Abstract Book

Endocrine Metabolic GPCRs Conference 2026

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Oral Communications

Oral Presentations 1
Monday 9 February 2026, 14:00 – 14:30

OC1

Profiling GIPR signalling mechanisms underlying lipid metabolism rewiring in adipocytes

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The glucose-dependent insulinotropic polypeptide receptor (GIPR), a class B GPCR involved in the control of glucose levels and body weight regulation, has emerged as a promising therapeutic target for obesity. However, there is no consensus on whether GIPR agonism or antagonism is the optimal strategy, as both approaches paradoxically promote weight loss through poorly understood mechanisms, nor is the main site of action of GIPR agonists clearly defined.

Here, we characterise the effects of acute and overnight GIP stimulation on lipid metabolism in stem cell-derived white and brown adipocytes and identify the existence of a spatially restricted GIPR-dependent cAMP/PKA signalosome at the interface between lipid droplets (LDs) and endoplasmic reticulum, and the mitochondria, using a genetically engineered split PKA biosensor. Our results confirm the presence of endogenous, signalling competent, GIPR in both adipocyte models, and further demonstrate the role of ER calcium and the SERCA pump in GIP-induced cAMP generation and lipolysis in these cells. We additionally unveil divergent effects of GIPR agonism in LD morphology in white *versus* brown adipocytes, with enlarged LD size and reduced LD number in white adipocytes, but increased abundance of small LDs in brown adipocytes following overnight GIP treatment, indicating adipocyte subtype-specific lipid metabolism effects.

We further employed Seahorse assays to assess oxygen consumption rates (OCR) with and without exposure to the CPT1a inhibitor, etomoxir, to demonstrate reduced fatty acid oxidation (FAO) in GIP-stimulated brown adipocytes, an effect that correlates with reduced LD-mitochondrial contacts, imaged with a fluorescent SPLICS membrane contact site sensor. Preliminary experiments using DGAT inhibitors appear to rule out a role for GIPR in lipid re-esterification in brown adipocytes, suggesting alternative destinations for the lipid molecules released by lipolysis in these cells. These findings provide new insights into how LD-localised GIPR signalling remodels adipocyte lipid metabolism and modulates energy expenditure, with implications for therapeutic targeting of the GIPR axis in obesity.

OC2

Two Succinate GPCR Pathways: Muscle Stem-Cell Sensing and Hypoxia-Induced Epithelial Signalling

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Metabolite-sensing GPCRs translate rapid changes in cellular energy state into coordinated tissue responses. Succinate, a mitochondria-derived stress metabolite, signals through SUCNR1, yet how this axis diversified across vertebrates and integrates metabolic stress at the tissue level remains unclear.

Using zebrafish, we identify three homologues (Oxgr1a.1, Oxgr1a.2, Oxgr1a.3) and show through molecular pharmacology, mutagenesis, and molecular simulations that Oxgr1a.2 and Oxgr1a.1 function as bona fide succinate sensors with conserved arginine-based ligand-binding determinants.

Oxgr1a.2 emerges as a metabolic-state sensor in skeletal muscle: it is enriched in muscle stem cells and dynamically regulated across their differentiation trajectory, linking succinate sensitivity to muscle metabolic adaptation. Cross-species analyses in mouse injury models and human spatial transcriptomics reveal that *SUCNR1* similarly tracks *MYOD/MYOG* expression during regeneration, indicating a conserved succinate-responsive programme in vertebrate muscle.

Oxgr1a.1, in contrast, is positioned at epithelial–ionocyte interfaces. To probe its role in metabolic-stress signalling, we exposed zebrafish to acute hypoxia and performed spatial metabolomics on gills. Succinate accumulated locally in ionocyte-rich regions, consistent with mitochondrial redox stress. Given *oxgr1a.1* expression in neighbouring epithelial cells, these data support a paracrine hypoxia-response circuit in which ionocyte-derived succinate is sensed via Oxgr1a.1 to coordinate hypoxia adaptation.

Together, these findings reveal two complementary metabolic-GPCR circuits—muscle stem-cell succinate sensing via Oxgr1a.2 and epithelial hypoxia sensing via Oxgr1a.1—and position the SUCNR receptors as conserved integrators of mitochondrial stress and tissue metabolic adaptation.

Oral Presentations 2
Monday 9 February 2026, 15:45 – 16:15

OC3

Circadian rhythm disruption reduces GLP1 and GIP agonist efficacy in pancreatic beta cells

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Background: Circadian disruption is associated with increased risk of developing type 2 diabetes. Circadian misalignment, such as that seen in shift-workers, is linked to reduced insulin secretion and impaired glucose tolerance. How circadian disruption influences pancreatic beta cell responses to anti-diabetic therapy remains poorly understood.

Materials and Methods: Bmal1$^{fl/fl}$ mice were crossed with a strain expressing Cre recombinase under the Ins1 promotor, giving beta-cell specific Bmal1 KO (β-cell$^{BMAL1-/-}$) and wild-type littermate controls (and β-cell$^{BMAL1+/+}$). cAMP levels were measured using the FRET-based sensor H188 (EpacVV), transduced using adenovirus. Insulin secretion was assessed in response to various stimuli using Promega Lumit assay. The F-actin cytoskeleton was visualised using phalloidin staining and granule docking investigated through transmission electron microscopy (TEM). GLP1R expression was determined using validated antibody and confocal microscopy. Glucose tolerance was assessed *in vivo* using intra-peritoneal glucose tolerance testing (IPGTT).

Results: β-cell$^{BMAL1-/-}$ islets displayed impaired insulin secretory responses to glucose, which could not be rescued using GLP1R or dual GLP1R/GIPR (dual) agonists. While GLP1R expression at the beta cell membrane was similar in β-cell$^{BMAL1-/-}$ and β-cell$^{BMAL+/+}$ islets, GLP1R and dual agonist-stimulated cAMP rises were ~ 2-fold lower in the circadian disruption model. Glucose-regulated F-actin cytoskeleton remodelling, needed for proper insulin release, was impaired in β-cell$^{BMAL1-/-}$ islets. Electron microscopy showed fewer docked insulin granules at the membrane in β-cell$^{BMAL1-/-}$ islets. β-cell$^{BMAL1-/-}$ mice were glucose intolerant, a phenotype that persisted following treatment with GLP1R and dual receptor agonists.

Conclusions: Circadian disruption reduces GLP1R/GLP1R (dual) agonist efficacy in vitro and in vivo. Mechanistically, circadian disruption leads to defective cAMP signalling as well as differences in cytoskeleton remodelling, reducing insulin granule docking and release.

