European Incretin Study Group Meeting
Lausanne, Sept 29th – Oct 1st, 2022

Venue:

University of Lausanne (UNIL),
Genopode Building,
Auditorium C
Quartier UNIL-Sorge,
CH-1015 Lausanne

Metro station (M1): UNIL-Sorge
https://planete.unil.ch/plan/?local=GEN-2012

Organisation:

Chairman:

Prof. Bernard Thorens, PhD
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Steering committee:

Bernard Thorens, Chairman, Lausanne, CH
Fiona Gribble, Vice-Chairwoman, Cambridge, UK
Remy Burcelin, Accountant, Toulouse, FR
Jens J. Holst, Member, Copenhagen, DK
Michael Nauck, Member, Bochum, DE
Francesco Giorgino, Member, Bari, IT
Mariana Monteiro, Member, Porto, P
Filip Knop, Member, London, UK
Stefan Trapp, Member, London, UK
Frank Reimann, Member, Cambridge, UK

Supporters:

Program:

Day 1 – Thursday 29th Sept. 2022

16:00 - 18:30 Registration and poster (A0, vertical) set-up

18:30 - 19:00 Welcome addresses

Bernard Thorens, Lausanne

Remy Burcelin, Toulouse

19:00 - 21:00 Poster viewing and cocktail dinner

Day 2 – Friday 30th Sept. 2022

Session 1: Incretin producing tissues

Chairperson: Filip Knop

08:30 -	08:45	**OP1**	**Single cell transcriptomic analysis of human and mouse enteroendocrine cells along the gut** ***Christopher Smith***, *Elisabeth O'Flaherty Rottenberger, Rula Bany Bakar, Lawrence Billing, Emily Miedzybrodzka, Deborah Goldspink, Pierre Larraufie, Van Lu, Alice Adriaenssens, Fiona Gribble, Frank Reimann, Cambridge*
08:45 -	09:00	**OP2**	**Mechanisms underlying nutrient sensing in human glucose-dependent insulinotropic peptide secreting cells** ***Nunzio Guccio***, *Emily Miedzybrodzka, Christopher Smith, Rula Bany Bakar, Frank Reimann, Fiona Gribble, Cambridge*
09:00 -	09:15	**OP3**	**Nutrient-induced electrical excitability of human duodenal K cells** ***Constanza Alcaino***, *Nunzio Guccio, Frank Reimann, Fiona Gribble, Cambridge*
09:15 -	09:30	**OP4**	**Enteroendocrine expression and morphology of biliopancreatic and alimentary limbs post RYGB surgery in high fat fed rats** *Peter Flatt, Ananyaa Sridhar,* ***Dawood Khan****, Jessie Elliott, Violetta Naughton, Charlotte Moffett, Coleraine*
09:30 -	09:45	**OP5**	**Fructose malabsorption modifies gut peptide expression profile and food intake behavior** ***Delphine Polvé***, *Véronique Douard, Sandy Ribes, Jouy-en-Josas*
09:45 -	10:00	**OP6**	**Low levels of "aberrant" processing of proglucagon and proGIP in pancreatic-cells and duodenal L- and K-cells – an observational study using human pancreatic and duodenal tissue** **Michael Nauck**, Sandra Ueberberg, Jakob Wefers, Nicolai Wewer-Albrechtsen, Andrea Tannapfel, Waldemar Uhl, Juris Meier, Jens Holst, Bochum

10:00 - 10:30 **Coffee break**

Session 2: Insights in GLP-1 and GIP signaling

Chairperson: Fiona Gribble

10:30 - 10:45 **OP7** **Visualizing and interrogating endogenous glucagon-like peptide-1 receptor expression and signaling**

Julia Ast, Nicholas Fine, Daniela Nasteska, Daniel Nieves, Zsombor Koszegi, Yann Lanoiselée, Federica Cuozzo, Katrina Viloria, Andrea Bacon, Nguyet Luu, Philip Newsome, Davide Calebiro, Dylan Owen, Johannes Broichhagen, ***David Hodson****, Oxford*

10:45 - 11:00 **OP8** **Regulation of β-cell GLP-1R responses by the lipid microenvironment**

Affiong Ika Oqua*, Lisa Casteller, Jorge Bernadino de la Serna, Wanling Song, Mark Sansom, Sarah Rouse, Ben Jones, Alejandra Tomas, London*

11:00 - 11:15 **OP9** **Characterisation of human gain-of-function GLP-1R variant Ala316Thr in pancreatic beta cells**

Liliane El Eid, Roxana Rujan, Giuseppe Deganutti, Ben Jones, ***Alejandra Tomas****, London*

11:15 - 11:30 **OP10** **Effects of ligand, genotype and tissue diversity on GIPR responses**

Yusman Manchanda*, Iyobel Kibreab, Matthew Coghlan, Kyle Sloop, Ben Jones, Alejandra Tomas, London*

11:30 - 11:45 **OP11** **Characterisation of a long-acting G protein-biased GLP-1R agonist**

Iona Davies*, Eleanor Peace, Charlotte Hinds, Shiqian Chen, Zayd Lakhi, James Minnion, Ben Jones, Tricia Tan, Steve Bloom, London*

Session 3: Incretins in glucose homeostasis

Chairperson: Mariana Monteiro

11:45 - 12:00 **OP12** **Recurring periods of NPY1R induced beta-cell rest together with GLP-1R stimulation improves diabetes control in high fat fed mice**

Nigel Irwin*, Neil Tanday, Ryan Lafferty, Peter Flatt, Coleraine*

12:00 - 12:15 **OP13** **Nephroprotective effects of semaglutide in a mouse model of hypertension-accelerated diabetic kidney disease**

Trine Porsgaard*, Louise Dalbøge, Michael Christensen, Thomas Secher, Nicole Endlich, Vedran Drenic, Martin Madsen, Henrik Hansen, Ida Rune, Lisbeth Fink, Mette Østergaard, Hørsholm*

12:15 - 13:30 Lunch break and poster viewing

13:30 - 13:45 **OP14** **Meta-Analysis of Head-to-Head Clinical trials Comparing Incretin-Based Glucose-Lowering Medications and Basal Insulin – An Update Including Recently Developed GLP-1 Receptor Agonists and the GIP/GLP-1 Receptor Co-Agonist Tirzepatide**

Daniel Quast*, Mirna Abd El Aziz, Michael Nauck, Bochum*

13:45 - 14:00 **OP15** **Oral smart control drug delivery systems for fine-tuning gut hormones**

Yining Xu*, J. Koehler, C. Michalowski, M. Hul, T. Darwish, I. Domingues, W. Zhang, V. Marotti, Emily Miedzybrodzka, P. Cani, D. Drucker, Frank Reimann, Fiona Gribble, A. Beloqui, Cambridge*

14:00 - 14:15 **OP16** **Reactive hypoglycaemia during the OGTT after gestational diabetes mellitus: metabolic implications and evolution**

Dan Quansah*, Sara De Giorgi, Le Dizes Olivier, Chiara Camponovo, Katrien Benhalima, Emmanuel Cosson, Jardena Puder, Lausanne*

Session 4: Pharmacological characterization of incretin-based drugs

Chairperson: Francesco Giorgino

14:15 - 14:30 **OP17** **GL0034, A novel long-acting glucagon-like peptide 1 receptor agonist exhibits significant efficacy in aged db/db mouse model of non-alcoholic fatty liver disease (NAFLD)**

Rajamannar Thennati, *Vinod Burade, Adolfo Garcia-Ocana, Richard E. Pratley, Guy A. Rutter, Tina Vilsboll, Bernard Thorens, Vadodara*

14:30 - 14:45 **OP18** **Dual GIP/GLP-2 analogues are capable of inducing bone strength in an animal model of disuse osteoporosis**

Aleksandra Mieczkowska, ***Guillaume Mabilleau****, Angers*

14:45 - 15:00 **OP19** **Phase I trial of a GLP-1/glucagon dual agonist for obesity**

Tricia Tan*, James Minnion, Reshma Malviya, Stephen Bloom, London*

15:00 - 15:30 Coffee break

15:30 - 15:45 **OP20** **Efficacy, Safety and Pharmacokinetics of Cotadutide, a Dual GLP1-glucagon Receptor Agonist, in Patients with Chronic Kidney Disease (CKD) and T2DM**

Victoria Parker*, Thuong Hoang, Heike Schlichthaar, Fraser Gibb, Barbara Wenzel, Maximillian Posch, Ludger Rose, Yi-Ting Chang, Lars Hansen, Phil Ambery, Lutz Jermutus, Hiddo Heerspink, Rory McCrimmon, Marcella Petrone, Cambridge*

15:45 - 16:00 **OP21** **GIPR agonism is a key contributor to tirzepatide modulation of metabolic adaptation**

William Roell*, Guillermo Sanchez-Delgado, Robbie Beyl, Julie Moyers, Kieren Mather, Axel Haupt, Zvonko Milicevic, Eric Ravussin, Tamer Coskun, Indianapolis*

16:00 - 16:15 **OP22** **The novel GIP, GLP-1, and glucagon triple receptor agonist LY3437943: From discovery to clinical proof-of-concept**

Tamer Coskun*, William Roell, Julie Moyers, Kieren Mather, Axel Haupt, Zvonko Milicevic, Indianapolis*

16:15 - 16:30 **OP23** **Discovery and selection of BI 456906 as a dual GCGR/GLP-1R agonist applying in vivo target engagement biomarkers**

Leo Thomas, Tina Zimmermann, Eric Simon, Wolfgang Rist, Ingo Uphues, Dieter Hamprecht, Heike Neubauer, ***Robert Augustin****, Biberach an der Riss*

16:30 - 16:45 **OP24** **The role of GIP in the regulation of food intake and body weight in mice**

Jo Lewis*, Tamana Darwish, Fiona Gribble, Frank Reinmann, Cambridge*

16:45 - 17:00 **OP25** **Glucose-dependent insulinotropic polypeptide receptor-expressing neurons employ distinct mechanisms to control feeding behaviour depending on their neuroanatomical location in mice**

Alice Adriaenssens*, Johannes Broichhagen, Julia Ast, A. De Bray, A. Hasib, Ben Jones, Alejandra Tomas, Orla Woodward, Jo Lewis, C. Jonathan, H. David, R. Samms, Tamer Coskun, Fiona Gribble, Frank Reimann, Cambridge*

17:00 - 17:15 **OP26** **The olfactory bulb GLP-1 system mediates the regulation of pre- and post-prandial energy metabolism in obesity**

Mireia Montaner Massana*, Jessica Denom, Wanqing Jiang, Marie Holt, Dan Brierley, Ewout Foppen, Claude Rouch, Christophe Magnan, Stéphanie Migrenne-Li, Stefan Trapp, Hirac Gurden, Paris*

17:15 - 17:30 **OP27** **Preproglucagon (PPG) neurons modulate physiological food intake and are not required for anorexigenic effects of naltrexone, rimonabant or nicotine**

Wanqing Jiang*, A. Adriaenssens, D. Nuzzaci, Fiona Gribble, Frank Reimann, Stephan Trapp, London*

18 :00 Bus departure for meeting dinner

Day 3 – Saturday 1st Oct. 2022

Session 5: Incretins and gut peptides in food intake and weight regulation

Chairperson: Stefan Trapp

08:30 - 08:45 **OP28** **5-HT2c receptor agonist lorcaserin requires preproglucagon neurons to reduce food intake in mice and acts additively to liraglutide-induced hypophagia**

Daniel Brierley, Alasdair Leeson-Payne, Stefan Wagner, Wanqing Jiang, Frank Reimann, Fiona Gribble, Lora Heisler, ***Stefan Trapp****, London*

08:45 - 09:00 **OP29** **Differential effects of biased GLP-1 receptor agonists on appetite versus blood glucose**

Maria Lucey, Maria Shchepinova, Shiqian Chen, Monica Imbernon, Carissa Wong, Tanyel Ashik, James Minnion, Ed Tate, Vincent Prevot, Guy Rutter, Tricia Tan, Steve Bloom, Alejandra Tomas, ***Ben Jones****, London*

09:00 - 09:15 **OP30** **Characterisation of G-protein coupled relaxin/insulin-like family peptide receptor 4 (Rxfp4)-expressing cells in the mouse hypothalamus**

Orla Woodward*, Jo Lewis, Alice Adriaenssens, Christopher Smith, Danae Nuzzaci, John Tadross, Sarah Kinston, Ernesto Ciabatti, Berthold Gottgens, Tripodi, David Hornigold, David Baker, Fiona Gribble, Frank Reimann, Cambridge*

09:15 - 09:30 **OP31** **Characterisation of VMH-RXFP4 neurons and their role in overfeeding**

Danae Nuzzaci*, Jo Lewis, Orla Woodward, Fiona Gribble, Frank Reimann, Cambridge*

09:30 - 09:45 **OP32** **Liraglutide decreases postprandial fibroblast growth factor 19 and glucagon-like peptide 2, and increases postprandial cholecystokinin in individuals with obesity**

Henriette Holst Nerild*, Andreas Brønden, Christina Nexøe-Larsen, Pernille H. Hellmann, Mille Bækdal, Ida Gether, Bolette Hartmann, Lotte Knudsen, Lisbeth Jacobsen, Jens Rehfeld, Jens J. Holst, David Sonne, Tiana Vilsbøll, Filip Knop, Hellenrup*

9:45 - 10:00 **OP33** **Exercise as weight loss maintenance strategy increases postprandial secretion of GLP-1**

Joachim Holt*, Simon Jensen, Charlotte Janus, Christian Juhl, Julie Lundgren, Anee Andresen, Bente Stallknecht, Jens J. Holst, Sten Madsbad, Signe Torekov, Copenhagen*

