tracer availability for GIPR binding. Native PAGE experiments confirmed concentration-dependent albumin binding of DOTA-GIP1234, which was absent for the DOTA-C803-GIP analogue (without the palmitic acid moiety), demonstrating that the interaction was mediated by the fatty acid modification. Albumin binding properties of DOTA-GIP1234 were retained after gallium-68 labelling. Interestingly, weaker interaction with rat serum albumin was observed, which may partly explain the faster tracer clearance in rats compared with pigs.

The modification did not impair radiolabelling or stability, but [^{68}Ga]Ga-DOTA-GIP1234 showed lower GIPR affinity than [^{68}Ga]Ga-DOTA-C803-GIP (K_d 40 ± 12.5 nM vs 18.3 ± 6.5 nM). Despite this, the tracer retained GIPR selectivity in vitro, with negligible binding to GLP-1R- and GCGR-expressing cells and a marked reduction in binding to GIPR-expressing frozen cell pellets following pre-incubation with tirzepatide.

In pigs, albumin binding properties markedly prolonged tracer blood circulation time, with more than four-fold higher blood activity than [68Ga]Ga-DOTA-C803-GIP. However, this was accompanied by increased hepatic uptake. Pancreatic uptake remained low and was not reduced by excess unlabelled peptide, indicating insufficient sensitivity for direct in vivo GIPR imaging. However, co-administration of unlabelled DOTA-GIP1234 increased

blood activity from approximately SUV 3 to 5.5, suggesting that some target-mediated binding may still have occurred but remained below the sensitivity of pancreatic PET detection.

Overall, the fatty acid modification successfully prolonged tracre's blood circulation time but introduced high hepatic retention and did not improve pancreatic GIPR imaging. Further development should therefore focus on increasing GIPR affinity while maintaining favourable pharmacokinetics and reducing liver uptake.

Paper II - Positron emission tomography to assess drug occupancy at peripheral and central incretin receptors

Background and Aim

Incretin-based therapies have transformed the treatment of type 2 diabetes (T2D) and obesity by improving glycaemic control while promoting weight loss (*70*). Recent advances have extended this therapeutic concept beyond selective glucagon-like peptide-1 receptor (GLP-1R) agonists to dual and triple agonists that simultaneously target the glucose-dependent insulinotropic polypeptide receptor (GIPR), GLP-1R, and glucagon receptor (GCGR) (*69*). Although these multi-receptor agonists demonstrate superior clinical efficacy, the contribution of individual receptors to their pharmacological effects, particularly within the central nervous system, remains incompletely understood (*71*). Direct assessment of receptor target engagement is therefore essential to elucidate their mechanisms of action and support dose optimisation during drug development. Positron emission tomography (PET) enables the non-invasive quantification of receptor occupancy in vivo using receptor-specific radiotracers and represents a valuable translational tool for evaluating novel therapeutics (*44*). Building on previous work establishing PET tracers targeting GLP-1R and GIPR (*44*,*45*), this study aimed to investigate the in vivo receptor occupancy of the dual GLP-1R/GIPR agonist tirzepatide and the triple GLP-1R/GIPR/GCGR agonist SAR441255. Using a translational pig model, PET imaging was employed to assess GLP-1R target engagement in the pancreas and selected brain regions with the GLP-1R-specific radiotracer [^{68}Ga]Ga-DO3A-Exendin-4, and to assess pancreatic GIPR occupancy using the GIPR-targeting tracer [^{68}Ga]S02-GIP-T4.

Results

In vitro autoradiography confirmed the specificity of the [^{68}Ga]Ga-DO3A-Exendin-4 and [^{68}Ga]S02-GIP-T4 PET tracers for their respective target receptors, GLP-1R and GIPR. [^{68}Ga]Ga-DO3A-Exendin-4 showed selective binding to GLP-1R, with negligible binding to GIPR and GCGR **(Figure 9A, B)**. Binding was reduced by 80 ± 16% following pre-incubation with unlabelled DO3A-Exendin-4, by 84.9 ± 7.0% with tirzepatide, and by 78.3 ± 11.1% with SAR441255, demonstrating competition for the GLP-1R binding site **(Figure 9A)**. Similarly, [^{68}Ga]S02-GIP-T4 exhibited specific binding to GIPR, which was reduced by 71.2 ± 1.0% following pre-incubation with SAR441255 and

by 82.5 ± 0.9% with unlabelled DOTA-S02-GIP-T4, confirming GIP receptor-specific binding **(Figure 9C, D)**.

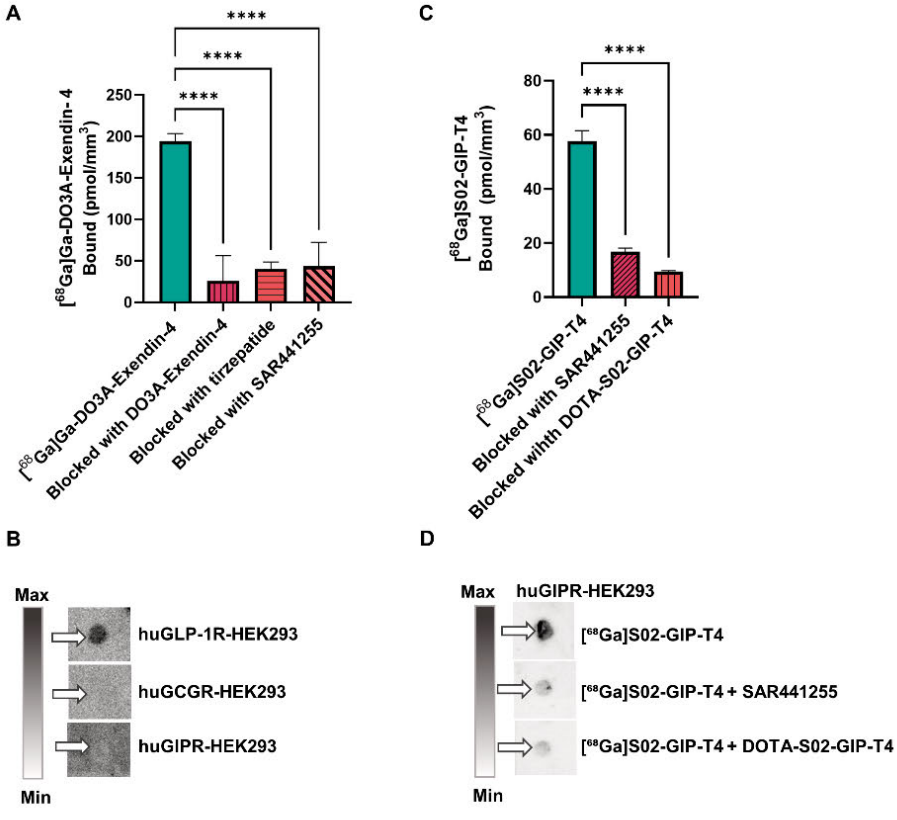

Figure 9. (A) Binding of [^{68}Ga]Ga-DO3A-Exendin-4 to huGLP-1R-HEK293 cell pellets when incubated alone or co-incubated with DO3A-Exendin-4, tirzepatide or SAR441255 (***$p < 0.0001$, $R^2 = 0.94$; one-way ANOVA). (B) Representative autoradiograms of [^{68}Ga]Ga-DO3A-Exendin-4 binding to frozen sections of huGLP-1R-,huGCGR, and huGIPR-expressing HEK293 cell pellets. (C) Binding of [^{68}Ga]S02-GIP-T4 to huGIPR-HEK293 cell pellets alone or following co-incubation with SAR441255 or DOTA-S02-GIP-T4 (***$p < 0.0001$, $R^2 = 0.99$; one-way ANOVA). (D) Representative autoradiograms showing [^{68}Ga]S02-GIP-T4 binding to frozen huGIPR-HEK293 cell pellet sections under the different experimental conditions. Normality was confirmed using the Shapiro–Wilk test ($p > 0.05$).