OC4

SSTR2 Receptor States and Recycling Dynamics Shape the Apparent Affinity of ^{177}Lu-DOTATATE

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Somatostatin receptors (SSTRs) regulate many endocrine and metabolic functions and are commonly overexpressed in tumours of endocrine origin making them suitable for targeted radionuclide therapy (TRT). The radiolabelled somatostatin analogue ^{177}Lu-DOTATATE, which primarily targets SSTR2, is approved for treating SSTR-positive gastroenteropancreatic neuroendocrine tumours (GEP-NETs).

This study provides a detailed characterization of how ^{177}Lu-DOTATATE interacts with SSTR2 expressed on live cancer cells. Real-time cell binding assays using LigandTracer revealed two main interaction populations distinguished by their retention times. High-affinity binding (low pM range) was associated with G-protein-coupled receptors, while lower-affinity binding (low nM range) corresponded to uncoupled receptors. Notably, high-affinity binding was also present in fixed cells, indicating a population of pre-coupled receptors. A minor third interaction was observed only in live cells, suggesting an intermediate receptor state with enhanced ligand binding accessibility.

At 37°C, ^{177}Lu-DOTATATE internalization was rapid and substantial, with more than 80% internalized ligands after 30 min. Cellular retention decreased with ligand exposure suggesting a shift from ^{177}Lu-DOTATATE being predominantly bound to coupled receptors to being bound mostly to uncoupled receptors after prolonged exposure. Displacement studies using unlabelled DOTATATE revealed that internalized ^{177}Lu-DOTATATE is rapidly recycled and released from the cells. Unlabelled somatostatin analogues (SSA) are used clinically for symptom management and their impact on internalized ^{177}Lu-DOTATATE warrants further investigation as this could inform optimal SSA administration timing to enhance TRT efficacy.

This study highlights the dynamic nature of ligand binding to GPCRs in a live cell environment. By employing cell-based real-time binding assays, we were able de-convolute the contribution of distinct receptor states to ^{177}Lu-DOTATATE affinity and retention and study the impact of internalization and recycling dynamics. Improved understanding of ligand/drug-GPCR interactions and how these are impacted by the cellular environment and metabolism can be used to optimize existing and new therapeutic approaches.

Oral Presentations 3
Tuesday 10 February 2026, 11:45 – 12:15

OC5

A GRK-Biased β_2-Adrenergic Receptor Agonist Induces Coordinated Fat Loss and Muscle Gain via Pathway-Selective Signaling

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Biased agonism at G protein–coupled receptors (GPCRs) enables selective modulation of intracellular signaling pathways, offering a strategy to enhance efficacy and safety. Building on our recent Cell publication, we now report a next-generation β_2-adrenergic receptor (β_2AR) agonist engineered for GRK-biased signaling. In cellular models, the compound exhibits limited β-arrestin recruitment and a more favorable cAMP/GRK-dependent signaling profile, resulting in sustained receptor responsiveness and reduced desensitization. In vivo, it drives a more pronounced remodeling of body composition, achieving greater reductions in fat mass and increases in lean mass than observed with earlier compounds, together with improved metabolic parameters. These effects, which occur independently of changes in food intake, are maintained with sustained efficacy and minimal cardiovascular impact. Furthermore, the compound is able to potentiate the fat loss from incretin analogues while preventing the associated loss of lean mass. Collectively, the findings demonstrate that pathway-selective β_2AR modulation can decouple the anabolic and metabolic benefits of β_2AR activation from its adverse effects, positioning this next-generation, GRK-biased β_2AR agonist as a promising therapeutic candidate for obesity, sarcopenia, and related metabolic disorders.

OC6

Ligand-PTH1R-βarrestin-complex stability dictates compartmentalized signaling

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The parathyroid hormone receptor (PTH1R) is a G protein–coupled receptor (GPCR) that mediates the actions of parathyroid hormone (PTH) and PTH-related peptide (PTHrP). Recent findings in vitro suggest that these two ligands elicit distinct physiological outcomes through spatially and temporally segregated signaling. Thus, PTH is thought to control systemic calcium homeostasis via sustained signaling from endosomes, whereas PTHrP regulates skeletal development through transient responses at the plasma membrane. Mutations in PTH1R that cause Eiken syndrome, an ultra-rare skeletal dysplasia, lead to delayed bone mineralization—consistent with enhanced PTHrP activity in growth plate chondrocytes—while simultaneously causing PTH resistance, indicative of impaired receptor function in bone and kidney. To dissect this paradoxical phenotype, we investigated two Eiken syndrome-associated variants, I237N and D241E, in cells and in mice. The mutated residues are located in transmembrane helix 2 and are predicted to participate in ligand-binding. In cell assays, both PTH1R mutants exhibited markedly weakened ligand binding affinity and thus formed destabilized ligand–receptor complexes. This instability did not impair initial Gαs activation at the cell surface, but compromised GRK recruitment and βarrestin engagement. The impaired βarrestin coupling reduced receptor desensitization at the plasma membrane and resulted in hyperresponsiveness to PTHrP. These effects were paralleled by delayed bone mineralization in D241E mice. Simultaneously with impaired βarrestin recruitment endosomal Gαs activation in response to PTH action was abolished in cells, which was paralleled by PTH resistance in D241E mice. These findings establish ligand–receptor complex stability dependent effector engagement as a key determinant of compartmentalized PTH1R signaling. They further highlight that relatively weak ligand-binding in vivo can cause a gain-of-function effect via a selective impairment of βarrestin-mediated desensitization relative to preserved Gαs activation.

Oral Presentations 3
Tuesday 10 February 2026, 14:00 – 14:30

OC7

In vivo role of β-arrestin 2 and GLP-1R and GIPR biased signalling in α-cells

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Glucagon-like peptide-1 receptor (GLP-1R) and glucose-dependent insulinotropic polypeptide receptor (GIPR) responses are adversely affected in a type 2 diabetes (T2D) diseased state, with GLP-1R retaining some activity but GIPR responses severely blunted despite unaffected levels of GIP secretion. However, it is now known that both GLP-1R and GIPR activities are restored in T2D patients after a period of blood glucose normalisation, making the both incretin receptors relevant targets for T2D treatment. Further, mechanisms of GIPR and GLP-1R modulation of α-cell glucagon secretion remain unclear.