10:00 - 10:30 Coffee break

Session 6: Incretin effects beyond glucose homeostasis and obesity

Chairperson: Frank Reimann

10:30 - 10:45 **OP34** **GIP infusion acutely decreases blood pressure and increases heart rate in men with type 2 diabetes, concomitantly with increased pro-atrial natriuretic peptide levels**

Nikolaj Sørum*, Laerke Gasbjerg, Maria Andersen, Sebastian Heimbürger, Liva Krogh, Jens Holst, Tina Visboll, Asger Lund, Natasha Bergmann, Filip Knop, Hellerup*

10:45 - 11:00 **OP35** **Endogenous glucose-dependent insulinotropic polypeptide (GIP) facilitates postprandial intestinal lipid uptake**

Lærke Gasbjerg*, Mads Helsted Signe Stensen, Liva Krogh, Alexander Sparre-Ulrich, Bolette Hartmann, Tina Vilsboll, Mikkel Christensen, Jens Holst, Christina Christoffersen, Filip Knop, Mette Rosenkilde, Copenhagen*

11:00 - 11:15 **OP36** **Incretins are neuroprotective in Alzheimer's and Parkinson's disease**

Christian Hölscher*, Zhengzhou*

11:15 - 11:30 **OP37** **Tmem117 in AVP neurons is a novel regulator of counterregulatory response to hypoglycemia**

Sevasti Gaspari, *Gwenaël Labouèbe, Alexandre Picard, Bernard Thorens, Lausanne*

11:30 - 11:45 **OP38** **Agpat5 in AgRP neurons is required for hypoglycemia-induced glucagon secretion**

Anastasiya Strembitska, *Gwenaël Labouèbe, Alexandre Picard, Xavier P. Berney, Bernard Thorens, Lausanne*

11:45 - 12:00 **OP39** **Molecular investigations of defective glucagon secretion in a murine model of type-2 diabetes: a multi-omics study**

Judit Castillo Armengol, *Ana Rodriguez Sanchez-Archidona, Christian Fledelius, Flavia Marzetta, Bernard Thorens, Lausanne*

12:00 End of meeting

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ABSTRACTS

1. Oral presentations:

OP1: Christopher Smith

Single cell transcriptomic analysis of human and mouse enteroendocrine cells along the gut

Smith C.[1], O'Flaherty Rottenberger E.[1], Bany Bakar R.[1], Billing L.[1], Miedzybrodzka E.[1], Goldspink D.[1], Larraufie P.[1], Lu V.[1], Adriaenssens A.[1], Gribble F.[1], Reimann F.[1],

University of Cambridge[1]

Enteroendocrine (EE) cells in the intestinal epithelium are of particular interest in the quest for effective anti-diabetic and weight loss drugs, due to their role in secretion of hormones involved in energy homeostasis in response to specific ingested nutrients. The benefits of drugs mimicking the synergistic effect of multiple hormones, targeting hormone receptors including those expressed in EE cells, are a popular focus of current study. However, understanding these benefits requires a fuller understanding of which cell types express which hormone receptors, and in which region of the gut these hormone receptors are expected. We previously published 10X single cell RNA sequencing (scRNAseq) data in mouse large intestinal cells that had expressed a common EE cell transcription factor NeuroD1, allowing us to evaluate sub-groups of both Gcg-expressing L-cells and Chga-expressing enterochromaffin cells (ECs). We present here scRNAseq datasets representing multiple regions along the human and mouse gut, including EE cells derived from human CHGA-expressing duodenum and ileum organoids, and EE cells from the stomach, upper and lower small intestine, and caecum of NeuroD1-Cre x EYFP mice. In line with previous literature, individual EE cells express multiple hormones, however cluster into distinguishable EE cell types based on their gene signatures, where differential expression analysis highlighted particular genes for secretory peptides, G-protein coupled receptors (GPCR), and transcription factors. Seurat-implemented integration of the human and mouse datasets, and subsequent plotting onto common UMAP coordinates, demonstrated that common mouse and human EE cell types broadly share gene markers, including cell type-specific genes encoding peptides involved in GPCR related-pathways, supporting the usefulness of *Mus musculus* as a model for testing effectiveness of candidate drugs in humans. This combined dataset provides an extensive resource for further detailed analysis of all EE cell types along the human and mouse gut.

OP2: Nunzio Guccio

Mechanisms underlying nutrient sensing in human glucose-dependent insulinotropic peptide secreting cells

Guccio N.[1], Miedzybrodzka E.[1], Smith C.[1], Bany Bakar R.[1], Reimann F.[1], Gribble F.[1],

Institute of Metabolic Science, University of Cambridge[1]

Background and aims: Glucose-dependent insulinotropic polypeptide (GIP) is a 42-amino-acid hormone secreted by K cells found in the proximal small intestine. While the sister incretin GLP-1 has been extensively studied, GIP physiology remains more elusive. Recent observations that dual GIPR/GLP-1R agonism promotes greater weight loss- than GLP-1R agonism alone, however, sparked new interest in GIP physiology. The aim of this study was to characterise mechanisms underlying GIP secretion from human K-cells in duodenum-derived organoids.

Materials and methods: A yellow-fluorescent protein (Venus), was inserted into the GIP locus via CRISPR-Cas9-mediated homology directed repair (HDR) in human duodenal organoids. Venus-expressing K cells were isolated using fluorescence-activated cell sorting (FACS) in order to perform bulk RNA-sequencing. The GIP-Venus reporter line was also used for live single-cell imaging of K cells, after loading with the Ca^{2+}-sensitive fluorescent indicator fura2, and for whole-organoid GIP secretion, which was measured in supernatants by ELISA.
Results: An array of G-protein coupled receptors (GPCRs) potentially involved in nutrient-sensing mechanisms were differentially expressed in K-cells (n=4), compared to the fluorescence-negative population (n=3). These include amino acid-sensing receptors CASR ($p<0.05$) and GPR142 ($p<0.05$), long-chain fatty acids (LCFAs) receptor FFAR1 ($p<0.05$), monoacylglycerol-sensing receptor GPR119 ($p<0.05$) and bile acid-sensing receptor GPBAR1 ($p<0.05$). K-cell intracellular calcium levels were increased upon stimulation with 20 mM L-phenylalanine ($p<0.001$, n=13), 20 mM L-tryptophan ($p<0.0001$; n=15) and 10 µM AM1638, a FFAR1 agonist ($p<0.0001$; n=22). These stimuli also induced a 2.5-fold increase in GIP secretion (p<0.05, n=8-12).Glucose (10 mM) also stimulated intracellular calcium levels (p<0.01; n=8), as well as eliciting 1.8-fold secretion of GIP (p<0.001; n=12) compared to baseline.
Conclusions: This newly generated human K-cell in vitro-model allows transcriptomic characterisation and identification of potential nutrient sensing pathways involved in GIP secretion and should aid identification of potential targets to modulate GIP secretion in diabetes and/or obesity.

OP3: Constanza Alcaino

Nutrient-induced electrical excitability of human duodenal K cells

Alcaino C.[1], Guccio N.[1], Reimann F.[1], Gribble F.[1],

University of Cambridge[1]

Introduction: K cells are a type of enteroendocrine cell (EEC), that release Glucose-dependent insulinotropic polypeptide (GIP) in response to nutrient stimulation. Activation of luminal receptors and transporters induces changes in membrane depolarisation that are known to activate voltage-gated channels, increasing the intracellular Ca^{2+} levels and causing the release of GIP into the blood stream. Electrical excitability has been linked to hormone release in other types of EECs, however no studies have directly demonstrated the biophysical profile of murine or human K cells. **Aim:** In this work we are using human intestinal organoids to determine whether nutrient stimulation can trigger changes in human K cell excitability. **Methods:** We developed intestinal organoids from human duodenum biopsies and used CRSPR-Cas9 to label K cells with the fluorescent protein venus (GIP-venus). Organoids were grown in 3D and plated as monolayers for electrophysiological recordings. We performed perforated patch clamp to assess the electrical excitability of GIP-venus cells, in the absence and presence of Glucose (0 to 10 mM), as well as by activation of cAMP using Forskolin and IBMX. **Results:** Current injection elicits fast action potentials in GIP-venus cells. Application of 10 mM Glucose induces membrane depolarisation followed by spontaneous action potentials. Application of Forskolin/IBMX significantly increases the frequency of the spontaneous action potentials. **Conclusions:** Our findings suggest that human K cell electrical excitability can be modulated by glucose, likely contributing to GIP release. Our future directions include testing other nutrients and characterising the ion channels and receptors potentially involved in this process. A deeper understanding of the molecular mechanisms of K cell excitability is fundamental for the development of pharmacological strategies to modulate GIP release from human intestinal K cells.

OP4: Peter Flatt

Enteroendocrine expression and morphology of biliopancreatic and alimentary limbs post RYGB surgery in high fat fed rats

Flatt P.[2], Sridhar A.[2], Khan D.[2], Elliott J.[1], Naughton V.[2], Moffett R.[2],

Department of Surgery, St James Hospital, Dublin, Ireland[1], School of Biomedical Sciences, Ulster University, Coleraine, UK[2]

Aims: Roux-en-Y gastric bypass (RYGB) induced alterations in gut hormone expression in the biliopancreatic limb (BPL) and alimentary limb (AL) have not been well characterised. This study assessed enteroendocrine expression and gut morphology of the BPL and AL to better understand effects of RYGB. **Methods:** 8-week-old female Wistar rats were fed a high-fat diet (HFD) or normal diet (ND) for 18-weeks prior to RYGB or sham surgeries. Three weeks post-surgery, excised tissues were taken for immunohistochemical analysis. **Results:** After surgery, BPL morphology of HFD-RYGB showed 44 % increase ($p<0.001$) in villous length, with ND-RYGB showing no difference compared to respective sham controls. AL villous length did not change after RYGB in either ND or HFD rats whereas villous length of HFD-sham group was increased by 21% ($p<0.001$). There were no changes in BPL crypt depth after RYGB. Contrastingly, AL crypt depth decreased by 15% ($p<0.05$) in the HFD-RYGB group, with no change after ND-RYGB. A 33% increase ($p<0.001$) in the AL crypt depth was observed in HFD-sham rats. The number of GLP-1 positive cells (per mm^2) of BPL villi was decreased by 38% in the HFD-RYGB group ($p<0.05$), whereas there was no change in the AL. The number of GLP-2 positive cells in the AL increased by 84% in the ND-RYGB ($p<0.05$). In contrast, the density of PYY or GIP positive cells in BPL did not change after RYGB in HFD or ND rats. However, in the BPL crypt, PYY cell density decreased by 49% ($p<0.01$) in the HFD-sham rats. PYY cell density decreased by 68% and 17% ($p<0.05$ to $p<0.01$) in the AL villous and its crypt respectively after RYGB in ND rats. **Conclusions:** BPL and AL maintain similar morphology despite being bypassed in RYGB surgery. Alterations in GLP-1, GLP-2 and PYY drive the phenotype towards improved metabolic state.

OP5: Delphine Polvé

Fructose malabsorption modifies gut peptide expression profile and food intake behavior

POLVÉ D.[1], DOUARD V.[1], RIBES S.[1],

Institut Micalis - INRAE AgroParisTech[1]

Fructose consumption has increased drastically over recent decades. However, the current average fructose intake is above the absorption capacity of intestinal transporter GLUT5. Therefore, a substantial fraction of fructose reaches the distal regions of the gut where it may affect the functions of various intestinal epithelial cells. We investigated the impact of fructose malabsorption on the enteroendocrine cells functions of the lower intestine and its consequences on food intake behavior.
GLUT5 knockout ($GLUT5^{-/-}$), a fructose malabsorption model, and wild type mice were fed 0% fructose diet (0F) from birth to 7 weeks of age and then subjected to either 0F or 10% fructose diet (10F) for 1 to 10 days.
Fructose malabsorption induced an increase in *Gcg* and *neurotensin* gene expression, 3-fold and 4-fold respectively, in the caecum of the $GLUT5^{-/-}$ as early as 1 day after the introduction of 10F diet. After 10d of 10F diet, $GLUT5^{-/-}$ mice also displayed a significant increase in cecal *cholecystokinin* (*Cck*) gene expression and in the circulating levels of GLP-1. These changes were associated with a decrease in food intake during the first 2 days following 10F diet introduction, which could be partially reversed by injecting the $GLUT5^{-/-}$ mice with extendin (9-39) (an antagosnist of GLP1-R) prior the introduction of the 10F diet. The reduction of food intake in $GLUT5^{-/-}$ mice was only transient and fully recovered after 2 days of 10F diet despite sustained increase in GLP-1 plasma levels and in expression of *Cck*, *Gcg* and *neurotensin*. This recovery was associated with an increase *Npy* and *Agrp* gene expression in the hypothalamus as early as 1d after introduction of the 10F diet.

Chronic fructose malabsorption was associated with a resistance to anorexigenic gut hormones at the central level. Given the prevalence of fructose in human diet our findings point to possible mechanisms responsible for negative consequences of fructose-rich diets on human food intake regulation.