PET imaging with [^{68}Ga]Ga-DO3A-Exendin-4 demonstrated dose-dependent GLP-1R occupancy by SAR441255 (**Figure 10A–H**). In the pancreas, occupancy increased from 16% at 1 µg/kg to 56% at 2.5 µg/kg and 73% at 4 µg/kg, reaching a maximum of 83% at 14 µg/kg, with no further increase at 100 µg/kg (**Figure 10A–C**). The estimated EC_{50} was 1.8 µg/kg. A similar dose-

dependent response was observed in the pituitary gland, where occupancy reached 77% at 14 µg/kg, with an EC_{50} of 3.9 µg/kg (**Figure 10D–F**). In the medial hypothalamic area, GLP-1R occupancy reached approximately 60%, with an estimated EC_{50} of 2.5 µg/kg (**Figure 10G**), demonstrating target engagement in appetite-regulating regions. Dose-dependent occupancy was also observed in the mammillary body (**Figure 10H**).

A direct comparison of [^{68}Ga]Ga-DO3A-Exendin-4 and [^{68}Ga]S02-GIP-T4 showed that pancreatic uptake of [^{68}Ga]S02-GIP-T4 was approximately 7–10-fold lower, while pituitary uptake was 3–4-fold lower, than that of [^{68}Ga]Ga-DO3A-Exendin-4 at 60 min after injection (**Figure 10I–L**). [^{68}Ga]S02-GIP-T4 also demonstrated higher hepatic retention and increased uptake in the vertebrae and bone marrow compared with the GLP-1R tracer **(Figure 10K, L**). Assessment of GIPR occupancy using [^{68}Ga]S02-GIP-T4 showed a 23 ± 8.5% reduction in pancreatic tracer uptake following administration of 100 µg/kg SAR441255 ($p < 0.05$) (**Figure 10M**), comparable to the 24% reduction observed after infusion of endogenous GIP(1–42). No significant changes in tracer uptake were detected in the brain or other tissues, indicating that GIPR target engagement could be demonstrated only in the pancreas under the conditions of this study.

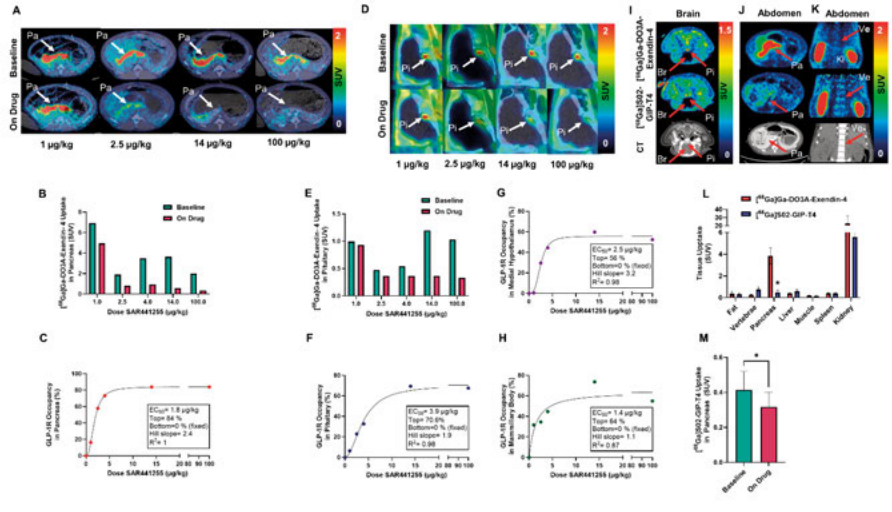

Figure 10.(A) Representative PET/CT images showing pancreatic (Pa) [^{68}Ga]Ga-DO3A-Exendin-4 uptake at baseline and following administration of different doses of SAR441255. (B) Individual pancreatic SUV values before and after SAR441255 treatment at 60 min post-tracer injection. (C) Pancreatic GLP-1R occupancy plotted against log_{10}-transformed SAR441255 dose. (D) Representative PET/CT images of [^{68}Ga]Ga-DO3A-Exendin-4 uptake in the pituitary gland (Pi) at baseline and during SAR441255 treatment. (E) Changes in pituitary SUV between baseline and post-treatment scans across the different doses of SAR441255. (F) Pituitary GLP-1R occupancy

as a function of SAR441255 dose. (G) GLP-1R occupancy in the medial hypothalamus and (H) mammillary body in respect to SAR441255 doses. (I–K) Direct comparison of the in vivo distribution of [^{68}Ga]Ga-DO3A-Exendin-4 and [^{68}Ga]S02-GIP-T4, with representative images of the brain and pituitary (I), pancreas in transaxial view (J), and abdominal distribution in coronal view (K). (L) Quantitative comparison of tracer uptake across selected tissues ($p < 0.05$). (M) Pancreatic [^{68}Ga]S02-GIP-T4 uptake before and after pre-treatment with 100 µg/kg SAR441255 ($p < 0.05$). Br, brain; Pi, pituitary gland; Pa, pancreas; Ki, kidney; Ve, vertebrae.

In contrast, tirzepatide exhibited lower GLP-1R occupancy under the experimental conditions in both the pancreas and pituitary (**Figure 11A, B**). Pancreatic [^{68}Ga]Ga-DO3A-Exendin-4 uptake decreased by 24%, 40%, and 51% following administration of 50, 150, and 450 µg/kg tirzepatide, respectively, while the corresponding reductions in the pituitary gland were 47%, 49%, and 55%. Thus, GLP-1R occupancy remained below 60% in target tissues and showed a less pronounced dose-dependent relationship than that observed with SAR441255.

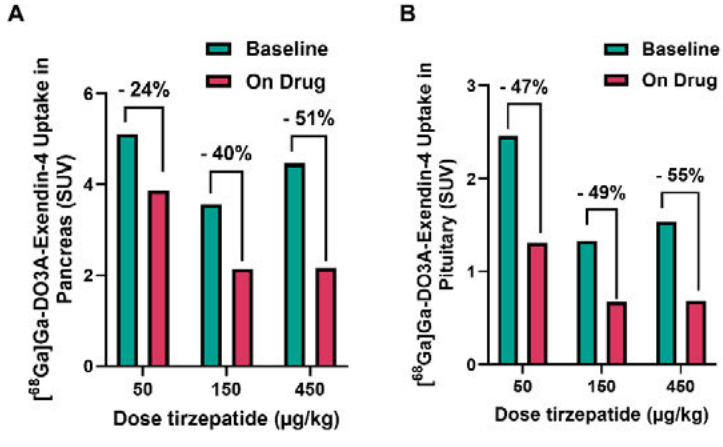

Figure 11. Dose-dependent reduction in [^{68}Ga]Ga-DO3A-Exendin-4 uptake in the pancreas (A) and pituitary gland (B) following tirzepatide administration.