Preliminary data has shown a G protein *versus* β-arrestin 2 (βArr2) recruitment bias for the GIPR in α- *versus* β-cells. We also previously described the glycaemic action of GLP-1R and dual GLP-1R/GIPR agonists in β-cell-specific βArr2$^{-/-}$ adult mice, with chow-fed KO females and mixed-sex animals under a high fat, high sucrose diet showing reduced acute glucose tolerance which improved 6 hours post-agonist exposure. Acute β-cell-specific cAMP generation in response to the pharmacological GLP-1R agonist exendin-4 (Ex-4) was reduced in KO islets but rescued by concomitant β-arrestin 1 (βArr1) knockdown. Here, we have assessed *in vivo* pharmacological incretin responses in an α-cell-specific βArr2$^{-/-}$ background using a PPG-Cre^{ERT}-βArr2 floxed mouse model. Chow-fed KO males showed no differences *versus* wildtype littermate controls in glucose tolerance following GLP-1R or GIPR agonist exposure. However, while chow-fed KO females also showed no difference in response to short-acting agonists, they displayed significantly improved glucose tolerance 24 hours post-administration of the long-acting GLP-1R/GIPR dual agonist tirzepatide, which was seemingly reversed at the 72-hour time point. Purified islets from α-cell-specific βArr2$^{-/-}$ mice expressing the cAMP biosensor ^{t}Epac^{vv} specifically from α-cells showed reduced GIP- and tirzepatide-mediated cAMP generation *versus* wildtype control islets, but negligible cAMP responses to Ex-4 in either wildtype or α-cell-specific βArr2$^{-/-}$ islets. βArr1 knockdown partially rescued the observed cAMP defect in α-cell βArr2-/- islets, implicating βArr1 action in the observed GIPR signal downregulation.

This work highlights the importance of understanding cell type-specific responses of incretin receptors within the islet, which may help underlie the rationale behind improved methods to target these receptors locally for the treatment of T2D, obesity and other associated disorders.

OC8

Spatiotemporally resolved GPCR interactome uncovers unique mediators of receptor agonism.

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Introduction.

G protein-coupled receptors (GPCRs) are the largest family of signalling membrane proteins. 30% of all the market drugs target GPCRs, making them highly attractive proteins to study[1]. GPCR signalling in cells in response to an agonist (small molecule, hormone, peptide to name just a few) is highly dynamic and underpinned by a diverse array of mechanisms. High-precision chemical biology approaches are required to decode GPCR interactions as they evolve in time and space, paving way for new therapeutics[1].

Here, we developed an analytical approach, integrating emerging proximity-based APEX2 method, quantitative multiplexed proteomics, bioinformatics and functional screening, providing a platform to interrogate the interaction network of the luteinizing hormone receptor (LHR) on a sub-minute timescale in the 20 nm proximity to LHR[2].

Methodology.

hLHR was tagged with APEX2 at the C-*terminus*. Upon activation with LH agonist, LHR initiates signalling cascades, followed by endocytosis within 2-3 minutes. APEX2 catalyzes the biotinylation of LHR interactome in the 20 nm proximity cloud in the presence of H_2O_2 and biotin-phenol. hLHR-APEX2 cells were activated with LH for 0, 1, 2, 3, 5 and 10 min, followed by proximity labelling, on-beads pull-down of biotinylated proteins, isobaric TMT-7plexing and quantitative MS/MS. We were able to resolve dynamic interactions of known LHR partners like G_as (GNAS), beta-arrestin (ARRB2), GIPC1 and others. We then developed a multi-layered quantitative interactome analysis pipeline, harnessing time-dependent LHR-GIPC1 association as a temporal reference, that allowed to identify multiple novel putative LHR interactors in time- and space-resolved manner. We have functionally validated 45 hits by performing an siRNA library screening and confocal microscopy, which led to discovery of two novel putative LHR interactors, RAP2B and RAB38, which may direct early endocytosis events.

Taken together, this is the first study to interrogate interactome of a poorly characterized GPCR, with minimal existing pharmacological tools and known protein interactions, providing a roadmap to accelerate fundamental discovery research for other GPCRs, and likely also other membrane receptors[2].

References:

1. Shchepinova MM, et al. Curr Opin Chem Biol. **2020**, doi: 10.1016/j.cbpa.2020.04.012.

2. Shchepinova MM, et al. Cell Chem Biol. **2025**, doi: 10.1016/j.chembiol.2025.04.006.

Poster Presentations

P1

Epicardial Adipose Tissue and Prokineticin Signaling: A Novel GPCR Axis in Obesity-Associated Heart Failure and Cardiometabolic Disease

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Epicardial adipose tissue (EAT), a visceral fat depot anatomically and functionally linked to the myocardium, has emerged as a dynamic endocrine organ influencing cardiovascular health. Its close proximity to the heart allows paracrine and mechanical interactions that drive cardiac remodeling, particularly in heart failure with preserved ejection fraction (HFpEF)—a condition closely associated with obesity and type 2 diabetes. Traditional obesity metrics, such as BMI, fail to capture the pathological impact of EAT, which contributes to cardiomyopathy through pro-inflammatory cytokine secretion, fibrosis, and metabolic stress.

Recent advancements in pharmacotherapy, including SGLT2 inhibitors and GLP-1 receptor agonists, have demonstrated the capacity to modulate EAT volume and function, thereby improving cardiometabolic outcomes. In parallel, emerging evidence positions the prokineticin/PKR1 signaling axis—an underexplored G protein-coupled receptor (GPCR) pathway—as a critical regulator of EAT homeostasis, adipose tissue remodeling, and systemic metabolic health. Prokineticin-2 (PK2), acting via PKR1, suppresses preadipocyte differentiation, promotes insulin sensitivity, reduces oxidative stress, and enhances cellular survival. Furthermore, PKR1 activation in epicardial progenitor cells supports angiogenesis while limiting pathological fat accumulation, positioning this pathway as a promising therapeutic target in the context of HFpEF, obesity, and insulin resistance.

This presentation will provide a comprehensive overview of EAT's multifaceted role in cardiovascular disease, the emerging therapeutic landscape targeting adipose tissue inflammation and remodeling, and the translational potential of prokineticin signaling. By elucidating a novel GPCR-regulated endocrine-metabolic axis, we propose a paradigm shift in the treatment of cardiometabolic disease rooted in visceral adiposity.