OP6: Michael Nauck

Low levels of "aberrant" processing of proglucagon and proGIP in pancreatic □-cells and duodenal L- and K-cells – an observational study using human pancreatic and duodenal tissue

Nauck M.[6], Ueberberg S.[6], Wefers J.[6], Wewer-Albrechtsen N.[2], Tannapfel A.[3], Uhl W.[4], Meier J.[1], Holst J.[5],

Augusta Kliniken, Bochum, Germany[1], Department of Clinical Biochemistry, Rigshospitalet, University of Copenhagen, Denmark[2], Department of Pathology, Ruhr-University Bochum, Germany[3], Department of Visceral Surgery, St. Josef Hospital, Ruhr-University Bochum, Germany[4], Novo Nordisk Foundation Center for Basic Metabolic Research, Department of Biomedical Sciences, University of Copenhagen, Denmark[5], St. Josef Hospital, Ruhr-University Bochum[6]

"Aberrantly" processed proglucagon and proGIP fragments (glucagon in intestinal L cells and GLP-1/GIP in pancreatic α-cells) have been claimed to be effective metabolic regulators. We quantified GLP-1, GIP and glucagon (frozen tissue extracts) and the expression of prohormone convertases (PC) 1/3 and 2 and GLP-1 or glucagon (immunofluorescence, formalin-fixed tissue) in human pancreatic and duodenal tissue from 8 subjects with and 12 without type 2 diabetes (pancreatic surgery). Target cell (α-cell, K- and L-cell) concentrations were estimated. In α-cells, glucagon concentrations were 6fold higher compared to $GLP\text{-}1_{total}$ ($p = 0.018$). In L-cells, there was 69fold more $GLP\text{-}1_{total}$ than glucagon ($p < 0.0001$). Pancreatic $GLP\text{-}1_{intact}$ and GIP_{total} concentrations were below the detection limit in 50.0 and 73.3 % of the specimens, respectively. >99 % of α-cells expressed PC2. Approximately 50 % of L cells expressed PC1/3, with aberrant expression in £5 % of α- and L-cells, however, unrelated to tissue concentrations of aberrantly processed peptides. Findings were similar for subjects with and without diabetes. In conclusion, aberrant expression of prohormone convertases and of proglucagon- and pro-GIP-derived peptides occurs in both pancreas and gut, however, at levels too low to support a paracrine role in modulating, e.g., ßcell secretion.

OP7: David Hodson

Visualizing and interrogating endogenous glucagon-like peptide-1 receptor expression and signaling

Ast J.[2], Fine N.[2], Nasteska D.[2], Nieves D.[2], Koszegi Z.[2], Lanoiselée Y.[2], Cuozzo F.[2], Viloria K.[2], Bacon A.[2], Luu N.[2], Newsome P.[2], Calebiro D.[2], Owen D.[2], Broichhagen J.[1], Hodson D.[3],

FMP Berlin[1], University of Birmingham[2], University of Oxford[3]

G protein-coupled receptors are fundamental to cell signaling and represent the largest druggable class of proteins. Despite this, nearly all of our information on GPCR signaling is obtained from pharmacological studies in heterologous cell lines stably-expressing the receptor of choice. Studying GPCRs in their native tissue environment has so far not been possible due to lack of reliable antibodies and models (1), especially for super-resolution imaging where specific dyes/buffers are needed (2). We are thus missing key information regarding GPCR signaling in the tissue context, where cell-cell interactions, cell heterogeneity and local signaling loops are all likely to influence and shape responses to ligand (3-6).
To circumvent this, we have generated mice harboring a SNAP-tag self-labeling enzyme knocked into the *N*-terminus of the glucagon-like peptide-1 receptor (GLP1R), a prototypical class B GPCR involved in obesity, diabetes and nonalcoholic steatohepatitis (NASH). Alongside these mice, we also designed and rationally tested a range of fluorescent SNAP labels for their performance in complex tissue and various imaging modalities.

Combining these approaches, we are able to show that; 1) SNAP labels commonly used for super-resolution imaging in cell lines perform poorly (or not at all) in complex tissue (i.e. Alexa Fluor 647); 2) restricting SNAP labels to the cell surface allows exclusive labelling of GPCRs for super-resolution imaging (dSTORM/SMLM); 3) subpopulations of pancreatic beta cells are recruited into GLP1R signalling, with agonists and dual agonists differentially affecting this process; 4) GLP1R possess higher organization at the cell membrane, arranging into nanodomains in a ligand-dependent manner; and 5) in the liver, GLP1R protein is only detected in CD4+ and CD8+ immune cells.
Using a multidisciplinary approach, we are thus able to show for the first time the distribution, organization, dynamics and signaling of an endogenous class B GPCR, revealing new tissue-level mechanisms through which therapeutically-relevant drugs might influence metabolic disease states.

OP8: Affiong Ika Oqua

Regulation of β-cell GLP-1R responses by the lipid microenvironment

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The glucagon-like peptide-1 receptor (GLP-1R) is a class B GPCR associated with the regulation of blood glucose levels and appetite through potentiation of insulin secretion and control of neuronal feeding centres, and a key pharmacological target for Type 2 Diabetes treatment. Buenaventura *et al*., (PLoS Biol 2019), demonstrated that changes in cholesterol levels affect GLP-1R plasma membrane organisation, dynamics, clustering, and function. We aimed to identify relevant GLP-1R cholesterol binding sites and their effects on its function in β-cells. Two independent molecular dynamic (MD) simulations of GLP-1R-cholesterol interactions, in its active *vs* inactive state, in an artificial lipid bilayer, identified 11 and 6 potential sites, respectively, with multiple GLP-1R amino acid residues involved. Some of these were mutated to alanine and screened for effects on GLP-1R internalisation and functionality in INS-1 832/3 β-cells. GLP-1R V229A and S163A mutants caused 38.35% and 43.06% (p value=0.005 and 0.001) decrease, respectively, in GLP-1R internalisation *vs* wild-type (wt) receptor. A photo-activatable click-cholesterol approach was used to investigate GLP-1R-cholesterol binding, showing that GLP-1R V229A had a decreased basal binding (41.09% decrease *vs* wt) but a higher fold-increase after stimulation with the pharmacological agonist exendin-4 (2.8+/-1.1 *vs* wt), in agreement with MD simulations of cholesterol binding to the V229A mutant. These changes were accompanied by increased GLP-1R plasma membrane recycling and receptor signalling bias in favour of mini-Gs *vs* β-arrestin-2 recruitment, showing that the GLP-1R-cholesterol interaction at the V229-containing binding site is important for β-cell GLP-1R functionality.

OP9: Alejandra Tomas

Characterisation of human gain-of-function GLP-1R variant Ala316Thr in pancreatic beta cells

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The glucagon-like peptide-1 receptor (GLP-1R) is an important pharmacological target for type 2 diabetes (T2D) due to its insulinotropic effects on pancreatic b-cells and weight lowering actions. GLP-1R action, including signalling and trafficking, is modulated by factors such as ligand bias and single nucleotide polymorphisms (SNPs) that alter receptor structure. Here we characterize the effect of the GLP-1R missense mutation Ala316Thr (A316T), associated with reduced T2D risk, in pancreatic beta cells by performing atomic level conformational modelling, signalling, and trafficking analyses of the mutant compared to wild type receptor. Consistent with

previous studies in HEK and CHO cells, the A316T mutation led to a significant loss of cell surface receptor expression in INS-1 832/3 pancreatic beta cells (p=0.0114). Moreover, MD simulations revealed that the mutant receptor exhibits altered mobility and flexibility of the extracellular domain (ECD) and important transmembrane regions pre-and post-activation, distinct to each agonist-bound state. Results also revealed a significant decrease in mutant receptor recycling (p=0.0431), associated with increased internalisation (p=0.024) and degradation (p=0.0323) upon GLP-1 binding. We also demonstrate significantly augmented mini-G_s recruitment in response to GLP-1 (p=0.0002), exendin-4 (p=0.0019), exendin-phe1 (p=0.0386), but not for the β-arrestin biased agonist exendin-asp3 by the Thr^{316} variant *vs* wild type. Strikingly, the variant also exhibits significantly increased efficacy (p=0.0083) and potency (p=0.0281) for endosomal over plasma membrane Gαs coupling. Assessment of Gαs coupling vs β-arrestin2 recruitment propensities reveal that mutant receptors exhibit a significant Gαs bias in response to GLP-1 (p<0.0001), exendin-4 (p<0.0003), and exendin-phe1 (p<0.0001), but not with exendin-asp3 (p=0.5640). The GLP-1R A316T polymorphism is therefore a determinant of pharmacological responses to GLP-1R-targeting compounds, including biased agonists, with varying responses associated to differentially biased compounds. The mutation causes significant changes in receptor conformational dynamics, signalling, and trafficking signatures highlighting the importance of the precise molecular characterization of GLP-1R variants to predict individual responses to specific GLP-1R therapies.

OP10: Yusman Manchanda

Effects of ligand, genotype and tissue diversity on GIPR responses

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The glucose-dependent insulinotropic peptide receptor (GIPR) is one of two incretin receptors promoting postprandial insulin release in healthy individuals, with its activity impaired in type 2 diabetes (T2D). GIPR-mediated release of glucagon from alpha cells, as well as its obesogenic action on adipose tissue, have resulted in GIPR agonism being side-lined as a T2D treatment. However, a revived interest in GIPR biology has challenged this view, with glucagon now being linked to stimulation of insulin secretion from beta cells and both GIPR agonism and antagonism linked to weight loss.
Here, we have aimed to dissect GIPR signalling and trafficking in alpha *versus* beta cells in response to GIP as well as two additional DPP-4-resistant GIP analogues with opposing bias characteristics (GIP-Aib2, GIP-Gly2). We observe robust cAMP responses in the aTC1 clone9 alpha cell line despite a ~200 fold reduction in GIPR mRNA levels relative to MIN6B1 beta cells (p<0.0001). Transient GIPR overexpression dampened GIPR cAMP responses in both cell lines in a ligand-specific manner. We have also investigated the responses of naturally occurring loss- and gain-of-function GIPR genetic variants. In an initial screen in HEK293T cells, we observed wide variation in surface expression, cAMP signalling and receptor internalisation responses from 40 GIPR variants. From this screen, we selected two variants for further characterisation in alpha *versus* beta cells: E354Q, a gain-of-function variant with the highest allele frequency; E288G, a loss-of-function variant, and a double mutant with both variants. cAMP responses to GIP agonists were increased in E354Q and absent in E288G *versus* WT GIPR, however these were partially rescued in the double mutant receptor despite reduced surface expression levels. Through these studies, we aim to answer critical questions about the physiological role of GIPR in alpha *versus* beta cells and its suitability a therapeutic target in the treatment of T2D.

OP11: Iona Davies

Characterisation of a long-acting G protein-biased GLP-1R agonist

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Signal bias in favour of G protein-dependent cyclic AMP signalling over β-arrestin recruitment may enhance the therapeutic effects of GLP-1R agonism. Here we characterise the pharmacology and physiological effects of two new GLP-1R agonist peptides showing variable bias between G protein and β-arrestin recruitment, with Semaglutide as a further comparator.
The pharmacological profile of Semaglutide, in addition to 2 acylated peptides with specific amino acid substitutions close to the N-terminus, SRB106 and SRB107, were tested in HEK293 cells. Signalling and insulin release responses were tested in beta cell and islet models. Food intake and body weight effects were tested in Wistar rats. Blood glucose effects were determined in C57Bl/6 mice.
Compared to native GLP-1 and Semaglutide, both SRB106 and SRB107 showed reduced mini-Gs and β-arrestin recruitment, i.e. were partial agonists. However, SRB106 responses were particularly attenuated, with β-arrestin being more dramatically affected, defining this ligand as a G protein-biased agonist. Despite its partial agonist profile, sustained *in vitro* insulin secretion from INS-1 832/3 cells and primary mouse islets was greater with SRB106 compared to SRB107 and Semaglutide, compatible with a role for β-arrestin recruitment in mediating GLP-1R desensitisation.
SRB106 maintained anti-hyperglycaemic efficacy over at least 72 hours after a single injection at 1 nmol/kg, whereas both SRB107 and Semaglutide ceased to be effective. SRB106 and SRB107 reduced food intake and body weight to a comparable extent after either a single injection at 1.5 nmol/kg or three 1 nmol/kg injections over a 7-day period. SRB106 and SRB107 surpassed the food intake and body weight reduction provided by Semaglutide at 4 nmol/kg.
These results are compatible with earlier studies showing that biased GLP-1R agonism may have therapeutic advantages for blood glucose lowering. Further work is needed to assess the effectiveness of this approach in humans.

OP12: Nigel Irwin

Recurring periods of NPY1R induced beta-cell rest together with GLP-1R stimulation improves diabetes control in high fat fed mice

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Background: Induction of pancreatic beta-cell rest can lead to improvements of insulin secretory responsiveness. Here we examine whether sequential administration of the NPY1R-specifc, beta-cell resting peptide, (D-Arg^{35})-sea lamprey PYY(1-36) (SL-PYY), and the GLP-1 mimetic liraglutide, can impart beneficial effects in high fat fed (HFF) mice with streptozotocin (STZ)-induced insulin deficiency, namely HFF-STZ mice.
Methods: Treatment regimens continued for 28-days and effects on energy intake, body weight, circulating glucose, insulin and glucagon as well as intraperitoneal glucose tolerance (18 mmol/kg) and insulin sensitivity (5 U/kg) were examined. Terminal analyses included assessment pancreatic insulin content and islet architecture.
Results: We have previously confirmed that 0.25 nmol/kg liraglutide exerts bioactivity over 8-12 h period in mice. Our current pharmacokinetic studies reveal that 75 nmol/kg SL-PYY yields a similar plasma drug profile. Thus, SL-PYY (75 nmol/kg) and liraglutide (0.25 nmol/kg) were administered sequentially at 08:00 h and 20:00 h, respectively, to HFF-STZ mice for 28 days. Combined alternating treatment reduced ($p<0.05$-0.01) energy intake, body weight, circulating glucose and glucagon whilst elevating insulin concentrations. Similar, but

slightly less striking benefits were observed with liraglutide only (0.25 nmol/kg at 08:00 and 20:00 h) therapy. Sequential SL-PYY and liraglutide treatment also improved ($p<0.05$) peripheral insulin sensitivity, glucose tolerance and insulin secretory responses, that was not apparent with twice-daily liraglutide only treatment. Combined therapy also elevated ($p<0.05$) pancreatic insulin and decreased pancreatic glucagon beyond that of liraglutide alone. Islet morphology was similar in all treatment groups, but numbers of central glucagon-positive islet cells were reduced ($p<0.01$) by sequential therapy.
Conclusion: Scheduled periods of beta-cell rest and stimulation requires further evaluation for the treatment of type 2 diabetes.