Discussion and Conclusion

This study demonstrates the utility of PET imaging for the non-invasive assessment of incretin receptor target engagement in vivo. Using a GLP-1 receptor-specific tracer, [^{68}Ga]Ga-DO3A-Exendin-4, dose-dependent GLP-1R occupancy by the triple agonist SAR441255 was quantified in the pancreas, pituitary gland, and selected brain regions involved in appetite regulation. In

contrast, tirzepatide showed lower GLP-1R occupancy under the experimental conditions. This may reflect its lower affinity for GLP-1R relative to GIPR, together with its delayed T_{max} of 8-72 hours, compared with SAR441255 T_{max} of 3 hours, resulting in incomplete receptor engagement within the imaging time window (*72*,*73*).

[^{68}Ga]S02-GIP-T4 showed specific binding to GIPR in vitro; however, its relatively low pancreatic uptake in pigs limited its sensitivity for GIPR occupancy measurements in vivo. Following SAR441255 administration, pancreatic tracer uptake decreased by 23 ± 8.5%, although the low baseline signal made it difficult to determine whether this reduction represented true GIPR occupancy or other physiological changes affecting tracer distribution. These results contrast with previously reported findings in NHPs where [^{68}Ga]S02-GIP-T4 showed a markedly higher pancreatic tracer uptake with SUV signal ∼1.2 at 60–90 minutes post-tracer administration (*44*). This finding likely reflects lower physiological GIPR expression in the porcine pancreas than in humans, highlighting an important limitation of the animal model for evaluating GIPR-targeted therapies.

Overall, PET enabled non-invasive assessment of GLP-1R occupancy in both peripheral and appetite-regulating brain regions, particularly for SAR441255. In contrast, GIPR occupancy assessment was limited to the pancreas, with no clear evidence of CNS GIPR engagement.

These findings show that PET imaging can provide quantitative information on receptor occupancy and target engagement that conventional pharmacodynamic endpoints alone cannot, highlighting its potential value in drug development, dose assessment, and evaluation of drug-target interaction in vivo.

Paper III - Radiosynthesis and preclinical evaluation of [^{11}C]Methyl-V-0219, a small molecule Glucagon-Like Peptide-1 allosteric modulator for PET brain imaging

Background and Aim

The remarkable clinical success of incretin receptor agonists has transformed the treatment of type 2 diabetes (T2D) and obesity. In particular, glucagon-like peptide-1 receptor (GLP-1R) agonists, such as semaglutide, and dual GLP-1R/glucose-dependent insulinotropic polypeptide receptor (GIPR) agonists, such as tirzepatide, have demonstrated substantial benefits in glycaemic control and body-weight reduction (*31*,*74*,*75*). These therapeutic advances have heightened interest in understanding the central mechanisms underlying their metabolic effects, particularly the role of GLP-1R in brain regions involved in appetite regulation, satiety, and energy homeostasis.

Positron emission tomography (PET) provides a unique opportunity to non-invasively investigate GLP-1R distribution, receptor availability, and drug-target engagement in vivo (*44*,*76*). As demonstrated in the preceding study of this thesis (Paper II), exendin-4-based PET tracers enable the quantification of GLP-1R occupancy in circumventricular organs that lack an intact blood-brain barrier (BBB). However, their peptide structure limits penetration across the BBB, preventing visualisation of GLP-1R in brain regions protected by an intact BBB (*77*). Consequently, there remains a need for small-molecule PET tracers capable of imaging GLP-1R throughout the brain.

To address this need, this study investigated V-0219, a small-molecule positive allosteric modulator of GLP-1R with physicochemical properties more compatible with BBB penetration than peptide-based ligands. The aim was to develop a carbon-11-labelled analogue of V-0219 and evaluate its potential for PET imaging of central GLP-1R.

Results

The resulting PET tracer, [^{11}C]Methyl-V-0219, was successfully evaluated in vitro and in vivo as a potential brain-penetrant PET tracer targeting GLP-1R. In vitro autoradiography demonstrated tracer binding to both human GLP-1R-expressing HEK293 cells and rat INS-1 cells **(Figure 12A, B)**. Pre-incubation with V-0219 reduced tracer binding by more than 65% in both cell types, whereas pre-incubation with Methyl-V-0219 reduced tracer binding by more than 75% in GLP-1R-expressing cells and by 44% in INS-1 cells **(Figure**

12C). However, [^{11}C]Methyl-V-0219 also exhibited blockable binding to GIPR- and GCGR-expressing cells, indicating cross-reactivity with other incretin receptors. In contrast, no appreciable or blockable binding was observed in exocrine pancreatic tissue **(Figure 12A-C)**.

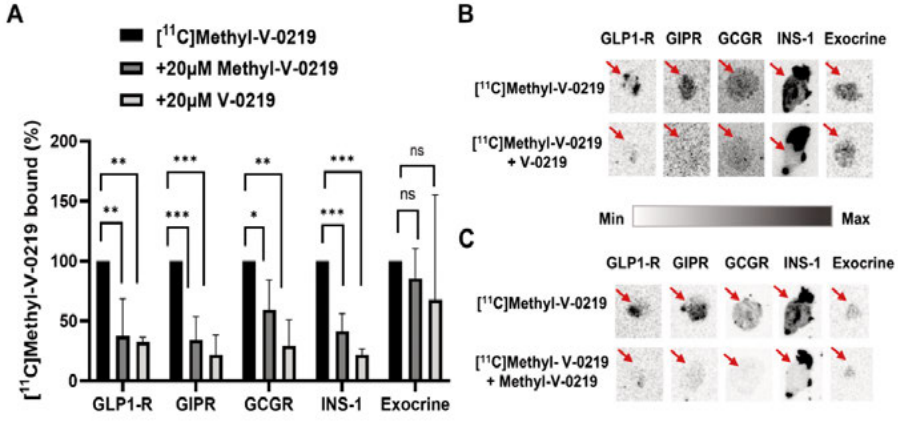

Figure 12. In vitro binding of [^{11}C]Methyl-V-0219. (A) Comparison of [^{11}C]Methyl-V-0219 binding to frozen sections of cells expressing GLP-1R, GIPR and GCGR, as well as INS-1 and exocrine pancreatic tissue, in the absence or presence of unlabelled Methyl-V-0219 or V-0219 (one-way ANOVA; $p < 0.05$, *$p < 0.01$, **$p < 0.001$; ns, not significant). (B) Representative autoradiograms showing reduced [^{11}C]Methyl-V-0219 binding following pre-incubation with V-0219. (C) Representative autoradiograms showing reduced tracer binding following pre-incubation with Methyl-V-0219.

Dynamic PET imaging in pigs demonstrated rapid brain uptake of [^{11}C]Methyl-V-0219, with SUV >2 within the first 5 min after injection, followed by progressive washout to SUV <1 by the end of the 60-min acquisition **(Figure 13A, B)**. Unlike the reference tracer [^{68}Ga]Ga-DO3A-Exendin-4, [^{11}C]Methyl-V-0219 showed no detectable retention in GLP-1R-rich tissues, including the pituitary gland and pancreas **(Figure 13C, D)**. Instead, high hepatic uptake followed by increasing intestinal activity indicated predominant hepatobiliary clearance **(Figure 13E)**.