P2

Unveiling a small-molecule modulator for Frizzleds: a structural and pharmacological breakthrough in Class F GPCRs

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Frizzleds (FZDs) belong to Class F of G protein-coupled receptors (GPCRs) and are well-known for their role in the WNT/β-catenin signaling pathway which has diverse roles in health and disease. Despite significant progress in understanding receptor activation, there are no current therapeutics on the market for this elusive class. Thus, further structureguided drug development is needed to identify how to target these receptors therapeutically. Recently, our lab has identified a putative small molecule that selectively binds FZDs albeit with limited efficacy in vitro. While this molecule has very low affinity, we have solved what is the first structure with a small molecule bound to a FZD and warrants further investigation into structure-based drug development. These findings significantly advance the field in not only understanding the basic pharmacology of FZDs but moreover the ability to fine tune and control signalling pathways linked to numerous pathologies.

P3

From Salt Imbalance to Tumour Discovery: A case of Hypernatremia and Hypokalemia Secondary to Neuroendocrine Tumour

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Background:

Electrolyte imbalance is a well-documented yet frequently underestimated feature of Cushing's syndrome, particularly when secondary to ectopic ACTH secretion by neuroendocrine tumors. While the majority of patients present with classical hormonal manifestations—such as central obesity, hypertension, and glucose intolerance—some may first exhibit severe metabolic disturbances, which can obscure the underlying diagnosis. Metabolic disturbances like Hypernatremia and Hypokalemia are medical emergencies that need urgent intervention and constant monitoring.

Case Presentation:

We present a case of a patient who initially presented to the acute medical unit with profound hypokalemia and hypernatremia. Early serological evaluation revealed elevated cortisol and ACTH levels, prompting further imaging. A CT scan of the thorax identified a suspicious mass in the lung, and a subsequent biopsy confirmed a neuroendocrine tumor. The patient was managed through a multidisciplinary approach involving endocrinology, oncology, and respiratory medicine which was initiated in the Acute medicine ward. Initial management focused on urgent correction of electrolyte imbalance, followed by planning for oncologic treatment. Histopathological examination confirmed the diagnosis of an ACTH-secreting pulmonary neuroendocrine tumor.

A key aspect of this case was the absence of cushingoid features, which may inadvertently divert clinical attention toward symptomatic correction of the electrolyte imbalance, potentially delaying investigation into the underlying etiology.

Discussion:

This case highlights the importance of maintaining a high index of suspicion for endocrine malignancy in patients presenting with unexplained electrolyte disturbances. Early serological testing and cross-sectional imaging can be instrumental in identifying the underlying cause. A multidisciplinary team (MDT) approach is crucial to manage not only the acute medical issues but also to coordinate long-term oncologic and endocrine care.

Conclusion:

Hypernatremia and hypokalemia are medical emergencies that demand prompt evaluation and management. However, when accompanied by atypical or unexplained features, clinicians must broaden the differential diagnosis. Early hormonal assays and radiologic imaging can be critical tools in uncovering occult neuroendocrine tumors, allowing for timely diagnosis and intervention that may significantly impact patient outcomes.

References: The Endocrine Society Clinical Practice Guideline, UK Best Practice Guidelines in Endocrinology, NICE Guidelines (NG12)

P4

Syndecan-3 modulates MC4R signaling

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Syndecans are a family of heparan sulfate proteoglycans, proposed to function as co-receptors for GPCR proteins. Overexpression of syndecan-1 in the mouse brain leads to hyperphagia and obesity, whereas syndecan-3 knockout mice show resistance to high-fat diet induced obesity. SDC3 is known to stimulate feeding behavior in mice by enhancing AgRP binding to MC4R. However, AgRP-independent mechanisms for SDC3-mediated MC4R signaling have not yet been investigated. In this study, we explored whether SDC3 affects downstream MC4R signaling independently of AgRP.

Analysis of human hypothalamic single-cell RNA sequencing data showed neuronal co-expression of MC4R and SDC3 at a ratio of 1:1. We therefore assessed the impact of SDC3 at this ratio on α-MSH-induced MC4R cAMP responsiveness in HEK293 cells, which lack AgRP. At a 1:1 ratio with MC4R, SDC3 suppressed Emax by 24±1.5% ($P<0.0001$), but had no significant effect on EC50. A previous mouse study showed that fasting causes hypothalamic SDC3 protein levels to increase >4-fold. To mimic this hunger state, we investigated the effect of SDC3 on α-MSH-induced cAMP production using an MC4R:SDC3 ratio of 1:5. At this ratio we observed stronger suppression of Emax (36±1.5%; $P<0.0001$), and an increase in EC50 (from 8±0.5 nM to 21±5.4 nM; $P<0.05$). Next, we investigated whether SDC3 modulates MC4R expression on the cell surface. We found that SDC3 had no significant effect at a ratio of 1:1, but a ratio of 1:5 markedly suppressed MC4R cell surface levels by 51±4.2% ($P<0.0001$), potentially explaining the reduction in cAMP production. Similarly, total expression levels of MC4R were not suppressed at a ratio of 1:1, but were suppressed by 33±5.8% ($P<0.05$) at a ratio of 1:5.

In conclusion, SDC3 suppresses downstream MC4R signaling independently of AgRP. The lack of effect on cell surface MC4R levels at a 1:1 ratio suggests that SDC3 can modulate intracellular signaling. The dose-dependency of this effect could be paralleled in vivo upon fasting when hypothalamic SDC3 levels are increased.

P5

Measuring GPCR signaling kinetics using biosensor and data analysis technologies

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The duration of GPCR signaling is a determinant of physiological and therapeutic responses to receptor activation. Routinely quantifying signaling over time requires robust kinetic assays and data analysis frameworks. Here signaling kinetics was measured for many hours using fluorescent biosensors for cAMP (cADDis) and arrestin recruitment (Borealis), for GPCRs including GLP-1, glucagon and GIP receptors. Profound differences of the duration of signaling were observed, e.g. transient cAMP generation by GLP-1 (7-36) and sustained wash-resistant signaling by orforglipron. Data were analyzed using two methods. The concentration response was determined at early, mid and late time points and the shift of activity over time used as a simple metric to rank compounds for persistence and onset of signaling. This was automated for rapid high throughput analysis in Excel. In addition the kinetic rate constants and other parameters were quantified by curve fitting in GraphPad Prism. Together the biosensor and data analysis technologies provide the means to rapidly and effectively quantify signaling kinetics, to provide new insight into physiological and therapeutic mechanisms.