OP13: Trine Porsgaard

Nephroprotective effects of semaglutide in a mouse model of hypertension-accelerated diabetic kidney disease

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Background: Obesity, hyperglycemia and hypertension are critical risk factors for development of diabetic kidney disease (DKD). Emerging evidence suggests that glucagon-like peptide-1 receptor (GLP-1R) agonists improve cardiovascular and renal outcomes in type 2 diabetes patients. Here, we characterized the effect of long-acting GLP-1R agonist semaglutide alone and in combination with an ACE inhibitor in a model of hypertension-accelerated, advanced DKD facilitated by adeno-associated virus-mediated renin overexpression (ReninAAV) in uninephrectomized (UNx) female *db/db* mice. **Methods:** Seven weeks after ReninAAV administration and six weeks post-UNx, *db/db* UNx-ReninAAV mice were administered (q.d.) vehicle, semaglutide (30 nmol/kg, s.c.) or semaglutide (30 nmol/kg, s.c.) + lisinopril (30 mg/kg, p.o.) for 11 weeks. Endpoints included blood pressure, plasma/urine biochemistry, kidney histopathology and RNA sequencing. **Results:** Semaglutide robustly reduced hyperglycemia, hypertension and albuminuria concurrent with notable improvements in glomerulosclerosis severity, podocyte filtration slit density, urine/renal kidney injury molecule-1 (KIM-1) levels and gene expression markers of inflammation and fibrogenesis. Co-administration of lisinopril further ameliorated hypertension and glomerulosclerosis. **Conclusion:** Semaglutide improves disease hallmarks in the db/db UNx-ReninAAV mouse model of advanced DKD. Renal outcomes were further improved by combined antihypertensive standard-of-care.

OP14: Michael Nauck

Meta-Analysis of Head-to-Head Clinical trials Comparing Incretin-Based Glucose-Lowering Medications and Basal Insulin – An Update Including Recently Developed GLP-1 Receptor Agonists and the GIP/GLP-1 Receptor Co-Agonist Tirzepatide

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Objective. To provide an update (since our previous meta-analysis published in 2017) on the comparative efficacy of injectable incretin-based glucose-lowering medications (IBGLM) versus basal insulin treatment in patients with type 2 diabetes, now including highly efficacious novel IBGLM like semaglutide and tirzepatide.

Research Design and Methods. We updated our previous meta-analysis of randomized clinical trials reporting head-to-head comparisons of IBGLM (with subgroups: Short- and long-acting GLP-1 receptor agonists [GLP-1 RAs] and GIP/GLP-1 receptor co-agonist tirzepatide) vs. basal insulin. Primary endpoint was the difference in the reduction of HbA_{1C} vs. baseline between pooled IBGLM (fixed-effects meta-analysis) and their subgroups (random-effects meta-analysis) compared to basal insulin treatment, calculating mean differences. Secondary endpoints included fasting plasma glucose, body weight, HbA_{1C} target achievement, hypoglycemic episodes, blood pressure and lipid-related parameters.
Results. 21 studies (8 more than previously available) were found eligible. Compared to basal insulin, IGBLM lowered HbA_{1c} by 0.48 [0.45-0.52] % more than did basal insulin treatment. This effect was driven by long-acting GLP-1 RAs (Δ HbA_{1c} -0.25 [-0.38;-0.11] %) and tirzepatide (ΔHbA_{1c} -0.90 [-1.06;-0.75] %), while short-acting GLP-1 RAs were equally effective compared to basal insulin ($p = 0.90$). All subgroups of IBGLM led to significantly lower body weight versus insulin treatment (-4.6 [-4.7;-4.4] kg), in particular tirzepatide (-12.0 [-13.8;-10.1] kg). IBGLM significantly reduced the proportion of patients reporting any or severe episodes of hypoglycemia. IBGLM had more favorable effects of blood pressure and lipid parameters.
Conclusions. The inclusion of recently introduced, highly effective IBGLM has elicited a more pronounced superiority of IBGLM over basal insulin treatment, reinforcing the recommendation that IBGLM should be considered as the first injectable treatment for most patients with type 2 diabetes.

OP15: Yining Xu

Oral smart control drug delivery systems for fine-tuning gut hormones

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Introduction:
More than twenty gut hormones are released into the bloodstream when enteroendocrine cells (EECs) respond to nutrients found in the gastrointestinal lumen. Different gut hormones act synergistically to achieve the modulation of physiological and pathophysiological actions in the body. Excipients used in drug delivery systems (DDSs) contain certain nutrients could activate G-protein coupled receptors (GPCRs) expressing on EECs. Great evidence has also demonstrated that DDS' physiochemical properties may affect the physiological interaction between nanoparticles and targeted areas in the gut. We herein hypothesize that rationally designed DDSs may target different EEC populations, increasing/inhibiting desired gut hormones for a targeted disease treatment.

Methods:
The comprehensive mechanism of interaction between developed DDSs with targeted EECs was unraveled by using human/mouse intestinal organoid models, via different approaches (e.g., genome editing). How precisely DDSs induces the release of different gut hormones and whether the gut microbiota is involved were evaluated in vivo. The therapeutic efficiency of drug-loaded smart DDSs was also tested in diabetic mice and in $Gcg^{DistalGut-/-}$ and $Gcg^{Gut-/-}$ mice that reduce the Gcg expression within the distal or entire gut.

Results:
Smart DDSs significantly increase glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) release in intestinal organoids in interaction with GPR 40 and 119. Smart DDSs secreting simultaneously three different hormones (GLP-1, GIP and PYY) can be obtained by changing the excipients in the formulation and getting a different particle size in vivo. Smart DDSs presenting different sizes can target different intestinal segments to regulate its multi-biological effects after oral administration in GcgDistalGut-/- and GcgGut-/- mice.

OP16: Dan Quansah

Reactive hypoglycaemia during the OGTT after gestational diabetes mellitus: metabolic implications and evolution

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Aims: Gestational diabetes (GDM) represents a state of increased insulin resistance and beta cell dysfunction and is associated with an increased cardio-metabolic risk. Reactive hypoglycaemia (RH) during the OGTT in pregnancy to establish the diagnosis of GDM is associated with adverse pregnancy outcomes. Although another OGTT is recommended in the postpartum in women after GDM, the occurrence and implications of RH during the OGTT are unknown. We investigated the prevalence, metabolic implications and longitudinal evolution of RH at 6-8 weeks postpartum in women with a history of GDM.
Methods: Between 2011-2021, we consecutively followed 1237 women with previous GDM undergoing an OGTT at 6-8 weeks postpartum. RH was defined as 2-h glucose <3.9mmol/l after the OGTT. Metabolic outcomes were compared in women with and without RH (RH+/RH-). We also included a subcohort of 191 women with data on insulin sensitivity/secretion indices (MATSUDA, HOMA-IR, insulin-adjusted-secretion ISSI-2).
Results: The postpartum prevalence of RH was 12%. RH+ women had a more favourable metabolic profile including 2-5-times lower prevalence of glucose intolerance and metabolic syndrome at 6-8 weeks postpartum compared to RH- (all $p \leq 0.034$). In the subcohort, women with RH+ had higher insulin sensitivity, higher ISSI-2 and an earlier glucose-peak after OGTT ($p \leq 0.049$) compared to RH- women at the same time point. Insulin resistance increased and ISSI-2 decreased over the first year postpartum in both groups. These changes were associated with a 50% reduction in overall RH prevalence at 1-year postpartum. Some of the favourable profiles of RH+ persisted at 1-year postpartum, without group-differences in the longitudinal metabolic changes.
Conclusions: At 6-8 weeks postpartum, RH was frequent in women after GDM and was associated with increased insulin sensitivity and higher insulin-adjusted-secretory capacity compared to GDM women without RH. RH might be related to an improved incretin function and could be a marker of favourable metabolic prognosis in women with a history of GDM.

OP17: Rajamannar Thennati

GL0034, A novel long-acting glucagon-like peptide 1 receptor agonist exhibits significant efficacy in aged db/db mouse model of non-alcoholic fatty liver disease (NAFLD)

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Background and aims: Obesity and type 2 diabetes are the major risk factors for non-alcoholic fatty liver disease (NAFLD) and advanced liver fibrosis. GL0034 is a novel long-acting human glucagon-like peptide 1 (GLP-1) receptor agonist, which selectively activates the GLP-1 receptor. Here, we evaluated the efficacy of GL0034 in aged db/db mice as a model for NAFLD.

Materials and methods: db/db mice (age: 22-24 week) were divided into vehicle control; GL0034 (20 nmol/kg) or semaglutide (20 nmol/kg). Animals were treated with vehicle or test item every third day up to day 27. On day 28, HbA1c, plasma triglycerides, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), liver transforming growth factor β(TGF-β) and liver malondialdehyde (MDA) were quantified. Liver steatosis was evaluated using Oil- Red O staining.
Results: Subcutaneous treatment with GL0034 led to a significant reduction in HbA1c (4.9%*** vs. day 0), body weight (8.9%***## vs. day 0) and plasma triglycerides (19.8%** vs. day 0), wherein semaglutide treatment resulted reduction of 4.0%***, 3.4%*** &6.9% in HbA1c, body weight and triglycerides respectively vs day 0. Thus GL0034 efficacy was higher than semaglutide under similar dosing conditions. Reduction in ALT/AST (3.3/2.8 folds vs. vehicle control) and liver TGF-β (3.8 folds vs vehicle control) was significant and better as compared to semaglutide. Reduction in steatosis was confirmed using Oil- Red O staining and liver MDA (2-fold decrease) level.
Changes in HbA1c (glycated haemoglobin), body weight, triglycerides, ALT (alanine aminotransferase) and AST (aspartate aminotransferase) on day 28. Mean ±SD using one-way ANOVA followed by Bonferroni's post-test respectively. *p<0.05, **p<0.01, ***p<0.001 vs. vehicle control and #p<0.05, ##p<0.01, ###p<0.001 vs. semaglutide. d0= day 0 and d28= day 28.
Conclusion: Hepatic steatosis and increases in triglycerides, ALT/AST in aged db/db mice was suppressed by GL0034, thereby reducing the risk of fibrosis that is accelerated during excessive lipid metabolism in the liver.

OP18: Guillaume Mabilleau

Dual GIP/GLP-2 analogues are capable of inducing bone strength in an animal model of disuse osteoporosis

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Several evidences pointed out to the crucial role of gut hormones, and especially GIP and GLP-2, in maintaining bone strength. Although these molecules modulate bone remodeling markers, investigations in preclinical models highlighted that the main action was through enhancement of bone material properties. Recently, we developed a series of dual GIP/GLP-2 analogues capable of increasing bone strength in a validated animal model of post-menopausal osteoporosis. The aim of the present study was to investigate whether dual GIP/GLP-2 analogues were capable of enhancing bone strength in an animal model of disuse osteoporosis, the second cause of bone fragility.
Botulinum toxin (BTX) was administered in the right quadriceps femoris of 24 male swiss mice to induce paralysis and disuse osteoporosis. Animals were randomized (n=8/group) to further receive saline, GL-0001, a dual GIP/GLP-2 analogues, or zoledronic acid (100µg/kg). Left hindlimb in saline-treated animals was used as control. Three-point bending, microCT and Fourier-transform infrared microspectroscopy were performed to evaluate bone strength, mass, microarchitecture and bone material properties. Analyses of variance have been performed and considered significant at $p<0.05$.
As expected, BTX administration led to a hindlimb paralysis, reductions in bone strength and bone mass, and alterations in bone microarchitecture and material properties. Administration of zoledronic acid, the gold standard in the treatment of osteoporosis, led to improvement in bone mass and bone microarchitecture, but failed to enhance either bone strength or bone material properties. On the other hand, administration of GL-0001 resulted in higher bone strength mostly by reversing alterations of bone material properties.
This study highlights the potential of dual GIP/GLP-2 analogues, and especially GL-0001, for the treatment of bone fragility. Further studies are required to assess whether all dual GIP/GLP-2 analogues exert such bone effects and whether these molecules are potent to increase bone strength in humans.