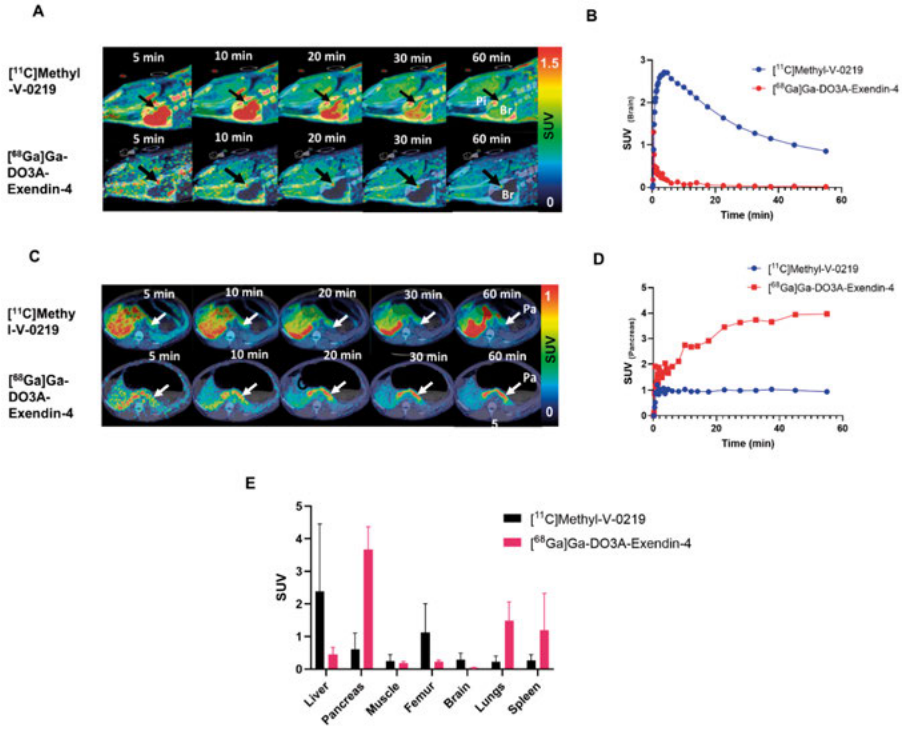

Figure 13. **Comparison of [^{11}C]Methyl-V-0219 and [^{68}Ga]Ga-DO3A-Exendin-4 biodistribution in pigs.** (A) Representative sagittal PET/CT images acquired at selected time points during dynamic brain imaging following intravenous administration of [^{11}C]Methyl-V-0219 (upper panel) or [^{68}Ga]Ga-DO3A-Exendin-4 (lower panel). Arrowheads indicate the pituitary gland. (B) Brain time–activity curves for both tracers over the 60-min dynamic PET acquisition. (C) Representative transverse PET/CT images of the pancreatic region obtained at selected time points after administration of [^{11}C]Methyl-V-0219 (upper panel) or [^{68}Ga]Ga-DO3A-Exendin-4 (lower panel). Arrowheads indicate the pancreas. (D) Pancreatic time–activity curves for both tracers during dynamic PET imaging. (E) Quantitative comparison of tracer uptake in selected organs at 60 min post-injection, expressed as mean SUV ± SD (n = 2). Br, brain; Pi, pituitary gland; Pa, pancreas.

PET/MRI studies in rats demonstrated a biodistribution pattern similar to that observed in pigs. The tracer rapidly penetrated the BBB but washed out continuously over time, with no detectable retention in GLP-1R-rich regions such as the pituitary gland or pancreas **(Figure 14A, B)**. Increasing tracer activity was again observed in the liver, intestines, Harderian glands, and salivary glands, consistent with the biodistribution observed in pigs **(Figure 14B)**.

To further evaluate the specificity of the brain signal, in vivo blocking and displacement studies were performed using Methyl-V-0219. Neither pre-administration of the blocking compound nor its administration during PET acquisition altered the brain uptake or kinetic profile of [^{11}C]Methyl-V-0219, indicating that the observed brain signal did not reflect specific, reversible GLP-1R binding **(Figure 14C)**.

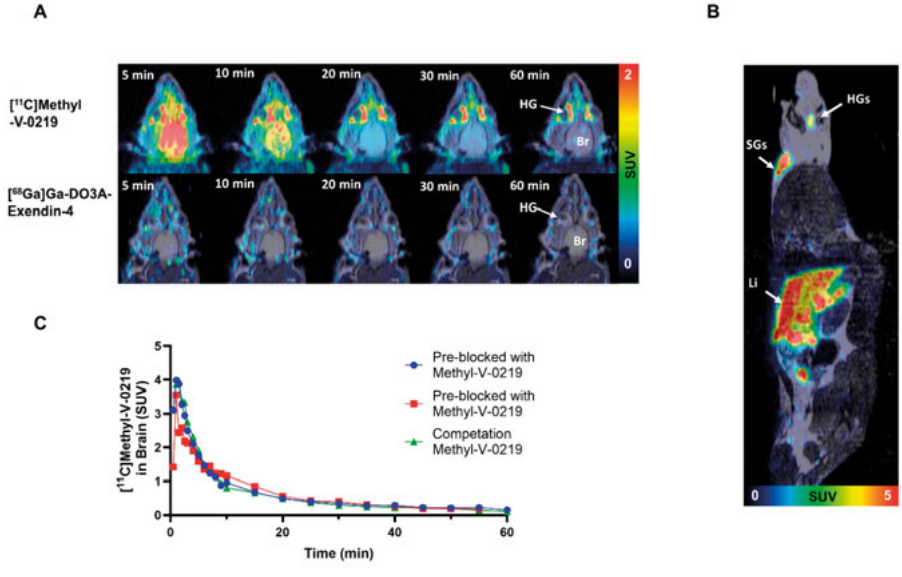

Figure 14. Rat PET imaging and blocking studies of [^{11}C]Methyl-V-0219. (A) Representative coronal PET/MRI images acquired at selected time points during dynamic brain imaging following intravenous administration of [^{11}C]Methyl-V-0219 (upper panel) or [^{68}Ga]Ga-DO3A-Exendin-4 (lower panel). Arrowheads indicate the Harderian glands. (B) Representative whole-body PET/MRI image obtained 60 min after administration of [^{11}C]Methyl-V-0219. (C) Brain time–activity curves for [^{11}C]Methyl-V-0219 under the different blocking conditions. Arrowheads indicate the salivary glands, liver and Harderian glands. Br, brain; HGs, Harderian glands; SGs, salivary glands; Li, liver. SUVs are displayed according to the indicated colour scale.

Discussion and Conclusion

This study evaluated the feasibility of using a small-molecule PET tracer to image central GLP-1R. In vitro, [^{11}C]Methyl-V-0219 demonstrated limited selectivity for GLP-1R, as binding was also detected at GIPR and, to a lesser extent, GCGR. The original pharmacological characterisation of V-0219 did not examine this broader receptor profile and focused primarily on GLP-1R-mediated effects. Given the homology within the incretin receptor family, some degree of cross-reactivity is nevertheless plausible.