P6

GPR75 in metabolic syndrome: role in adipogenesis

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Metabolic syndrome (MetS) is a group of conditions including insulin resistance, hypertension, and obesity, which can lead to cardiovascular disease, type 2 diabetes and steatotic liver disease. The global increase in MetS means there is an urgent need for new drug targets. Genetic deletion of the orphan G protein-coupled receptor, GPR75, protects against high-fat-diet (HFD)-induced MetS (Leeson-Payne *et al.,* 2024), however the mechanism and cellar targets by which GPR75 deficiency leads to reduced fat accumulation remains unknown. We aimed to investigate the role of GPR75 in adipogenesis. WT- and GPR75-SGBS (human preadipocyte cell line) and primary preadipocytes isolated from brown adipose tissue (BAT) from WT- and CRISPR-GPR75 mice were differentiated for 8-12 days to induce adipogenesis and lipid formation confirmed by oil-red staining. Markers of adipogenesis, lipid metabolism and mitochondrial function were determined by qPCR and western blot. Real-time oxygen consumption rate of adipocytes was assessed using Seahorse XF HS Mini Analyzer. Deletion of GPR75 enhanced brown mouse adipocyte differentiation and induced markers of lipolysis (pHSL) and mitochondrial function (PHB2) [pHSL: 0.27+/-0.02 vs. 0.71+/-0.08, $P<0.05$; PHB2: 1.1+/-0.3 vs. 2.1+/-0.11, $P<0.05$, relative expression WT vs. CRISPR-GPR75, respectively, n=3]. Differentiated brown adipocytes isolated from CRISPR-GPR75 mice showed increased basal and maximal mitochondrial respiration compared to WT [basal: 9.27+/-3.23 vs. 19.01+/-2.23, $P<0.05$; maximal, 23.1 +/-16.3 vs. 65.1 +/-7.5, $P<0.01$, WT vs. CRISPR-GPR75, respectively, n=3]. BAT from HFD-CRISPR showed increased expression of the thermogenic marker UCP1 [1.7 +/- 0.12 vs. 2.53 +/- 0.07, HFD-WT vs. -CRISPR-GPR75, respectively, n=3, ($P<0.01$)]. In contrast, FABP4, and Adiponectin, and lipid droplet formation, which increased during differentiation in WT-SGBS (FABP4: Day 0: 1.6 +/- 0.81, Day 8: 17902 +/- 6588, n=3; Adiponectin, Day 0: 0.6 +/- 0.1, Day 8: 1685 +/- 429, n=3) were blunted in SGBS overexpressing GPR75 (FABP4, Day 0: 1.1 +/- 0.27, Day 8: 31.4 +/- 8.5; n=3, $P<0.05$: Adiponectin, Day 0: 0.6 +/- 0.2, Day 8: 3.2 +/- 1.1, n=3, $P<0.05$). Our data show GPR75 to have a key role in the regulation of adipogenesis and brown adipocyte function, which may contribute to its protective role in MetS and validate GPR75 as a therapeutic target.

P7

GPR75 and GPR151: novel orphan GPCRs therapeutic targets for drug discovery in obesity

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Orphan G protein-coupled receptors - with no identified endogenous ligands - represent an untapped reservoir of therapeutic potential, offering promising avenues for developing innovative treatments across a spectrum of diseases. However, drug development for these ~100 uncharacterized GPCRs is challenging due to the absence of validated ligands and limited understanding of their signaling, complicating drug screening assay design. Several recent findings using whole exome sequencing and association of variants with clinical phenotypes have identified GPR75 and GPR151 as therapeutic targets for obesity and other metabolic disorders. Combining Domain's bioSens-All® platform and GPCR expertise, offers the opportunity to go after new and challenging GPCR targets with high therapeutic relevance. We will discuss applications of our enhanced bystander bioluminescence resonance energy transfer (ebBRET) platform, bioSens-All®, to develop high-throughput screening assays for orphan GPCR drug discovery efforts (e.g., GPR75 and GPR151). Using different strategies taking advantage of a receptor's constitutive activity a screening campaign was performed to identify agonist, inverse agonists and potential antagonists targeting GPR75. In addition, we have revealed that GPR151's constitutive activity is able to trans-inhibit the activity of a surrogate receptor offering a screening assay for antagonists in the absence of a known ligands. The identification of screening tools and therapeutics targeting novel orphan genetically validated GPCR target, such as GPR75 and GPR151 in metabolic disorders, expands the obesity targets beyond incretin receptors.

P8

Investigating the impact of binding kinetics on biased signalling at GLP-1R

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Introduction. Tirzepatide is a dual-GIP/GLP-1 receptor agonist that has demonstrated superior efficacy in terms of weight loss and glycemic control compared to mono-GLP-1R agonists. The contribution of GIPR activation to this superior efficacy is currently unknown. Tirzepatide also acts as a partial agonist at GLP-1R compared to GLP-1 in terms of G protein signalling and is reported to cause less receptor desensitization and internalization relative to GLP-1.

Aims. This project aimed to investigate the binding kinetics and signalling properties of the non-lipidated precursor of tirzepatide (NLT) compared to native GLP-1 at GLP-1R.

Methods. G protein and arrestin recruitment to GLP-1R, as well as agonist-mediated receptor endocytosis, were assessed using BRET-based methods. Binding assays were performed using HEK-293 cells stably expressing Nluc-GLP-1R and fluorescently labelled LUXendin645. The affinities of GLP-1 and NLT were calculated using the Cheng-Prusoff method, and association and dissociation rates were calculated using the Motulsky-Mahan method.

Results. NLT recruited mini-Gs to GLP-1R with a lower Emax (P=0.037) than GLP-1. Unlike GLP-1, NLT-mediated arrestin recruitment to GLP-1R was undetectable, and NLT-stimulated receptor endocytosis occurred at a slower rate (P=0.026) and to a lesser extent (P=0.0049) than GLP-1. In contrast, NLT had a higher affinity (P=0.0005) for Nluc-GLP-1R compared to GLP-1. There was no difference in their rate of dissociation; however, NLT's rate of association was faster (P=0.0008) than that of GLP-1.

Discussion. NLT is a G protein-biased agonist at GLP-1R and a partial agonist in terms of G protein recruitment relative to native GLP-1. A faster rate of association drives NLT's higher affinity for Nluc-GLP-1R relative to GLP-1.