OP20: Victoria Parker

Efficacy, Safety and Pharmacokinetics of Cotadutide, a Dual GLP1-glucagon Receptor Agonist, in Patients with Chronic Kidney Disease (CKD) and T2DM

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Background: Cotadutide is a dual GLP1-glucagon receptor agonist under development for NASH and CKD with T2DM.
Methods: In this randomised, double-blind, phase 2a study, patients with T2DM on insulin and/or oral therapy (HbA1c ≥6.5 and ≤10.5%), eGFR ≥30 and <60 ml/min/1.73m^2 and BMI ≥25 to ≤45 kg/m^2 were randomised to receive once-daily SC cotadutide titrated up to 300 µg (n = 21) or placebo (n = 20) for 32 days. Study endpoints evaluated glucose lowering via mixed meal tolerance test (MMTT) and continuous glucose monitoring (CGM), body weight, eGFR (CKD-Epi), urinary albumin creatinine ratio (UACR) and exploratory renal biomarkers.
Results: The primary endpoint was met; a significant reduction in MMTT glucose AUC -26.71% (90% CI -34.58, -18.83) was observed on cotadutide vs placebo (P <0.001). In addition, cotadutide resulted in significant reductions from baseline in body weight (-3.69%), HbA1c (-0.65%) and more time spent in target range on CGM over 32 days (78.7%) (all P <0.001). This was achieved alongside a 35.2% reduction in insulin dose on cotadutide, p=0.012. For renal-related endpoints, eGFR was unchanged after 32 days of dosing at 1.17 ml/min/1.73m^2 (90% CI -1.29, 3.62), p=0.268, and in subjects with baseline micro or macroalbuminuria (N=18) a numerical reduction in UACR of 51.00% (SD 0.77, p= 0.057) relative to placebo was observed. No changes in renal inflammatory markers were apparent, but significant reductions in serum ammonia and NT-proBNP were noted. SAEs were balanced across groups and tolerability and PK profiles were comparable to those observed in patients without CKD.
Conclusions: In patients with CKD with T2DM, cotadutide promoted clinically important effects on glycaemic control and body weight. The observed reduction in albuminuria suggests cotadutide has potential to deliver benefit on renal outcomes, but this needs to be evaluated in larger and longer studies.

OP21: William Roell

GIPR agonism is a key contributor to tirzepatide modulation of metabolic adaptation

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Body weight management via dietary restriction (DR) and exercise or pharmacological intervention is limited as the weight loss achieved with these interventions is accompanied by metabolic adaptation—reduction in metabolic rate as a compensatory mechanism to counter reduced caloric intake. The dual GIP and GLP-1 receptor agonist Tirzepatide (TZP) has demonstrated profound weight loss in clinical trials. However, the contribution of GIPR agonism to this effect is incompletely understood. Here, we investigate TZP's ability to interrupt metabolic adaptation and the mechanistic role of GIPR agonism in a mouse model of DR. Additionally, we investigated GIPR agonism regulation of adipose tissue function and transcriptional profile using both ex vivo tissue and cell-based models. TZP reduced metabolic adaptation and increased lipid oxidation in the mice, a process which required combined action of GIPR and GLP-1R agonism. Additionally, GIPR agonism modulated transcription of genes associated with lipid storage and release in both adipose tissue and cell-based adipocyte models. Functionally, GIP and TZP, but not GLP-1 signaled in adipocytes increasing lipolysis, an effect that was suppressed by insulin. These findings demonstrate GIPR agonism is a key contributor to TZP

interruption of metabolic adaptation and the associated elevation of lipid oxidation. These experiments suggest GIPR agonism enhances the ability to mobilize lipid from adipose tissue subsequently fueling the elevated energy expenditure stimulated by the dual agonism of TZP during DR. The findings presented here better elucidate key metabolic mechanisms by which TZP stimulates reduction in bodyweight and the potential benefits of dual GIPR/GLP-R agonism.

OP22: Tamer Coskun

The novel GIP, GLP-1, and glucagon triple receptor agonist LY3437943: From discovery to clinical proof-of-concept

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With an increasing prevalence of obesity, there is a need for new therapies to improve body weight management and metabolic health. Multi-receptor agonists in development may provide approaches to fulfill this unmet medical need. LY3437943 is a novel, unimolecular triple agonist peptide at the glucagon receptor (GCGR), glucose-dependent insulinotropic polypeptide receptor (GIPR), and glucagon-like peptide-1 receptor (GLP-1R). *In vitro*, LY3437943 shows balanced GCGR and GLP-1R activity, but more GIPR activity. In obese mice, administration of LY3437943 decreased body weight and improved glycemic control. Body weight loss could be attributed to a combination of energy expenditure, primarily mediated by GCGR, and food intake, driven primarily by GLP-1R and GIPR. In a randomized, double-blind, placebo-controlled, Phase 1 proof-of-concept study, we assessed the safety and tolerability of multiple ascending doses of LY in patients with type 2 diabetes (T2D). Vital signs, laboratory data and adverse events (AEs) were monitored to assess safety and tolerability. Efficacy was assessed by monitoring change in glycated hemoglobin (HbA_{1c}) and body weight at week 12. The most common treatment-emergent AEs were gastrointestinal (nausea and diarrhea), which were mostly mild in severity. By week 12, mean HbA_{1c} decreased from baseline in all groups, with higher doses of LY showing statistically significant baseline-adjusted decreases of up to 1.90%. Dose-dependent decreases in mean baseline-adjusted body weight of up to 8.65 kg were observed with LY.
In conclusion, LY3437943 showed a safety and tolerability profile similar to other incretins. Its pharmacokinetic profile supported once-weekly dosing. Promising glycemic and body weight loss efficacy within these studies highlights the potential for LY to provide additional benefit versus existing therapies in treatment of T2D and obesity.

OP23: Robert Augustin

Discovery and selection of BI 456906 as a dual GCGR/GLP-1R agonist applying in vivo target engagement biomarkers

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Obesity represents a global health challenge requiring treatment interventions. Glucagon (GCG) and Glucagon-like peptide 1 (GLP-1) are peptide hormones that pharmacologically regulate body weight by increasing energy expenditure and reducing energy intake, respectively. We describe the discovery and selection of BI 456906 as a dual GCGR/GLP-1R agonist. In vitro potencies for the GCGR and GLP-1R were determined in cells of recombinant and endogenous receptor expression. Upon acute, single dosing to lean mice, engagement of the GCGR and the GLP-1R was determined by liver NNMT mRNA expression and plasma FGF-21 and oral glucose tolerance tests, respectively. Body weight and glucose lowering (HbA1c) efficacies were investigated in diet-

induced obese (DIO) and diabetic (db/db) mice, respectively. Nineteen GCGR/GLP-1R dual agonists were tested for improving oral glucose tolerance (30 nmol/kg) and to increase plasma FGF-21 and liver NNMT mRNA expression (100 nmol/kg). Specificity of the biomarkers was confirmed applying the selective GLP-1R agonist semaglutide and GLP-1R knockout mice. Based on their acute in vivo profiles, BI 456906, BI456908 and BI 456897 were selected to confirm dose-dependent engagement of both, the GLP-1R and GCGR. In DIO mice, BI 456906 (30 nmol/kg), BI 456908 (30 nmol/kg) and BI 456897 (10 nmol/kg) achieved a body weight lowering efficacy from baseline of 25%, 27%, and 26%, respectively. In db/db mice, BI 456906 and BI 456908 (10 and 20 nmol/kg, qd) significantly lowered HbA1c (0.4-0.6%), while no significant effect was observed for BI 456897 (3 and 7 nmol/kg qd). Due to its balanced *in vivo* efficacy profile BI 456906 was further characterized to provide superior weight lowering compared to a maximally effective dose of the GLP-1R agonist semaglutide (32% vs. 27%; $p<0.05$) which was attributed to an increase in energy expenditure. Selected as clinical candidate, BI 456906 is currently investigated in Ph2 clinical trials in people with obesity or NASH.

OP24: Jo Lewis

The role of GIP in the regulation of food intake and body weight in mice

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Background and Aims: Hormones from the gut that signal nutrient uptake and availability to the brain are key elements in the control of appetite. Indeed, GLP-1 based pharmacotherapies are licensed for the treatment of type 2 diabetes and obesity, with dual agonist peptides based on GLP-1 and GIP, demonstrating improved glucose homeostasis and reduced body weight. GIP regulates blood glucose via its insulinotrophic and glucagonotrophic action on the pancreas. However, the role of GIP in the regulation of food intake, body weight and whole-body physiology remains controversial.
Materials and methods: Utilising a novel rodent model (GIP-Cre x Dq), in which the of GIP expressing cells are activated by a hM3Dq Designer Receptor Activated by Designer Drugs (Dq-DREADD), we measured intraperitoneal glucose tolerance and food intake in the lean and diet induced obese state, as well as whole-body physiology and feeding behaviour in metabolic cages (n = 14, cross-over design).
Results: In lean mice, activation of the Dq results in an increase in plasma GIP, akin to that in the postprandial state in mice. In lean and diet induced obese mice, the increase in GIP is associated with improved glucose tolerance ($p<0.0001$) and a reduction in food intake both in the ad lib fed state at the onset of the dark phase ($p<0.01$) and in the fast-refeed paradigm ($p<0.001$). The reduction in food intake is a consequence of reduced feeding time ($p<0.05$) and an increase in the time between meals ($p<0.001$). No change in energy expenditure or activity was detected following GIP cell activation, however, a transient reduction in body weight was apparent ($p<0.05$). In lean mice, the effects on glucose tolerance and food intake were blocked by pre-treatment with a GIPR antagonistic antibody, suggesting the effects were specific to GIP.
Conclusion: We propose these studies will result in new insights into the physiological role of GIP.

OP25: Alice Adriaenssens

Glucose-dependent insulinotropic polypeptide receptor-expressing neurons employ distinct mechanisms to control feeding behaviour depending on their neuroanatomical location in mice.

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Though central glucose-dependent insulinotropic polypeptide (GIP) receptor (GIPR) signalling is necessary for GIP-based therapeutics to lower body weight, pathways leveraged by GIPR agonism in the brain remain poorly understood. We explored the role of *Gipr* neurons in the hypothalamus and dorsal vagal complex (DVC)—brain regions critical to the regulation of energy balance.
Brain areas accessible to peripherally-dosed GIPR agonists (GIPRAs) were mapped using fluorescent GIP-based peptides. Effects of chemogenetic manipulation of *Gipr* neurons in identified target regions were measured using behavioural assays and metabolic cages. Neurocircuit mapping defined projections and relay centres of *Gipr* neurons. scRNAseq of purified DVC *Gipr* cells was used in combination with RNAscope to characterise transcriptomic profiles defining subpopulations of *Gipr* neurons.
Peripherally-dosed fluorescent GIPRAs localised to the hypothalamus and DVC. Chemogenetic stimulation of both hypothalamic and DVC *Gipr* neurons suppressed food intake. While activation of DVC *Gipr* neurons reduced ambulatory activity and induced conditioned taste avoidance (CTA), peripheral GIPRA administration did not induce CTA. DVC *Gipr* neuron stimulation evoked c-Fos activation in brain centres controlling meal size, with *Gipr* neurons of the nucleus tractus solitarius (NTS), but not the area postrema (AP), projecting to the parabrachial nucleus and paraventricular hypothalamic nucleus. scRNAseq and RNAscope analysis revealed that *Gipr* neurons in the NTS and AP are transcriptomically distinct, with enrichment of *Th* and *Dbh* in the NTS, and *Penk* and *Npy2r* in the AP.
The hypothalamus and DVC are key sites of *Gipr* expression and GIPRA access. While activation of *Gipr* neurons in both sites reduces food intake, DVC *Gipr* neurons are regulators of activity and taste avoidance. Within the DVC, *Gipr* neurons of the NTS and AP differ in connectivity and gene expression, and may engage separate pathways that control appetite.

OP26: Mireia Montaner Massana

The olfactory bulb GLP-1 system mediates the regulation of pre- and post-prandial energy metabolism in obesity

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Central circuits involved in the regulation of energy homeostasis by GLP-1 and GLP-1 receptors (GLP-1R) remain unclear. Within the brain, it was shown that cells in the olfactory bulb (OB) express GLP-1R and a subset of interneurons are preproglucagon (PPG) positive. This sensory structure codes olfactory food information, which is crucial for the estimation of food quality (palatable or toxic) and energy content even before ingestion. In this context, our study focuses on the roles of GLP-1 in the regulation of pre- and postprandial energy metabolism *via* the OB in health and obesity.
Lean and obese mice underwent olfactory as well as glucose tolerance tests after acute injections of GLP-1, Exendin-4 or Exendin-9 in the OB or chemogenetic modulation of bulbar GLP-1R-expressing neurons, and insulin secretion was assessed.
We report that during the preprandial state of lean mice, food odors trigger a cephalic phase of insulin secretion (CPIR), which is blocked by OB-injected Exendin-9. Odor-evoked CPIR is lost in obese mice and improved by OB-injected Exendin-4.
Interestingly, chemogenetic inhibition of GLP-1R-expressing cells in the OB of lean mice induces an increase in food intake whereas an activation in obese mice downregulates food intake.
In the postprandial state, DREADD activation of GLP-1R-expressing cells in the OB strongly improves glucose homeostasis by increasing insulin secretion *via* a hypothalamic relay in obese mice. Vagotomy does not affect the beneficial effect of GLP-1R activation on insulin release. In contrast, pancreatic noradrenaline levels are

decreased in response to GLP-1R activation, suggesting a decrease in sympathetic activity. Since sympathetic activity is regulated by GABAergic tone in paraventricular nucleus (PVN), we injected bicuculline ($GABA_AR$ antagonist) into the PVN to prevent sympathetic inhibition. Under these conditions, the beneficial effect of GLP-1 in the OB on insulin secretion is lost.
Our results unravel the GLP-1 system in the OB as a new actor in the regulation of glucose homeostasis.