[^{11}C]Methyl-V-0219 rapidly penetrated the BBB in both rats and pigs, in contrast to peptide-based GLP-1R tracers such as [^{68}Ga]Ga-DO3A-Exendin-4. However, brain uptake was followed by rapid washout, and little retention was observed in other GLP-1R-rich tissues, including the pancreas and pituitary gland. The tracer instead showed prominent hepatic and intestinal uptake, together with accumulation in salivary and Harderian glands, suggesting non-specific distribution and hepatobiliary clearance.

Crucially, neither pre-blocking nor displacement with a Methyl-V-0219 analogue altered the brain time–activity curves in rats, indicating that the brain signal could not be attributed to measurable GLP-1R-specific binding. The discrepancy between the clear receptor binding observed in vitro and the lack of target engagement in vivo may partly reflect the high receptor expression in the cell-based models used for autoradiography. However, the weak pancreatic retention suggests that low receptor density in the brain was not the sole limitation. Moreover, a compound can produce pharmacological effects at receptor occupancy levels that are insufficient to generate a detectable PET signal. Thus, although [^{11}C]Methyl-V-0219 successfully penetrated the brain, its limited receptor selectivity and lack of detectable specific binding in vivo restricted its suitability as a GLP-1R PET tracer. Nevertheless, the study provided important guidance for future tracer development, demonstrating that BBB penetration alone is insufficient and that successful central GLP-1R imaging will require an optimised balance of receptor affinity, selectivity, lipophilicity, and specific in vivo retention.

Paper IV - A DPP6-targeted PET tracer for non-invasive imaging of transplanted human islets

Background and Aim

Type 1 diabetes (T1D) is characterised by autoimmune destruction of pancreatic β-cells, resulting in insulin deficiency and lifelong insulin dependence (*4*). As therapies aimed at preserving or restoring β-cell mass continue to evolve, and as islet replacement strategies based on the transplantation of donor-derived and stem cell-derived islets advance, there is a growing need for non-invasive imaging techniques capable of directly quantifying islet-cell mass, monitoring graft survival, and evaluating therapeutic efficacy over time (*9*,*78*–*80*). Current clinical biomarkers, such as C-peptide, insulin secretion, and HbA1c, provide only indirect measures of β-cell function and cannot accurately assess the mass of transplanted islets (*80*).

Positron emission tomography (PET) provides a highly sensitive method for the non-invasive imaging of transplanted islets, yet its use has been constrained by the lack of sufficiently specific molecular targets (*43*,*53*,*81*,*82*). Dipeptidyl peptidase-like protein 6 (DPP6) has recently emerged as a promising biomarker because of its high expression in human pancreatic β-cells, and to a lower extent in α-cells (*63*). A DPP6-targeting nanobody, 4hD29, labelled with ^{99m}Tc previously enabled visualisation of transplanted human islets using SPECT, with tracer uptake correlating with graft size and significant detection achieved for grafts containing approximately 3000 IEQ (*63*,*64*). Building on these findings, this study aimed to evaluate the DPP6-targeting PET tracer [^{68}Ga]Ga-NOTA-4hD29 for imaging transplanted human and stem cell-derived islets (SC-islets)and to characterise its specificity, biodistribution, dosimetry, and ability to detect small islet grafts.

Results

[^{68}Ga]Ga-NOTA-4hD29 was successfully radiolabeled with high radiochemical yield (97.5 ± 0.5%) and radiochemical purity (99.8 ± 0.1%), resulting in a molar activity of 11.5 ± 0.5 MBq/nmol. The tracer remained stable in both the formulation buffer and human plasma for at least 60 min after radiolabelling. In rat plasma, 65–70% of the tracer remained intact 1 h following administration, demonstrating acceptable in vivo stability **(Figure 15)**.

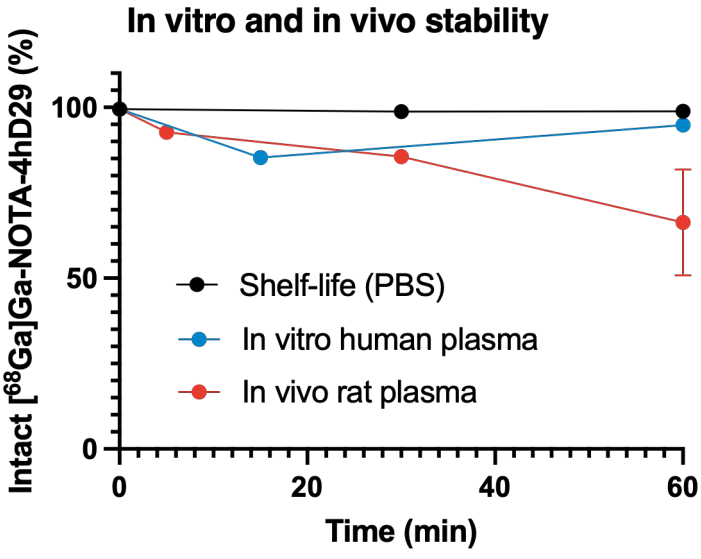

Figure 15. The stability of [^{68}Ga]Ga-NOTA-4hD29 in formulation buffer, *in vitro* in human plasma, or in vivo in rat plasma, expressed as the percentage of intact tracer over time.

In vitro autoradiography demonstrated strong and specific binding of [^{68}Ga]Ga-NOTA-4hD29 to DPP6-expressing islets, with negligible uptake in exocrine pancreatic tissue. Tracer binding was highest in purified SC-islets and progressively decreased as islet purity declined, demonstrating a strong correlation between tracer uptake and islet purity **(Figure 16A, C)**. Pre-incubation with excess unlabelled 4hD29 almost completely abolished tracer binding, confirming its specificity for DPP6 (**Figure 16B**). [^{68}Ga]Ga-NOTA-4hD29 binding showed a strong positive correlation with islet preparation purity ($R^2 = 0.96$, $p < 0.05$), whereas no significant correlation was observed following blocking with excess unlabelled 4hD29 ($R^2 = 0.65$, n.s.) **(Figure 16A, B)**. Furthermore, negligible binding was observed in extra-pancreatic tissues, including the heart, liver, spleen, and lungs **(Figure 17)**.