P9

Exploring apelinergic system signaling in autosomal dominant polycystic kidney disease

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The apelinergic system (AS), composed of the apelin receptor (APLNR), a GPCR, and its ligands apelin and apela, has emerged as a compelling therapeutic target in various pathophysiological contexts, including hypertension, heart failure, oncogenesis and nephropathies, owing to its anti-inflammatory and anti-apoptotic properties. However, the knowledge of its precise function within the human kidney remains limited. We aim to investigate the role of the AS in the human kidney, focusing on autosomal dominant polycystic kidney disease (ADPKD). ADPKD is a hereditary, progressive nephropathy characterized by cystogenesis, parenchymal destruction, and eventual kidney failure. Currently, no curative treatment is available and mechanistic insight in the process of cyst formation is limited. Enhanced cAMP signaling is a hallmark of cyst cells and a principal driver of cyst expansion. It has been shown that cAMP signaling can be attenuated by AS signaling. Moreover, important signaling cascades (including Akt/mTOR, AMPK, ERK) are shared by the AS and aberrant cell signaling in ADPKD cells. Further, elevated circulating apelin levels in ADPKD patients with preserved renal function have been observed.

Using human kidney tissue samples from both healthy individuals and ADPKD patients and human kidney epithelial cell lines generated from nephrectomy or urine samples, our findings indicate endogenous AS expression in the human kidney and APLNR upregulation in ADPKD collecting duct cells. Single-cell perfusion experiments corroborated the AS ability to attenuate cAMP signaling. Moreover, AS activation mediated pro-proliferative effects and a putative influence on cyst growth in a 3D cyst assay using patient-derived cystic cells.

These findings provide novel insights into the AS in the human kidney and its involvement in ADPKD pathology. We further aim to elucidate the signaling intricacies of AS activity in human kidney epithelial cells and to explore its translational potential in clinical nephrology.

P10

The lactate receptor enhances thermogenesis in human and murine brown adipose tissue

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Brown adipose tissue (BAT) is a thermogenic organ that generates heat via uncoupling protein 1 (UCP1) to maintain body temperature during cold exposure. The presence of BAT is associated with improved cardiometabolic health, as such BAT activation holds therapeutic potential against obesity and associated diseases. BAT thermogenesis is fuelled by energy substrates such as glucose, which undergoes glycolysis and is released primarily as lactate. We hypothesized that lactate enhances BAT thermogenesis through its receptor GPR81 (encoded by *HCAR1*). GPR81 is a Gi-coupled protein receptor which inhibits lipolysis in white adipose tissue (WAT), however its role in BAT is not clearly understood.

The effect of lactate and the GPR81 agonist 3,5-dihydroxybenzoic acid (DHBA) on cellular respiration, lipolysis, and thermogenic gene expression were investigated in primary human and murine brown and white adipocytes. Basal and stimulated mitochondrial respiration was also measured in adipose tissue explants from mice with and without global disruption of *Hcar1*. In human adipocytes, *HCAR1* mRNA levels were expressed ~3-fold higher in brown than white adipocytes. Both lactate and DHBA increased *UCP1* expression in brown but not white adipocytes, while siRNA knockdown of *HCAR1* tended to decrease *UCP1* expression and cellular respiration in brown adipocytes. In mice, *Hcar1* knockout (KO) reduced mitochondrial respiration in both interscapular BAT and inguinal beige adipose tissue explants, with more enhanced decrease in BAT. These data revealed sex-specific differences, as *Hcar1* KO reduced mitochondrial respiration to a greater extent in BAT in female mice, but in inguinal adipose in male mice. In murine primary adipocytes, glycerol release (a marker of lipolysis) was increased in white adipocytes from *Hcar1* KO animals, but was decreased in brown adipocytes, highlighting depot-specific differences in GPR81 function.

These preliminary data suggest that GPR81 enhances brown adipose tissue thermogenesis, potentially through activation of UCP1. These data also reveal novel depot- and sex-specific differences in the role of GPR81 in thermogenic adipose tissue. Further work is ongoing to determine the thermogenic importance of adipose GPR81 in vivo.

P11

Regulation of β-cell GLP-1R function by SUMOylation

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The glucagon-liked peptide-1 receptor (GLP-1R) is a class B1 G protein–coupled receptor (GPCR) that enhances glucose-dependent insulin secretion in pancreatic β-cells and regulates appetite in the brain, with drugs targeting this receptor being major therapeutic options for type 2 diabetes and obesity. Although several post-translational modifications (PTMs), most notably phosphorylation and ubiquitination, are known to modulate GPCR activity, the possible contribution of SUMOylation in GPCR signal regulation has received far less attention. SUMOylation is a reversible PTM involving the covalent attachment of Small Ubiquitin-like Modifier (SUMO) proteins to lysine residues, which is dynamically regulated by SUMO-specific proteases. While the SUMO-dependent regulation of insulin secretion via the direct effect of the SUMO protease SENP1 on the β-cell exocytotic machinery is recognised, the influence of SUMOylation on GLP-1R signalling and trafficking remains poorly understood. Earlier work from our laboratory using mass spectrometry–based interactomic analysis of INS-1 832/3 pancreatic β-cells revealed that the GLP-1R is constitutively SUMOylated and undergoes ligand-dependent deSUMOylation upon its activation. Building on this, we profiled receptor signalling and trafficking characteristics following either modulation of SENP1 activity or mutation of predicted GLP-1R SUMOylation sites. Results indicate that alanine substitution of three intracellular lysine residues (K437, K434, K342) predicted as GLP-1R SUMOylation sites leads to reduced plasma membrane diffusion rates, β-arrestin 2 recruitment, cAMP production and receptor surface expression following stimulation with the pharmacological GLP-1R agonist (GLP-1RA) exendin-4 in INS-1 832/3 cells. Conversely, SENP1 knockdown produced largely opposite effects, leading to enhanced GLP-1R plasma membrane diffusion, cAMP generation, and surface expression, as well as decreased receptor internalisation. Importantly, preliminary *in vivo* studies in β-cell-specific SENP1 KO mice indicate impaired oral glucose tolerance and glucoregulatory responses to pharmacological GLP-1RAs only present in female mice, implying a sex dimorphic effect of this PTM in regulating β-cell GLP-1R responses. Together, these findings reveal an important and previously unappreciated role for receptor SUMOylation in the regulation of GLP-1R function which might be amenable to pharmacological exploitation.