OP27: Wanqing Jiang

Preproglucagon (PPG) neurons modulate physiological food intake and are not required for anorexigenic effects of naltrexone, rimonabant or nicotine

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Glucagon-like peptide-1 (GLP-1) is a gut-derived incretin and an anorexigenic neuropeptide. Neuronal GLP-1 is produced by PPG neurons located in the nucleus tractus solitarius (NTS) and intermediate reticular nucleus (IRT) of the brainstem. Chemogenetic activation of NTS PPG neurons reduces *ad libitum* eating, while ablation has no effect. However, these studies ignored IRT PPG neurons which form ~40% of the population. Additionally, we asked if PPG neurons can be activated pharmacologically for anti-obesity therapy.
Here, we ablated NTS and IRT PPG neurons in PPG-Cre transgenic mice via bilateral injection of an adeno-associated virus Cre-dependently encoding diphtheria toxin subunit A. PPG-ablated mice showed significantly higher body weight 6 month after surgery and higher dark phase *ad libitum* food intake than mice injected with control virus due to an increase in meal size and duration. To identify receptor targets on PPG neurons, we then performed single-nucleus RNA sequencing (snRNAseq) analysis on nuclear-tagged PPG neurons. We found expression of receptors linked to food intake regulation, including the mu opioid receptor, the cannabinoid receptor 1 and the a7 nicotinic acetylcholine receptor in 21%, 9.8% and 11.6% of PPG neurons, respectively. We targeted these receptors with naltrexone, rimonabant and nicotine and found a similar degree of food intake reduction in both control and PPG-ablated mice, indicating that these receptors on PPG neurons are not essential in food intake control.
In conclusion, NTS and IRT PPG neurons modulate physiological eating behaviour. Whether this is achieved by the population as a whole, or a distributed subpopulation is currently unclear. Despite expressing their receptors, PPG neurons are not required for the anorexigenic effect of naltrexone, rimonabant or nicotine. Our study demonstrates the combination of targeted snRNAseq with PPG ablation is a powerful tool to identify drugs requiring PPG neurons for their action *in vivo*.

OP28: Stefan Trapp

5-HT2c receptor agonist lorcaserin requires preproglucagon neurons to reduce food intake in mice and acts additively to liraglutide-induced hypophagia

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The most efficacious obesity treatment to date is GLP-1 receptor agonists (GLP-1RAs) producing widespread activation in the CNS despite limited brain penetration. Previous work suggests that systemic GLP-1RAs and GLP-1 from preproglucagon (PPG) neurons reduce food intake via separate pathways and might have additive effects. Here, we explore activation of PPG neurons by the $5\text{-}HT_{2C}$ receptor agonist lorcaserin and assess combination therapy with the GLP-1RA liraglutide. First, we showed that lorcaserin increased c-fos immunoreactivity in PPG neurons and that PPG neurons express $5\text{-}HT_{2c}$ receptors. We then ablated nucleus

tractus solitarius (NTS) PPG neurons in Glu^{cre} mice by cre-dependent viral expression of diphtheria toxin subunit A. The anorexigenic effect of lorcaserin was significantly attenuated in mice lacking NTS PPG neurons. We next used $5\text{-}HT_{2C}R^{Cre}$ mice virally expressing hM3Dq in the NTS to demonstrate that selective activation of NTS $5HT_{2C}$ receptor expressing cells with CNO reduces food intake, but that this effect is lost when NTS GLP-1R expression is knocked down with virally delivered GLP-1R shRNA. Collectively, this suggests that NTS PPG neurons are necessary for the anorexigenic effect of lorcaserin and may produce this effect by activation of GLP-1Rs in the NTS. In contrast, the hypophagic action of liraglutide was retained under NTS GLP-1R knockdown, suggesting that NTS GLP-1Rs are primarily a target for PPG neurons, not systemic GLP-1RAs. Finally, we examined the effect of liraglutide and lorcaserin in combination. A greater reduction in food intake was observed with the drug combination than either monotherapy. We conclude that lorcaserin mediates its hypophagic effect partly through PPG neurons, and that lorcaserin can be combined with liraglutide for more effective appetite suppression. These findings support that combination of GLP-1RAs with pharmacological activation of PPG neurons has potential to improve current anti-obesity therapies.

OP29: Ben Jones

Differential effects of biased GLP-1 receptor agonists on appetite versus blood glucose

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Biased agonism could be a way to alter the balance between GLP-1 receptor agonist glucoregulatory, anorectic and nauseating effects. Here we have compared the physiological effects of a partial GLP-1R agonist with virtually undetectable β-arrestin recruitment, exendin-phe1 (ExF1), with the equivalent full agonist exendin-asp3 (ExD3).
We profiled signalling and trafficking effects of ExF1 and ExD3 in HEK293 cells using pharmacological and imaging techniques. Physiological tests were performed in mice. Agonist uptake into the brain was assessed using fluorescent agonist conjugates.
ExF1 showed moderately reduced Gαs recruitment and cAMP signalling, along with virtually absent β-arrestin recruitment and GLP-1R endocytosis. However, blood glucose-lowering was much greater with ExF1 than with ExD3, especially when assessed after a delay to allow desensitisation effects to emerge. In contrast, both ligands showed broadly similar effects on appetite reduction, albeit with a slower rate of onset with ExF1 compared to ExD3. This amounted to a ~300-fold selectivity advantage for glucose-lowering with ExF1. In case this difference in appetite regulation kinetics reflected delayed entry into the brain with ExF1, e.g. via GLP-1R-mediated endocytic uptake through hypothalamic tanycytes, we quantified brain accumulation of fluorescent ExF1 and ExD3 conjugates in mice using optical projection tomography, but did not observe any difference between each agonist. Uptake and release of each conjugate in a primary tanycyte model was also similar.
These studies highlight how a partial GLP-1R agonist with preferential coupling to Gαs signalling yields physiologically specific effects on blood glucose *versus* appetite suppression. The endocytic profile of each ligand did not predict uptake into brain appetite centres.

OP30: Orla Woodward

Characterisation of G-protein coupled relaxin/insulin-like family peptide receptor 4 (Rxfp4)-expressing cells in the mouse hypothalamus

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Relaxin/insulin-like family peptide receptor 4 (RXFP4) is the cognate receptor for the orexigenic hormone insulin-like peptide 5 (INSL5), co-secreted with GLP-1 from enteroendocrine L-cells in the distal gastrointestinal tract. RXFP4, a $G\alpha_{i/o}$-coupled receptor, has been implicated in feeding behaviour as *Rxfp4* knock-out mice have altered meal patterns and food preferences. Here, we implemented the novel *Rxfp4*-Cre reporter mouse as a functional tool to identify and characterise *Rxfp4*-expressing cells in the central nervous system. Using immunohistochemistry techniques, *Rxfp4*-expressing cells were identified in multiple brain regions, with a distinct population observed in the ventromedial hypothalamus (VMH). Single-cell RNA-sequencing of hypothalamic *Rxfp4*-expressing cells identified clusters of *Rxfp4*-expressing neurons exhibiting transcriptomic markers previously associated with food intake and body weight regulation (*Glp-1R, Cckar, Cnr1, Mc4r*, *Nmur2, Esr1, Tac1)*. Employing pseudotyped rabies and AAV-ChR2 viral tracing techniques, we found that VMH *Rxfp4*-expressing neurons ($RXFP4^{VMH}$) receive projections from regions associated with homeostatic food intake including the paraventricular hypothalamus, arcuate nucleus and lateral hypothalamus, and project to regions of the reward system including the bed nucleus of the stria terminalis, paraventricular thalamus, substantia innominata and central nucleus of the amygdala. Intra-VMH infusion of INSL5 and chemogenetic inhibition of $RXFP4^{VMH}$ neurons increased intake of a high fat diet and highly palatable meal, while chemogenetic activation of these neurons had the opposite effect. These findings indicate that VMH *Rxfp4*-expressing neurons may regulate the reward system to influence ingestive behaviours and may represent targets to develop treatments for appetite-related disorders such as obesity and anorexia nervosa.

OP31: Danae Nuzzaci

Characterisation of VMH-RXFP4 neurons and their role in overfeeding.

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INSL5 is a hormone co-released with GLP-1 and PYY from L-cells in the distal colon and rectum. We recently identified a cluster of central neurons expressing the INSL5 receptor, RXFP4, in the ventromedial hypothalamus (VMH). Central administration of INSL5, which activates G_i-signalling downstream of RXFP4, increased high fat diet intake and chemogenetic activation of Rxfp4-expressing neurons ($Rxfp4^{Dq}$ treated with CNO) reduced intake of high fat diet and ensure liquid meals, but not standard chow consumption.
Our goal is to explore the physiological role of this neuronal population, during short-term and long-term overfeeding. Using Rxfp4-Cre mice crossed with reporters for Ca^{2+} ($Rxfp4^{GCaMP3}$) or cAMP ($Rxfp4^{CAMPER}$), for imaging in acute brain slices, confocal microscopy and electrophysiology, our aims are to characterize VMH-RXFP4 neurons, explore synaptic plasticity in different nutritional conditions and study the connection with other relevant circuits regulating food intake.
ScRNAseq data from FACsorted $Rxfp4^{EYFP}$ neurons revealed that VMH-RXFP4 neurons express receptors for other important nutritional cues such as CCK, ghrelin and aMSH. In acute brain slices, 29% and 47% of $Rxfp4^{GCaMP3}$ neurons exhibited Ca^{2+}-responses to hexarelin (100 nM; 8 slices) and CCK (100 nM; 9 slices), respectively, and 25% of $Rxfp4^{CAMPER}$ neurons showed an elevation of cytosolic cAMP in response to aMSH (200 nM; 8 slices), whilst the remaining neurons showed no apparent changes. 3D-reconstruction of confocal images of $Rxfp4^{GCaMP3}$ neurons in the VMH stained immunohistochemically for VGluT1(glutamatergic marker) and VGAT (GABAergic marker) demonstrated a predominance of glutamatergic synaptic input into these neurons in ad lib chow fed mice.
Mirroring other gut/brain signalling pathways, the central RXFP4 axis is closely linked to the control of food intake. This in vitro work in conjunction with viral tracing will characterise the neuronal networks of Rxfp4-neurons, with the hope to manipulate these in the future for the treatment of obesity.

OP32: Henriette Holst Nerild

Liraglutide decreases postprandial fibroblast growth factor 19 and glucagon-like peptide 2, and increases postprandial cholecystokinin in individuals with obesity

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Background and aims
Treatment with liraglutide as well as other glucagon-like peptide 1 (GLP-1) receptor agonists is associated with slightly increased risk of gallbladder-related disorders, which have been proposed to be a consequence of altered gallbladder motility; thus, liraglutide seems to delay postprandial gallbladder refilling. The gut hormones cholecystokinin (CCK), fibroblast growth factor 19 (FGF19) and glucagon-like peptide 2 (GLP-2) are known to regulate gallbladder motility, and changes in postprandial concentrations of these hormones could explain the altered gallbladder motility.
Methods
In a single-centre, double-blinded, 12-week trial 52 participants with obesity were randomised 1:1 to once-daily subcutaneous liraglutide (escalated from 0.6 mg to 3.0 mg with 0.6-mg weekly increments) or placebo. We evaluated gallbladder dynamics using ultrasonograpy during 4-hour liquid meal tests (600 kcal, 23.7 g fat) at baseline, after the first dose of study drug and following 12 weeks of treatment and showed a liraglutide-induced deceleration of postprandial gallbladder refilling. Postprandial plasma responses of hormones known to modulate gallbladder motility (CCK, FGF19, GLP-2) were secondary endpoints. The primary endpoint of the study, maximum postprandial gallbladder ejection fraction, was reported previously.
Results
Baseline characteristics were similar between groups (50% male, age 47.6±10.0 years, body weight 99.0±15.7 kg, BMI 32.6±3.4 kg/m^2 (mean±SD)). Compared to placebo, liraglutide reduced postprandial FGF19 responses after first dose (AUC 24.8 vs 48.0 ng/ml×min with treatment ratio (TR) [95% CI] 0.52 [0.39; 0.69]) and following 12 weeks of treatment (AUC 33.7 vs 48.5 ng/ml×min, TR 0.69 [0.52; 0.93]). Liraglutide also reduced postprandial GLP-2 responses (AUC 3,650 vs 4,894 pmol/l×min, TR 0.75 [0.62; 0.90]) following first dose as well as after 12 weeks (AUC 3,760 vs 4,882 pmol/l×min, TR 0.77 [0.60; 0.99]). Compared to placebo, liraglutide increased postprandial responses of CCK after first dose (AUC 762 vs 670 pmol/l×min (TR 1.14 [0.97; 1.33]) and following 12 weeks of treatment (AUC 873 vs 628 pmol/l×min (TR 1.39 [1.12; 1.73]).
Conclusion
Treatment with liraglutide caused increased postprandial plasma CCK concentrations and decreased plasma FGF19 and GLP-2 concentrations compared to placebo, which may explain the delayed postprandial gallbladder refilling observed in individuals with obesity treated with liraglutide.
ClinicalTrials.gov ID NCT02717858. Sponsor: Novo Nordisk

OP33: Joachim Holt

Exercise as weight loss maintenance strategy increases postprandial secretion of GLP-1

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Background: Postprandial secretion of the appetite-inhibiting hormone GLP-1 is attenuated in people with obesity, but weight loss seems to increase this response. It is unknown how aerobic exercise program after weight loss affects GLP-1 meal responses.
Methods: Participants with obesity but not diabetes (BMI range 32-43) were enrolled in this randomized placebo-controlled study. All participants (n=195) were, after an 8-week low calory diet (800 kcal/day), randomized (1:1:1:1) to one of four 1-year interventions; placebo, the GLP-1 receptor agonist liraglutide (3.0 mg/day) (liraglutide), moderate to vigorous exercise (exercise only), or the combination of liraglutide and exercise (combination). Plasma levels of GLP-1 were measured during liquid meal tests (600 kcal) before and after diet induced weight loss and after 1 year of intervention.
Results: The low calory diet induced a mean weight loss of 13.1 kg. After one year the placebo group regained weight, while the exercise and liraglutide groups maintained their weight loss and the combination group decreased body weight further. Fasting GLP-1 levels after weight loss fell 11% ($p<0.05$), but both postprandial total area under the curve ($_{t}$AUC) and incremental AUC ($_{i}$AUC) of GLP-1 increased significantly ($p<0.01$). After 1 year of exercise program (exercise only), the postprandial GLP-1 response was further increased by 16% ($_{t}$AUC, $p<0.05$) and 35% ($_{i}$AUC $p<0.01$), whereas fasting levels of GLP-1 were unchanged.
Conclusion: Acute weight loss decreases fasting levels of GLP-1, but increases postprandial GLP-1 secretion. A one-year exercise program after weight loss further increased the postprandial GLP-1 response. This may explain part of the success of exercise as a weight loss maintenance strategy after an initial weight loss.