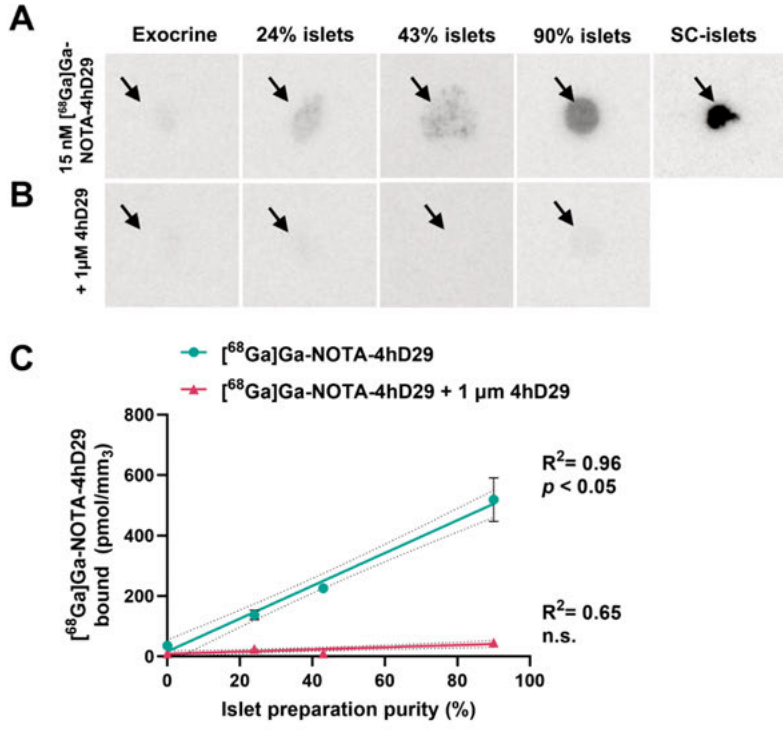

Figure 16. Autoradiograms of [^{68}Ga]Ga-NOTA-4hD29 uptake in exocrine pancreatic tissue, islet preparations with increasing purity (24%, 43% or 90%) and pure stem cell-derived islets (SC-islets) (A). Co-incubation with excess unlabeled 4hD29 blocks uptake, demonstrating high binding specificity (B). Black arrows indicate tissue locations. Quantification of [^{68}Ga]Ga-NOTA-4hD29 binding and correlation with islet purity (C).

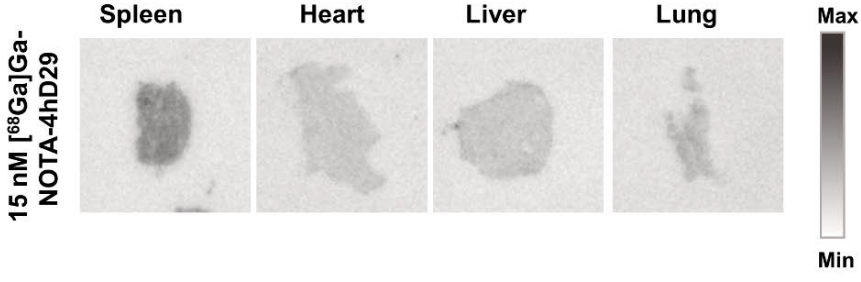

Figure 17. Representative autoradiograms demonstrating low or negligible binding of [^{68}Ga]Ga-NOTA-4hD29 to spleen, heart (myocardium), liver and lung.

Ex vivo biodistribution studies in rats and dynamic PET/MRI demonstrated rapid renal clearance of [^{68}Ga]Ga-NOTA-4hD29, with minimal retention in non-target tissues **(Figure 18A-B)**. These findings were confirmed in pigs, where the tracer similarly showed predominant kidney uptake and rapid elimination, resulting in low background activity in other organs **(Figure 18C-D)**.

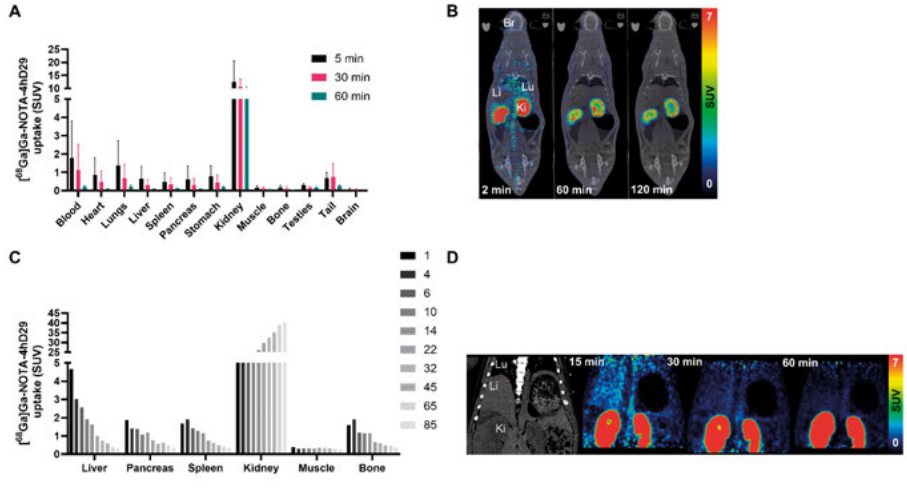

Figure 18. (A) Ex vivo biodistribution of [^{68}Ga]Ga-NOTA-4hD29 in rats at 5, 30, and 60 min post-injection, expressed as standardised uptake values (SUV). (B) Representative PET/MRI images showing the in vivo biodistribution in rats at 2, 60, and 120 min post-injection. (C) Quantification of in vivo tracer uptake in pigs by PET imaging over 85 min. (D) Representative PET/CT images showing the in vivo biodistribution of [^{68}Ga]Ga-NOTA-4hD29 in a pig at 15, 30, and 60 min post-injection. Li, liver; Lu, lungs; Ki, kidney.

PET/MRI successfully visualised both stem cell-derived and human islet grafts in vivo. Mice transplanted with 400-800 IEQ of stem cell-derived islets showed significantly higher tracer uptake than non-transplanted controls, and the PET signal correlated with graft survival **(Figure 19A-D)**. Similarly, transplanted human islets were clearly detectable, with tracer uptake corresponding to graft viability as determined by post-mortem analyses **(Figure 19E-F)**. Histological evaluation confirmed insulin-positive, DPP6-expressing cells in both stem cell-derived and human islet grafts **(Figure 20G-H)**.

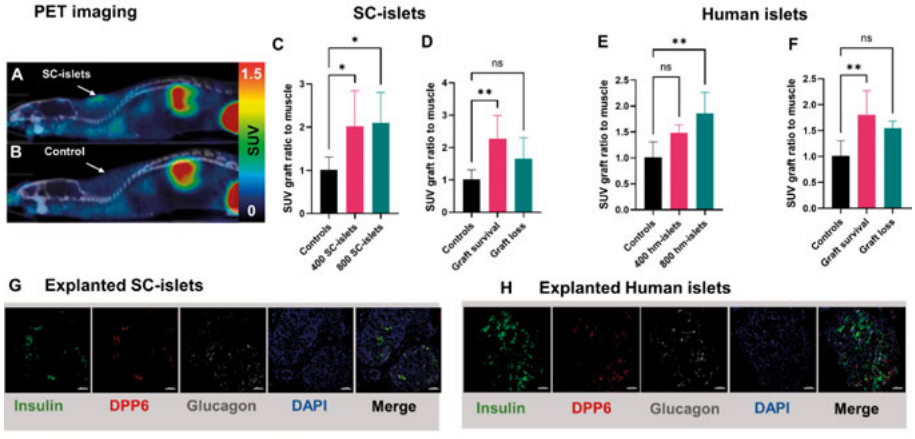

Figure 19. Representative sagittal PET/MRI image of [^{68}Ga]Ga-NOTA-4hD29 signal in an SC-islet graft in the neck muscle (A, white arrow) compared to a non-transplanted control (B, white arrow). (C) Quantification of PET signal in SC-islet grafts (400 or 800 islets) versus controls and (D) correlation between PET signal intensity and graft survival outcome. (E) Quantification of PET signal in human islet grafts (400 or 800 islets) versus controls and (F) correlation between PET signal intensity and graft survival outcome. Uptake is expressed as the graft-to-muscle SUV ratio. (G, H) Representative post-mortem histology of SC-islet and human islet grafts confirming DPP6 immunostaining.