P12

Development of Tools for the elucidation of GPR75 signalling and identification of GPR75 modulators

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G protein coupled receptors (GPCR) play a vital role in the regulation of metabolic processes, making them ideal therapeutic targets for treating metabolic associated disorders. These include conditions such as type 2 diabetes (T2D), obesity, and dyslipidaemia that together impose a major burden on healthcare systems worldwide. Orphan receptor GPR75 has attracted a great deal of attention since its emergence as a potential druggable target for metabolic disorders. GPR75 is expressed in a range of tissues with high expression in the brain, where it is believed to play a key role in regulating appetite, body weight and energy metabolism.

Chemokine CCL5 and 20-HETE have been reported to bind GPR75. However, challenges in reproducing consistent ligand responses across different cell-based systems have hindered target validation and drug discovery efforts. Advancing our understanding of GPR75 signalling is crucial to realising its therapeutic potential in neurometabolic disorders.

To this end, a suite of GPR75 expressing cell lines and functional assays were generated to interrogate GPR75 signalling pathways and support the identification of novel ligands. These tools allow assessment of second messenger/G protein signalling, cell surface receptor expression, and β-arrestin interaction.

β-arrestin recruitment and CRE reporter assays were established to measure constitutive GPR75 activity as well as ligand-induced receptor signalling. A high-throughput,384-well plate format was subsequently optimised to support the detection of GPR75 modulating molecules.

A screening workflow was created utilising the β-arrestin recruitment and CRE reporter assay systems alongside GPR75 receptor cell-surface/internalisation assays. Together, these tools form a solid foundation for the identification of GPR75 modulators and provide unique opportunities to investigate GPR75 signalling. Development of these functional assays fulfils a critical need for hit-finding solutions that can lead to the discovery of novel therapeutic molecules for metabolic associated disorders.

P13

Impact on Kisspeptin Receptor (KISS1R) Regulation from its Interaction with G Protein-Coupled Estrogen Receptor (GPER)

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Introduction: The kisspeptin receptor (KISS1R) and G-protein-coupled oestrogen receptor (GPER) are pivotal for reproductive health. We previously showed oestrogen potentiates gonadotrophin secretion following KISS1R agonism. GPER heterodimerises with KISS1R, yet the impact on KISS1R pharmacology remains unclear. We aimed to characterise KISS1R/GPER interaction effects on expression, signalling and internalisation.

Methods and Results: In HEK293 cells transfected with human KISS1R and/or GPER, BRET confirmed constitutive heteromer formation, unaffected by ligands (KP10, KP54, TAK-448). Flow cytometry indicated GPER co-expression reduced KISS1R surface expression, unreversed by ligands. While maximum internalisation occurred at 10 minutes for all ligands, kinetics differed. KP54 induced maximal KISS1R internalisation (23.6%, n=5), which was reduced by GPER co-expression (17.6%, n=5). Furthermore, GPER shifted the transient KP54-induced internalisation to a sustained profile. Functionally, GPER-expressing cells showed high basal IP1 accumulation. Co-expression lowered basal IP1 and, unexpectedly, prevented further increases following treatment with any KISS1R ligand, even with oestrogen pretreatment (50pg/ml).

Conclusion: These findings suggest KISS1R/GPER heteromerisation dampens KISS1R surface expression and Gq-signalling while uniquely modulating agonist-induced endocytic trafficking. This highlights a regulatory role for heterodimerisation in fine-tuning KISS1R activity.

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P14

Leptin Gene Polymorphism in Under, Normal, Over Weight and Obese Depressed Patients.

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Background: Initially identified as anti-obesity gene, leptin gene is believed to play a role in mood control. The current study was planned to monitor leptin gene polymorphism in different BMI groups of male and female depressed patients.

Methods: Diagnosed depressed patients were divided into groups according to their BMI and severity of depression. Their leptin gene was sequenced to learn the relationship of leptin gene polymorphism with BMI and severity in depression.

Results: Leptin gene polymorphisms rs36219260, rs17151914 and rs17151922 were found to be related with BMI and depression.

Conclusion: Further studies are required to learn the relationship of these SNPs with the hormonal levels and the frequency of these SNPs in undepressed population.

P15

Free fatty acid receptor 4 (FFAR4) variants associated with disease have distinct signalling profiles

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The free fatty acid receptor 4 (FFAR4) is activated by medium- to long-chain fatty acids, including omega-3 fatty acids, and regulates metabolic processes. Two human FFAR4 variants, R67C and R254H/R270H, are associated with an increased risk of obesity and diabetes, yet their signalling profiles are poorly defined. This study characterised the signalling of these variants in model and adipocyte cell lines.

HEK293T cells and immortalised brown mouse preadipocytes differentiated into brown adipocytes were transfected with wild-type (WT), R67C, or R254H FFAR4-Nluc constructs together with Venus-tagged mini-G proteins (mG12, Gq, Go, Gs, Gi) or β-arrestin 2. Agonist-induced recruitment was quantified by BRET following stimulation with the tool agonist TUG891 or endogenous ligand α-linolenic acid (αLA). Additional assays examined responses to intracellular FFAs generated by lipolysis. Signalling differences between WT and variants were evaluated by paired t-tests (AUC, $p < 0.05$). Receptor localisation was assessed by immunofluorescence and HILO microscopy.

In HEK293T cells, R67C showed reduced recruitment of mGq, mGo, and mGi to both ligands. R254H had diminished mGq and mGo recruitment but increased mGi and mGs interactions. In brown adipocytes, WT and R67C responded similarly to TUG891 and αLA, whereas R254H exhibited enhanced mGq, mGi, and β-arrestin 2 recruitment. Lipolysis-derived intracellular FFAs produced comparable patterns, with R254H consistently showing elevated mGi and β-arrestin 2 coupling. Imaging indicated that both variants and the WT receptor were localised intracellularly and associated with lipid droplets.

Two FFAR4 variants exhibit distinct signalling profiles and may mediate an increased risk of obesity and impaired insulin sensitivity through different mechanisms. Greater recruitment of local inhibitory coupling partners, such as mGi and β-arrestin 2, by the R254H variant may attenuate stimulated lipolysis and potentially contribute to lipid dysregulation in obesity.

P16

Intracrine FFA4 signaling controls lipolysis at lipid droplets

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Metabolites not only act as building blocks for biosynthetic pathways but also as ligands for G protein-coupled receptors (GPCRs)[1]. With the emerging paradigm of GPCR signalling at intracellular sites[2], an intriguing possibility is that metabolite-sensing GPCRs may signal from intracellular membranes to locally regulate metabolite functions. We tested whether the free fatty acid receptor 4 (FFA4), a GPCR activated by medium- and long-chain fatty acids, inhibits lipolysis via intracellular signalling, applying real-time BRET, live-cell imaging, HA-tagged FFA4 knock-in mice, and functional assays to map FFA4 signalling in adipocytes.