OP34: Nikolaj Sørum

GIP infusion acutely decreases blood pressure and increases heart rate in men with type 2 diabetes, concomitantly with increased pro-atrial natriuretic peptide levels

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Background and aims. While dual glucagon-like peptide 1 (GLP-1) receptor / glucose-dependent insulinotropic polypeptide (GIP) receptor agonists have shown promising results regarding glycaemic control and weight loss in people with type 2 diabetes (T2D), the independent cardiovascular effects of GIP remain unclear. In this study we examined the effect of acute GIP infusion on blood pressure (BP), heart rate (HR) and circulating pro-atrial natriuretic peptide (proANP) in overweight / obese men with metformin and long-acting GLP-1 receptor agonist-treated T2D. ProANP results were compared with results from a cohort of healthy men also receiving acute GIP infusion (NCT02747472).
Materials and methods. Thirteen men with metformin and long-acting GLP-1 receptor agonist-treated T2D received a double-blind continuous infusion of GIP (6 pmol/kg/min) and saline (placebo), respectively, on separate experimental days. After 60 minutes of infusion, the participants ingested a standardised liquid mixed meal (300 kcal). BP, HR, and plasma levels of proANP (measured as mid-regional proANP) were measured regularly throughout the 270 minutes infusion period. Plasma from 10 healthy men undergoing 60 minutes of GIP infusion (1.5 pmol/kg/min) was analysed for proANP levels at baseline and regularly during infusion.
Results. Compared to placebo, GIP infusion decreased mean arterial pressure (MAP) (95±1.8 vs 89±1.5 mmHg p=0.004), mean systolic BP (SBP) (132±2.2 vs 125±2.9 mmHg, p=0.017) and mean diastolic BP (DBP) (77±2.0 vs 72±1.4 mmHg, p=0.003) and increased HR (67±2.2 vs 76±3.4 beats per minute, $p<0.001$). Plasma proANP increased following GIP infusion (baseline-subtracted AUC 262±79 vs 988±342 minutes×pmol/l, p=0.002). A similar trend was seen in the healthy cohort (316±51 vs 576±159 minutes×pmol/l, p=0.065)

Conclusions. Infusion of GIP in overweight / obese men with metformin and long-acting GLP-1 receptor agonist-treated T2D, decreased MAP, SBP, and DBP and increased HR pointing to potentially additive haemodynamic effects of GIP receptor and GLP-1 receptor co-agonism. As plasma levels of proANP increased following GIP infusion, the BP reduction could be mediated by proANP.

OP35: Lærke Gasbjerg

Endogenous glucose-dependent insulinotropic polypeptide (GIP) facilitates postprandial intestinal lipid uptake

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The gut incretin hormone glucose-dependent insulinotropic polypeptide (GIP) increases triglyceride deposition in subcutaneous adipose tissue when administered to healthy individuals but these effects are attenuated both in patients with type 2 diabetes (T2D) and obesity. Using the selective GIP receptor antagonist GIP(3-30)NH_2, we investigated the effect of endogenous GIP on circulating lipoproteins during a meal in healthy men and patients with T2D.
We measured apolipoprotein B48 (ApoB48) as a marker of chylomicron count and plasma lipoprotein (chylomicrons, VLDL, LDL, HDL) triglyceride and cholesterol content during two four-hour liquid meal tests (1,894 kJ) with double-blind infusions of GIP(3-30)NH_2 or placebo (saline) in randomized order in 12 healthy men (19-65 years, BMI 20.3-25.5 kg/m^2) and in 10 men with T2D (44-72 years, HbA1c 37-70 mmol/mol (6.2-11%), BMI 27.4-41.2 kg/m^2). Increment AUC (iAUC) values were compared with Wilcoxon paired test.
GIP(3-30)NH_2 reached ~ 1,000 higher plasma levels than GIP(1-42). Compared to placebo, the GIP(3-30)NH_2 infusion lowered postprandial levels of ApoB48 ($iAUC_{0\text{-}180\ min}$ (median (95% CI)): 215 (156-258) vs 404 (363-541) mg $\times l^{-1}$ $\times$min, p=0.0049), chylomicron-triglyceride content ($iAUC_{0\text{-}270\ min}$: 32.7 (-2.32-72.1) vs 49.5 (1.21-170) mmol $\times l^{-1}$ $\times$min p=0.083) and HDL-triglyceride content ($iAUC_{0\text{-}270min}$: -0.90 (-1.95-0.18) vs 0.95 (-0.72-3.95) mmol $\times l^{-1}$ $\times$min, p=0.016). In patients with T2D, infusion of GIP(3-30)NH_2 did not change postprandial levels of ApoB48 or lipoprotein-triglyceride content. In both groups, there were no differences in lipoprotein-cholesterol contents.
In conclusion, we show that GIP receptor antagonism reduces postprandial circulating chylomicrons (ApoB48 levels) and HDL-triglyceride content in healthy individuals suggesting that endogenous GIP facilitates intestinal lipid uptake during a meal; an effect which seems to be reduced in patients with T2D.

OP36: Christian Hölscher

Incretins are neuroprotective in Alzheimer's and Parkinson's disease

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Alzheimer's (AD) and Parkinson's disease (PD) are chronic neurodegenerative disorders for which there are no disease-modifying drugs on the market. GLP-1 mimetics such as Exendin-4 (exenatide, Bydureon), and Liraglutide (Victoza, Saxenda) showed very good protective effects in phase II clinical trials in PD patients. Motor activity was improved by both drugs in two clinical trials. The Liraglutide study furthermore showed clear improvements in everyday activity such as walking, eating, talking, getting dressed, etc.. The drugs improved additional parameters such as mood and well being. Furthermore, liraglutide showed good protective effects in our phase II trial in AD patients (ELAD study). The drug group scored better in cognitive tests (ADASexec),

and a reduction in brain shrinking was observed as shown in MRI brain scans. Several other clinical trials testing different GLP-1 mimetics such as semaglutide (Ozempic, Rybelsus) and lixisenatide (Lyxumia, Adlyxin) are currently ongoing in PD or AD patients. Newer GLP-1 mimetics and dual GLP-1/GIP receptor agonists have been developed for diabetes and for the treatment of AD and PD. Preclinical research demonstrates that these drugs are more effective compared to the older drug generation. One finding was that not all drugs designed to treat diabetes can readily cross the blood-brain barrier (BBB) well. The longer they stay in the blood stream, the lower the influx into the brain. Novel drugs that have been developed to cross the BBB easier are the most effective ones in direct comparisons in animal models of AD or PD. In the MPTP mouse model of PD, the dual GLP-1/GIP receptor agonist KP405 showed superiority over exendin-4, liraglutide, and lixisenatide. In the 6-OHDA rat model of PD, KP405 was superior to exendin-4 or semaglutide. In the APP/PS1 mouse model of AD, KP404 was superior to exendin-4 and liraglutide. We conclude that dual GLP-1/GIP receptor agonist peptides that have been developed to cross the BBB show the most promise in stopping disease progression in AD and PD. KP405 is currently in Phase 1 tests. An overview will be given of the latest clinical and preclinical trials in this new research area.

OP37: Sevasti Gaspari

Tmem117 in AVP neurons is a novel regulator of counterregulatory response to hypoglycemia.

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The counterregulatory response (CRR) to hypoglycemia, which aims at maintaining sufficient provision of glucose to the brain, is an essential survival function. It constitutes a brain-orchestrated neuroendocrine reflex characterized by the secretion of multiple hormones (glucagon, cortisol, norepinephrine, epinephrine) that minimize glucose utilization and stimulate endogenous glucose production to restore normoglycemia. Glucose responsive neurons of the central nervous system (CNS) play a crucial role in triggering the CRR. However, the molecular mechanisms underlying this coordinated response remain largely unexplored.
To identify hypothalamic genes implicated in the control of the CRR, we previously performed a genetic/genomic screen using recombinant inbred BXD mice for insulin-induced glucagon (GCG) secretion. We identified Tmem117, a transmembrane protein with unknown function, as a potential regulator of GCG secretion. Using immunofluorescence microscopy analysis (IF) we observed that Tmem117 is localized in vasopressin (AVP) magnocellular neurons of the hypothalamus. c-Fos IF revealed that these AVP neurons were activated by insulin-induced hypoglycemia and ex vivo slice electrophysiological recordings verified that a subpopulation of them is activated by low glucose levels (glucose-inhibited neurons). In line with these observations, plasma levels of copeptin (CPP ; a surrogate for AVP) were elevated 1 hour after insulin-induced hypoglycemia. We then generated $Tmem117^{fl/fl}$ mice and used a viral mediated approach to conditionally knock-out *Tmem117* only in AVP neurons ($AVP^{TM117KO}$). This led to higher hypoglycemia-induced CPP and GCG secretion. When studied over the longer term, $AVP^{TM117KO}$ mice exhibited progressive loss of AVP neurons. This was accompanied by the loss of the CPP and GCG hypoglycemia-induced hypersecretion phenotype. Interestingly, the described secretory phenotype was observed in male mice and only during the proestrus phase in females, suggesting a role of sex hormones in Tmem117-regulated AVP secretion.
Overall, our study identified *Tmem117* as a novel hypothalamic regulator of CRR that affects AVP secretion and neuronal survival, and its action appears to be sexually dimorphic.

OP38: Anastasiya Strembitska

Agpat5 in AgRP neurons is required for hypoglycemia-induced glucagon secretion

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The counter-regulatory response to hypoglycemia restores blood glucose levels by stimulating glucagon secretion and hepatic glucose release. Yet, the mechanisms of brain hypoglycemia sensing, which control autonomic nervous activity and the hypothalamus-pituitary-adrenal axis, remain poorly characterized. In a previous genetic and genomic screen for hypothalamic regulators of hypoglycemia-induced glucagon secretion we identified *Agpat5* (mouse, chr8), as a candidate gene. Here we aimed at characterizing the role of Agpat5, a mitochondrial membrane-associated enzyme that uses fatty acyl-CoAs and lysophosphatidic acid to produce phosphatidic acid.
We found that mice lacking *Agpat5* expression in AgRP neurons of the hypothalamus arcuate nucleus (ARC) (*AgRP*^{Agpat5KO}, KO) had reduced glucagon secretion upon insulin-induced hypoglycemia (IIH) as compared to control mice (29.0±4.1pM (Ctrl) vs 16.1±1.7pM (KO), $p<0.001$). This was not observed in mice lacking *Agpat5* in PVN neurons or in ARC astrocytes. c-Fos immunostaining showed reduced number of AgRP neurons activated by hypoglycemia in KO vs Ctrl mice. Patch clamp analysis performed on acute brain slices revealed that inactivation of *Agpat5* reduced by half the number of glucose inhibited (GI) AgRP neurons (72.2% (Ctrl) vs 33.3% (KO), $p<0.05$). Reduced responsiveness to hypoglycemia of AgRP neurons form KO mice was further confirmed by *in vivo* fiber photometry Ca^{++} measurements; this was also associated with defective activation of the vagal nerve by hypoglycemia. Using a murine hypothalamic cell line, we found that silencing *Agpat5* expression increased mitochondrial fatty acid β-oxidation (FAO), oxygen consumption rate (OCR), and ATP production. This increased mitochondrial activity was suppressed by inhibiting Cpt1a function or expression. Similarly, inactivating *Cpt1a* in AgRP neurons of *AgRP*^{Agpat5KO} mice restored the number of hypoglycemia-activated AgRP neurons as determined by c-Fos immunostaining and patch clamp analysis.
Collectively, our data show that limiting FAO in AgRP neurons is necessary for effective hypoglycemia detection. This process is ensured by Agpat5, which partitions fatty acyl-CoAs away from mitochondrial FAO and ATP generation. This ensures that the hypoglycemia-dependent fall in ATP production, which triggers AgRP neuron firing through inhibition of the Na^+/K^+ATPase, is not prevented by increased FAO. This mechanism is especially important in the fasted state, when circulating free fatty acid concentrations increase and glucagon needs to be secreted.