Human dosimetry estimates indicated an effective dose of 0.025 mSv/MBq, with the urinary bladder identified as the dose-limiting organ at 0.41 mSv/MBq. These estimates suggest that approximately 400 MBq of [^{68}Ga]Ga-NOTA-4hD29 could be administered annually while remaining within commonly accepted effective dose limits for research participants.

Discussion and Conclusion

This study provides an extensive preclinical evaluation of [^{68}Ga]Ga-NOTA-4hD29 for PET imaging of transplanted human islets.

A key finding included the ability of [^{68}Ga]Ga-NOTA-4hD29 PET to detect intramuscular human and stem SC-islet grafts containing 400–800 IEQ. This represents a substantial improvement over the previously developed ^{99m}Tc-labelled SPECT tracer, for which reliable detection required approximately 3000 IEQ. Tracer binding also correlated with islet purity in vitro. Importantly, grafts with low PET signal in vivo subsequently lacked both insulin and DPP6 expression, suggesting that tracer uptake may also provide information on graft survival. Evaluation at additional clinically relevant transplantation sites, including the liver, will be important for further validation.
[^{68}Ga]Ga-NOTA-4hD29 also showed favourable biodistribution and preliminary dosimetry profiles, supporting the feasibility of repeated PET

examinations for longitudinal graft monitoring. Since DPP6 cross-reactivity is restricted to primates, cynomolgus macaques represent an important model for subsequent biodistribution, dosimetry and toxicology studies before clinical translation. Translation is further supported by the established clinical use of sdAb-based platforms, the possibility of GMP production, and the compatibility of NOTA/^{68}Ga labelling with existing nuclear medicine workflows.

To conclude, [^{68}Ga]Ga-NOTA-4hD29 enabled sensitive and specific PET detection of transplanted human and SC-islet grafts within the 400–800 IEQ range. Its favourable biodistribution, preliminary dosimetry and limited off-target tissue binding support further translational development for non-invasive and longitudinal monitoring of transplanted islet grafts.

Concluding Remarks, Future Perspectives and Limitations

The increasing prevalence of diabetes and the rapid development of new incretin-based and β-cell replacement therapies create a growing need for non-invasive methods that can assess both drug target engagement and pancreatic islet biology in vivo. The tracers evaluated in this thesis address these questions from different perspectives.

In **Papers I** and **II**, we evaluated GIPR- and GLP-1R-targeted PET tracers for receptor imaging and pharmacological studies. [^{68}Ga]Ga-DOTA-GIP1234, a GIPR tracer, demonstrated that albumin binding can prolong systemic exposure, but the resulting high blood and liver activity limited pancreatic contrast. [^{68}Ga]Ga-S02-GIP-T4 was more suitable for evaluating pancreatic GIPR target engagement and is therefore the more promising of the two GIPR tracers investigated here. However, further optimisation is still required, particularly considering the relatively low physiological expression of GIPR (compared to GLP-1R expression) and the resulting challenge of imaging a low-density receptor target. In contrast, [^{68}Ga]Ga-DO3A-Exendin-4 provided a robust readout of GLP-1R occupancy and demonstrated that PET can quantify dose-dependent target engagement of dual and triple incretin agonists in vivo. Such approaches could be incorporated into future drug-development studies to support dose selection and receptor-occupancy characterisation.

In **Paper III**,[^{11}C]Methyl-V-0219 demonstrated rapid brain penetration but no measurable specific GLP-1R signal in vivo. These findings suggest that further optimisation of affinity, specificity and retention is required before clinical translation of this tracer concept. Additionally, several novel GLP-1R small-molecule agonists have recently entered clinical-phase drug development. Potentially, some of these candidates may be repurposed as PET imaging agents, if they are found suitable for carbon-11 or fluorine-18 radiolabelling.

In **Paper IV**, [^{68}Ga]Ga-NOTA-4hD29 enabled specific PET imaging of small amounts of transplanted human and stem SC-islets by targeting DPP6, demonstrating high sensitivity and a favourable biodistribution profile with minimal uptake in off-target tissues. Among the tracers developed in this thesis, it represents a particularly promising candidate for further clinical

translation. Further preclinical evaluation should focus on safety, dosimetry, biodistribution, and pancreatic uptake, followed by clinical studies assessing whether DPP6 PET can distinguish differences in islet mass and monitor transplanted islets longitudinally.

A longer-term application of these methods would be longitudinal natural-history studies. DPP6 PET could potentially be used to follow changes in islet mass during diabetes progression or following β-cell replacement therapies, while incretin receptor PET could be used to investigate whether GLP-1R or GIPR availability changes with disease progression or treatment. Ultimately, combining islet imaging with receptor occupancy PET may provide complementary information on disease biology and therapeutic target engagement that cannot currently be obtained non-invasively in humans. Such approaches could support further drug development, facilitate dose optimisation, and provide new tools for evaluating and monitoring islet replacement therapies.

Several limitations of the studies included in this thesis should be acknowledged. In **Paper I**, the in vivo evaluation of [^{68}Ga]Ga-DOTA-GIP1234 was limited by the small number of animals, particularly the pig study, which was performed in a single animal. The comparison with [^{68}Ga]Ga-DOTA-C803-GIP was also based partly on previously generated data rather than a fully matched head-to-head study. In addition, given the observed species-dependent differences in albumin binding and tracer pharmacokinetics, extrapolation of these findings to humans should be made with caution.

In **Paper II**, the relatively small sample size reduced statistical power. The study design was also constrained by the limited time window during which the pigs could remain under anaesthesia. This was particularly relevant for tirzepatide, since the follow-up PET scan was performed before the drug reached its expected maximal plasma concentration (8-72 h), making the design suboptimal for evaluating its full receptor occupancy. Furthermore, porcine pancreatic GIPR expression is substantially lower than in humans, limiting the suitability of this model for assessment of GIPR occupancy. The inability to detect GIPR occupancy in the brain also restricted conclusions regarding central GIPR engagement.

In **Paper III**, the in vivo evaluation of [^{11}C]Methyl-V-0219 was exploratory and performed in a limited number of animals. In particular, the blocking experiments included only two rats for pre-blocking and one rat for displacement. Therefore, although no measurable displaceable brain binding was observed, the study did not allow a robust quantitative assessment of specific binding under a wider range of experimental conditions.

In **Paper IV**, the major limitation is that the sensitivity experiments were performed using transplanted islets concentrated at a defined intramuscular

location. This model does not fully reproduce the more difficult situation of endogenous pancreatic islets, which are small and dispersed throughout the pancreas. The animal groups were also relatively small, and the large-animal biodistribution evaluation was preliminary. In addition, the species specificity of 4hD29 limits conventional preclinical evaluation because the nanobody does not recognise murine DPP6 in commonly used rodent models.

Despite these limitations, the studies in this thesis provide important proof-of-concept data and identify both the potential and the current boundaries of PET imaging for incretin receptors and pancreatic islets. Together, they build a foundation for further tracer optimisation, improved study designs, and ultimately clinical translation.