Herein, we found that FFA4 localized to intracellular membranes, specifically endoplasmic reticulum subdomains surrounding lipid droplets, in both adipocytes and mouse adipose tissue[3]. During lipolysis, released fatty acids activated intracellular FFA4, inducing $G\alpha_{i/o}$ signalling, and preferential reduction of cAMP levels near lipid droplets. Local inhibition with mini-$G\alpha_{i/o}$ proteins revealed that FFA4 signalling at lipid droplet–associated membranes is required for FFA4 suppression on lipolysis[3].

Our study identifies a previously unrecognized intracrine signalling modality for a prototypical metabolite-sensing GPCR. It further reveals a specialized intracellular signalling hub closely associated with lipid droplets, providing a mechanism for rapid and efficient regulation of lipolysis in adipocytes[3].

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P17

Activation of Gs-coupled GPCR signaling in mouse K-cells improves obesity and diabetes-related metabolic deficits

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Background: Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are the two major incretin hormones that are secreted from specialized enteroendocrine cells (L and K cells, respectively) following a meal. While the beneficial metabolic activities of GLP-1 have been studied in detail, much less is known about GIP-dependent physiological processes. However, strategies aimed at stimulating the secretion of endogenous GIP hold the potential of potentiating insulin secretion from pancreatic beta cells to correct hyperglycemia in obesity and type-2 diabetes. Like other cell types, K-cells express dozens of G protein-coupled receptors (GPCRs) that are coupled to different functional classes of heterotrimeric G proteins. The role of Gs-coupled GPCRs expressed by K-cells in endogenous GIP secretion, and the associated effects on systemic glucose homeostasis remain unexplored.

Methods: To address this question, we generated and analyzed two novel mouse models: First, we used a chemogenetic strategy involving the use of a Gs-coupled designer GPCR (Gs DREADD) to generate a mouse strain that allowed us to selectively stimulate K-cell G_s signaling. Second, we generated a complementary mouse strain harboring an inactivating mutation of *Gnas*, the gene encoding the a-subunit of Gs, selectively in K-cells.

Results: We found that acute and chronic receptor-mediated Gs activation in K-cells led to pronounced improvements in glucose tolerance and overall glucose homeostasis in lean and diet-induced obese mice. More strikingly, chronic Gs activation in K-cells significantly hampered streptozotocin-induced diabetes in mice. These beneficial metabolic effects were due to enhanced GIP secretion, since blockade of GIP receptor signaling by a monoclonal GIP-receptor antibody reversed the improvements in glucose homeostasis caused by K-cell Gs signaling. Mice with K-cell-specific *Gnas* knockout showed a strong reduction in circulating plasma GIP levels and hyperglycemia under certain experimental conditions.

Conclusions: Selective agonists for Gs-coupled GPCRs expressed by K-cells hold therapeutic potential as novel anti-diabetic drugs.

P18

The RFamide receptor agonist, NN501, requires both PRLHR and NPFFR2 for full efficacy on food intake and fatty acid oxidation

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Both mice and humans with mutations in the G protein-coupled receptor, GPR10 (gene name *Prlhr*), have early-onset obesity. The only known ligand for GPR10 is prolactin-releasing peptide (PrRP) which is a member of a larger family of RF-amide peptides having a conserved role in metabolic regulation across phyla. When injected into the brain of rodents, PrRP causes a decrease in food intake and an increase in adaptive thermogenesis which are dependent on GPR10. The newly developed PrRP analogue, NN501, has moderate effects on both feeding and energy expenditure, but has a significant and sustained effect on fatty acid oxidation when administered systemically. Importantly, both PrRP and NN501 also have affinity for another receptor of the RFamide family, neuropeptide FF receptor 2 (NPFFR2). We have previously described the phenotype of both mice and humans with mutations in PRLHR/GPR10 (humanised mice develop late onset obesity due primarily to reduced energy expenditure). In null *Prlhr*$^{-/-}$ mice the effects of NN501 on body weight, food intake and fat utilisation were attenuated, demonstrating dependence on this receptor. However, there was not a total reversal of effect. We describe a new *Npffr2*$^{-/-}$ knock-out model, which itself does not have a strong metabolic phenotype when maintained on either chow or high-energy diet. We find that weight loss in response to NN501 is significantly reduced in *Npffr2*$^{-/-}$ mice when compared with their wild-type *Npffr2*$^{+/+}$ littermates. Thus, both GPR10 and NPFFR2 are required for the full effects of NN501, involving actions on both the hypophagia and increased lipid utilisation.

P19

Systemic RFamide receptor agonist, NN501, accesses the brain and engages brainstem and forebrain circuitry

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Prolactin-releasing peptide (PrRP) gene (*Prlh*) expression in the brainstem nucleus of the tractus solitarius (NTS) and dorsomedial hypothalamic nucleus (DMH) is down regulated in stages of negative energy balance, while the genetic deletion of *Prlh* in mice also results in an obese phenotype. Together, these findings suggest an important role for PrRP in the homeostatic control of body weight. Systemic administration of the PrRP analogue, NN501, can cause significant body-weight loss in mice without causing any obvious adverse effects. Here, mice were injected intravenously with fluorescent VivoTag750-labelled NN501 and compared with vehicle-injected animals to demonstrate that NN501 can access the brain via circumventricular organs (CVO) that lack a blood-brain barrier. Potential receptors for NN501, GPR10 and NPFFR2, are expressed in regions within or adjacent to these CVO. NN501 increased FOS significantly in the area postrema, nucleus of the tractus solitarius and parabrachial nucleus of the brainstem, as well as the parasubthalamic nucleus (PSTN), the paraventricular thalamic nucleus, central amygdala and supraoptic nucleus of the forebrain, but not in the other brain regions investigated: including the arcuate and paraventricular nuclei of the hypothalamus. The PSTN is a region recently described has having an important role in regulating metabolism and, in particular mediating the effects of several anorectic stimuli. We demonstrate that both NN501 and the glucagon-like peptide-1 receptor agonist, exendin-4, activate a population of neurons in the PSTN that contain tachykinin 1. Interestingly, disabling $Tac1^{PSTN}$ neurons blocked the weight-reducing effects of sub-chronic NN501, but not of semaglutide. These data suggest that the two classes of drug, administered systemically, utilise distinct central mechanisms of action.