Popular science summary

Светлячки, которые помогают увидеть невидимое

Представьте, что вы оказались ночью в лесу.
В темноте невозможно разглядеть, какие растения растут вокруг. Но вдруг в лес выпускают тысячи крошечных светлячков. Это необычные светлячки: каждый из них ищет только один-единственный вид цветка. Найдя его, он начинает светиться. Постепенно лес озаряется множеством маленьких огоньков, и становится ясно, где растут именно эти цветы.
Именно так можно представить себе работу ПЭТ-визуализации (позитронно-эмиссионной томографии).
Вместо светлячков мы используем крошечные молекулы — ПЭТ-трейсеры. Каждый такой «светлячок» запрограммирован искать только одну определённую цель в организме — определённый рецептор или тип клеток. Когда он находит свою цель, он словно загорается. Самого света, конечно, нет, но слабый радиоактивный сигнал улавливает ПЭТ-сканер, позволяя построить карту происходящего внутри живого организма.
Почему это важно?
Наши клетки постоянно обмениваются сигналами с помощью рецепторов — своеобразных молекулярных «замков», к которым подходят только определённые «ключи». Через них регулируются уровень сахара в крови, чувство голода, работа гормонов и многие другие процессы. Однако сами рецепторы настолько малы, что увидеть их обычными методами невозможно.
Моя работа заключается в создании новых молекулярных «светлячков», которые находят рецепторы GLP-1 и GIP — важнейшие участники обмена веществ. Именно на эти рецепторы воздействуют современные препараты для лечения сахарного диабета и ожирения, такие как Оземпик и другие инкретиновые препараты.
С помощью ПЭТ мы можем буквально увидеть, достигло ли лекарство своей цели. Достаточно ли рецепторов занято препаратом? Какие органы он действительно затрагивает — только поджелудочную железу или также головной мозг? В какой части мозга? Ответы на эти вопросы

помогают понять, почему лекарства работают именно так и как сделать их ещё эффективнее.

Ещё одно направление моей работы — визуализация островков Лангерганса, небольших скоплений клеток поджелудочной железы, вырабатывающих инсулин. При диабете первого типа эти клетки погибают. Одним из перспективных методов лечения является трансплантация островков. Однако после пересадки врачам практически невозможно определить, сколько клеток выжило и продолжают ли они функционировать. Разрабатывая новые ПЭТ-трейсеры, мы стремимся создать «светлячков», которые смогут находить пересаженные островки и позволят наблюдать за их судьбой без операций и биопсий.

Создать такого «светлячка» совсем непросто. Он должен достаточно долго циркулировать в крови, не теряться по пути, не садиться на «неправильные» клетки и быстро находить именно свою цель. Иногда изменение всего одной молекулы может сделать изображение значительно лучше — или полностью лишить трейсер способности находить нужный рецептор.

В конечном счёте моя работа — это не просто разработка новых методов диагностики. Это создание молекулярных «светлячков», которые освещают процессы, скрытые глубоко внутри организма. Следуя за их светом, мы можем увидеть то, что раньше было невидимым: как работают лекарства, где находятся важные рецепторы и что происходит с пересаженными клетками. А значит — сделать диагностику точнее, лечение эффективнее, а жизнь людей с диабетом лучше.

كيف نرى ما لا يُرى داخل الجسم؟

تخيّل مدينةً كبيرة انقطع عنها التيار الكهربائي، فأصبحت غارقة في الظلام. إذا نظرت إليها من الأعلى، فلن تستطيع أن تميّز المستشفيات أو المدارس أو المنازل؛ فكل المباني موجودة، لكنها مخفية في الظلام.
الآن تخيّل أننا نرسل ملايين الأشخاص، يحمل كلٌّ منهم فانوسًا صغيرًا جدًا. لكن هؤلاء ليسوا أشخاصًا عاديين؛ فكل واحدٍ منهم متتجه الى عنوان مبنى واحد فقط، ولن يتوقف إلا عنده.
عندما ننظر إلى المدينة من الأعلى، لا نرى الأشخاص أنفسهم، بل نرى الفوانيس التي يحملونها .وكلما ازداد عدد الفوانيس المتجمعة في مكانٍ ما، ازداد ذلك المكان إشراقًا، فأصبح من السهل معرفة المبنى الذي كنا نبحث عنه، رغم أن المدينة بأكملها ما زالت غارقة في الظلام.
بهذه الفكرة يعمل التصوير المقطعي بالإصدار البوزيتروني.(PET)
في أبحاثي، يقوم المتتبّع الإشعاعي (PET tracer) بدور أحد هؤلاء الأشخاص. وهو جزيء صغير جدًا صُمِّم للوصول إلى هدف واحد محدد داخل الجسم، ويحمل معه كميةً ضئيلة جدًا من مادة مشعة. وهذه المادة لا تُصدر ضوءًا مرئيًا كما في المثال، وإنما تُطلق إشارات إشعاعية دقيقة يستطيع جهاز PETالتقاطها. لذلك، فالفانوس في هذه الرواية ليس إلا تشبيهًا لهذه الإشارة الإشعاعية.
وكما أن كل شخص يتجه إلى عنوان محدد فقط، فإن كل متتبّع إشعاعي صُمِّم للبحث عن هدف محدد، مثل مستقبل معين أو نوع معين من الخلايا. وعندما يصل إلى هدفه وتتجمع أعداد كبيرة من هذه الجزيئات في المكان نفسه، يلتقط جهاز PET الإشارات الإشعاعية الصادرة عنها، ويرسم خريطة تُظهر لنا مكان وجود هذا الهدف داخل الجسم.
أعمل على تصميم هذه الجزيئات لتتعرف على مستقبلات GLP-1 وGIP، وهي مستقبلات تؤدي دورًا محوريًا في تنظيم سكر الدم والشهية، كما أنها الهدف الذي تعمل عليه أدوية حديثة لعلاج السكري والسمنة، مثل أوزمبيك.(Ozempic)
وباستخدام PET نستطيع الإجابة عن أسئلة لم يكن من الممكن الإجابة عنها من قبل: هل يصل الدواء فعلًا إلى المستقبلات التي صُمِّم لاستهدافها؟ وهل يقتصر تأثيره على البنكرياس، أم يصل أيضًا إلى الدماغ وأعضاء أخرى؟ إن رؤية هذه المستقبلات داخل الجسم تساعدنا على فهم آلية عمل الأدوية بصورة أدق، وقد تسهم في تطوير علاجات أكثر فاعلية في المستقبل.
ويمتد عملي أيضًا إلى تطوير جزيئات تستطيع العثور على جزر لانغرهانس المزروعة، وهي تجمعات من الخلايا المنتجة للإنسولين تُزرع لبعض مرضى السكري من النوع الأول. وبعد عملية الزراعة، يصعب معرفة ما إذا كانت هذه الخلايا ما تزال حية وتؤدي وظيفتها. أما إذا تمكنت هذه الجزيئات من الوصول إلى الجزر المزروعة، فإنها ترسل إشاراتها الإشعاعية، فنستطيع رؤيتها ومتابعة بقائها وعملها داخل الجسم دون الحاجة إلى جراحة أو أخذ خزعات.
وتصميم هذا المتتبّع الإشعاعي ليس بالمهمة السهلة؛ إذ يجب أن يصل إلى العنوان الصحيح بدقة، وألا يضل طريقه إلى أهداف أخرى، وأن يحمل إشارة واضحة يستطيع جهاز PET رصدها.
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Preclinical PET Platform

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