OP39: Judit Castillo Armengol

Molecular investigations of defective glucagon secretion in a murine model of type-2 diabetes: a multi-omics study

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Repeated hypoglycaemic episodes in insulin-treated diabetic patients lead to impaired secretion of counter-regulatory hormones. In order to identify hypothalamic mechanisms that underlie the development of defective counter-regulatory response (CRR), in particular reduced glucagon secretion, we induced acute (AH) or recurrent hypoglycaemia (RH) by i.p. injection of insulin in a mouse model of type-2 diabetes (T2D). We measured plasma glucagon after the last insulin injection and isolated the hypothalamus and cortex of mice from the AH and RH group to perform single nuclei RNA sequencing (snRNAseq). Insulin-induced hypoglycemia was associated with a lower glucagon secretion in the RH than in the AH group. Analysis of snRNAseq data

revealed a total of 2373 differentially expressed genes (adj.p< 0.05) in neurons of RH vs. AH mice. Functional enrichment analysis revealed terms related to synapse organization, cell projections and to the mitochondrial electron transport chain. Analysis of cortex snRNASeq data showed similar deregulated gene expression. Analysis of differentially expressed neuronal hypothalamic genes (RH vs. AH) with (adj.p< 0.05 and fold change>1.2; n=41 genes) showed marked downregulation of *Avp* mRNA expression, and of two mRNAs associated with T2D susceptibility, *Kcnq1ot1* and *Pcsk1n*. A proteomic profiling of the hypothalamus and of its synaptosomal fractions has been performed and data are being analyzed. Collectively, this study provides a mouse model of T2D with defective CRR. It shows that impaired CRR is related to genes controlling synaptic function and organization both in the hypothalamus and cortex, suggesting a general deregulation of brain neuronal activity induced by hypoglycemia. The reduced expression level of *Avp* supports a role for AVP magnocellular neurons in CRR. This study further highlights the potential role of two genes (*Kcnq1ot1* and *Pcsk1n*) previously associated with T2D in defective CRR.

ABSTRACTS

2. Poster presentations (A0, vertical):

PP1: Ananyaa Sridhar

Sub-chronic PYY (3-36) administration alters enteroendocrine and pancreatic islet cell population in high-fat fed female mice

Sridhar A.[1], Khan D.[1], Flatt P.[1], Moffett R.[1],

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Aims

Low concentrations of circulating Peptide-YY(PYY) predispose individuals to develop obesity. Direct effects of PYY on enteroendocrine cells and pancreatic islets along with their influence on other gut-hormone distribution is yet to be explored in detail. In the present study, we have evaluated how sub-chronic PYY administration after prolonged high-fat diet influences morphology together with differential expression of key gut and pancreatic hormones.

Methods

After 14-weeks on high-fat diet, female TO-mice received saline and PYY(3-36) i.p injections twice daily for 21-days. Another saline group was maintained on normal chow for the same period, following which they were euthanized. Excised pancreatic and intestinal ileal tissues were used for detailed immunohistochemical analysis.

Results

Significant ($p<0.05$) reductions in body weight and blood glucose were observed after sub-chronic PYY administration. A significant ($p<0.01$) increase in ileal GLP-1 content was observed in the PYY group compared to high-fat saline with no change in GIP and PYY contents. Both villi length and crypt depth increased significantly ($p<0.001$) after PYY administration. In the pancreas, PYY significantly ($p<0.05$) decreased alpha cell area while islet, beta, PYY and delta cell areas remained unchanged. PYY also significantly ($p<0.001$) increased the percentage of beta cells while causing significant ($p<0.001$) reduction in alpha cell percentage. Co-localization of PYY per total glucagon ($p<0.01$) and somatostatin ($p<0.001$) cells were significantly higher compared to high-fat saline. Subsequently, there was no change in GLP-1 and glucagon co-expression in islets of the PYY group. PYY administration caused significant ($p<0.05$) reductions in both beta cell proliferation and apoptosis frequencies. However, PYY did not alter ileal GIP, GLP-1 and PYY cell counts in high-fat group.

Conclusion

PYY administration induces prominent changes in morphology and cellular composition of enteroendocrine and pancreatic islet cell population. These changes suggest NPY receptor modulation as a promising therapeutic target for the treatment and prevention for diet-induced obesity and related disorders.

PP2: Andrei Tarasov

Distinct effect of chronic GLP-1 agonism on glucose metabolism in pancreatic islet β-cells

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Ulster University[1]

Background and Aims:
Antidiabetic medications based on GLP-1 receptor agonism provide efficient amplification of glucose-induced exocytosis of insulin, accomplished via an elevation of cytosolic cAMP in pancreatic islet β-cells. At the same time, elevation of cAMP has a proven inhibitory effect on glycolytic flux in other systems, such as hepatocytes, which is likely to impair the stimulus-secretion coupling in β-cells. We therefore aimed to investigate the impact of GLP-1 treatment on glucose metabolism in β-cells, focusing on long-term (48h) effects of the incretin that model the administration of long-lasting GLP-1 analogues.
Materials and Methods:
We investigated the effect of 48-h exposure to GLP-1 on glycolytic and oxidative metabolism using fluorescent recombinant sensors for ATP (Perceval) or fructose 1,6-bisphosphate (HYlight) that were delivered into isolated mouse or human islets of Langerhans or BRIN-BD11 β-cell line. The cell responses to metabolic stimuli were then imaged using a robotised wide-field microscope, equipped with a 20×/1.0 objective. Data was analysed using Fiji, IgorPro and R.
Results:
Chronic exposure to 10 nM GLP-1 significantly attenuated the response of primary and clonal β-cells to acutely added high glucose, at the level of FBP (83.95%±10.38) and ATP (92.38%±0.21). Similarly, other compounds known to elevate cytosolic cAMP levels, GIP (10nM) and IBMX (100μM), demonstrated a decreased ATP response (GIP –91.50%±0.42; IBMX –99.04%±0.12) to the glucose step. However, FBP response to glucose was significantly increased in these groups (GIP -107.68%±6.58; IBMX –109.46%±2.2). GLP-1 and GIP demonstrate augmented, whereas IBMX attenuated cell viability. Glucose-induced insulin secretion was significantly upregulated after culturing in chronic 10nM GLP-1. However, culturing in chronic 10nM GIP and 100μM IBMX incurred no significant effects in insulin secretion.
Conclusions:
GLP-1 has a distinct molecular mechanism of regulating the glycolytic metabolism, different to that of GIP and IBMX, hypothetically preventing stress associated with glucotoxicity in β-cells and thus restoring their functionality after chronic hyperglycaemia.

PP3: Andrej Janez

Once-weekly semaglutide delays late digestive period of gastric emptying in women with PCOS and obesity

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Context: Semaglutide could contribute to reduced energy intake and weight loss by delaying gastric emptying (GE). However, the evidence for notable effects of semaglutide is inconclusive and compromised by the use of indirect methodology.
Objective: to evaluate the effect of once-weekly subcutaneous (s.c.) semaglutide 1.0 mg on late digestive period of GE after ingestion of a standardized solid test meal by using technetium scintigraphy, the reference method for this purpose.
Design/Participants/Main Outcome Measures: We conducted single-blind, placebo-controlled trial with 20 obese women with PCOS (BMI 37 (30.7-39.8) kg/m^2) randomized to s.c semaglutide 1.0 mg QW (S) or placebo

(P) for 12 weeks. GE was assessed after ingestion of [^{99mT}c] colloid in pancake labelled with radiopharmaceutical by scintigraphy using sequential static imaging and dynamic acquisition at baseline and at week 13. Estimation of GE was obtained by repeated imaging of remaining [^{99mT}c] activity (RA) at fixed time intervals over 4 hours after ingestion.

Results: From baseline to study end, semaglutide increased the estimated retention of gastric contents for 3%, 25,5%, 32% and 29% at 1st, 2nd, 3rd and 4th hour after ingestion of the radioactively labelled standard meal. At study end, there was a significant difference between S and P group at all measured time-points, with 37% RA in S vs. 0% in P (p=0,002)) 4 hours after meal ingestion, $T_{1/2}$ was significantly longer in S as compared to P (171 min vs 118 min, (p<0.001)).
Conclusion: Semaglutide markedly delayed late digestive period of GE in women with PCOS and obesity.

PP4: Anna Kowalka

The post-prandial secretion of PeptideYY1-36 and 3-36 secretion in obesity is differentially increased after gastric bypass versus sleeve gastrectomy

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Imperial College Healthcare NHS Trust[1], Imperial College London[2], North West London Pathology[3], Raigmore Hospital[4], University College London[5]

Objectives
Peptide tyrosine tyrosine (PYY) exists as two active species, PYY_{1-36} and PYY_{3-36}, which have distinct effects on insulin secretion and appetite regulation. The detailed effects of bariatric surgery on PYY_{1-36} and PYY_{3-36}secretion are not known as previous studies have used non-specific immunoassays to measure total PYY. Our objective was to characterise the effect of sleeve gastrectomy (SG) and Roux-en-Y gastric bypass (RYGB) on fasting and post-prandial PYY_{1-36} and PYY_{3-36} secretion using a newly developed liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay.
Design and subjects
Prospective observational study in 28 participants with obesity who underwent RYGB (n=20) or SG (n=8) at the Imperial Weight Centre [NCT01945840]. Participants were studied using a standardised mixed meal test (MMT) before and 1 year after surgery. The outcome measure was PYY_{1-36} and PYY_{3-36} concentration, by LC-MS/MS.
Results
Pre-surgery, the fasting and post-prandial levels of PYY_{1-36} and PYY_{3-36} were very low, with minimal responses to the mixed meal, unlike previous studies utilising total PYY immunoassays. The postprandial secretion of both PYY_{1-36} and PYY_{3-36} was amplified by surgery, with the response being significantly higher in RYGB compared to SG.
Conclusions
In obesity, the deficient post-prandial secretion of PYY_{3-36} reduces the feedback inhibition of appetite after eating. Both RYGB versus SG are associated with increased post-prandial secretion of PYY_{1-36} and PYY_{3-36}, but this is less marked after the latter, which may account for long-term differences in efficacy and adverse effects between the two types of surgery.

PP5: Ben Jones

Using whole exome sequencing (WES) data to establish the effects of GLP1R coding variation on random glucose levels and GLP-1R function

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Genetic imputation has been useful in enlarging the number of testable variants identified by genotyping arrays in genome-wide association studies (GWAS). However, rare variants (minor allele frequency, MAF <0.1%) are less reliably detected by imputation and may require resource-intensive sequencing instead. In this study, we compared the estimated effects on random glucose (RG) of missense single nucleotide polymorphisms (SNPs) in the glucagon-like peptide-1 receptor *(GLP1R)* gene, determined using imputed/array genotype or whole exome sequencing (WES) data from UK Biobank participants. We then performed functional and structural studies to explain these effects and identify how patients with type 2 diabetes might respond differently to different GLP-1R agonist drugs.
We used the latest WES data release from the UK Biobank to test for association between *GLP1R* coding variants and RG in 165,818 unrelated European individuals. Additionally, we run a GWAS of RG in the UK Biobank (401,810 individuals) using the Haplotype Reference Consortium (HRC) imputed panel. For the selected *GLP1R* variants we experimentally assessed G protein coupling in response to FDA-approved GLP-1R agonists and performed molecular dynamics simulations to explain observed effects.
853 *GLP1R* coding variants were detected using WES data while 18 variants were detected in the GWAS data. RG effects estimated in the larger imputed data and the WES data were directionally consistent with higher effect size similarity/consistency at commoner variants (rs10305492; protein coding consequence A316T, MAF = 1.5%, $BETA_{WES}$ = -0.011, $BETA_{Genotyped}$ = -0.011). The RG-lowering A316T variant showed increased coupling to stimulatory G proteins, which was explained by a structural alteration in the nearby hydrogen bond network. This observed gain-of-function applied more to certain GLP-1R agonists (e.g., tirzepatide) than to others (e.g., exenatide).
Sensitivity analysis using WES data confirmed imputed data can still provide useful insights into variant effects in complex phenotypes such as RG. Functional and structural characterisation of RG-associated *GLP1R* coding variants provides a possible framework for T2D treatment stratification.

PP6: Carolina Lobato

Protocol of HypoBar I: Delaying intestinal glucose absorption to ameliorate post-bariatric hypoglycaemia. A randomized cross-over clinical trial.

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Roux-en-Y gastric bypass (RYGB) is one of the most effective and popular surgical treatments for severe obesity and related diseases. Post-bariatric hypoglycaemia (PBH) is a rare yet disabling clinical condition, mostly reported after RYGB. PBH aetiology remains unclear, and PBH treatment options are empirical, limited in effectiveness and/or with considerable side-effects. Acarbose inhibits carbohydrate digestion and thus its absorption. It is the most popular pharmacological treatment for PBH but its gastrointestinal side effects limit patient compliance. Canagliflozin, a commercially available drug approved for the treatment of type 2 diabetes, inhibits sodium-dependent glucose absorption in the gut and kidney, and may provide stable blood glucose levels in PBH, by altering incretin hormones postprandial profile and reducing glycaemic excursions.

The study aims at investigating how blood glucose levels in the outpatient setting and after a meal test are affected by the chronic treatment of PBH with canagliflozin and to study entero-endocrine mechanisms implied in the observed responses.
In a double-blinded, randomized, cross-over clinical trial, we will investigate the effectiveness, safety and entero-endocrine mechanisms of treatment of PBH with canagliflozin 300mg twice daily during a one-month intervention, compared with acarbose 50mg thrice daily and placebo.
If effective and safe, canagliflozin could prove a novel and well-tolerated treatment option for patients with PBH, increasing our understanding of this disabling condition and improving its standard of care.
Registry: EudraCT number 2022-000157-87. Funding: Hvidovre Hospital's Strategic Research Foundation has supported the project with DKK 350,000. This work is supported by a research grant from the Danish Diabetes Academy (grant-ID PhD013-20), which is funded by the Novo Nordisk Foundation, grant nr. NNF17SA0031406; and by a grant from the "la Caixa" Foundation (ID 100010434, code LCF/BQ/EU21/11890081).

PP7: Charlotte Moffett

GLP-1 receptor agonist regulates enteroendocrine adaptation and female reproductive function in diet-induced obese mice.

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Background/Aims:
GLP-1R is expressed in components of the reproductive system. Most hormones regulating feeding behaviour within the central nervous system also contribute to the actions of gonadal function. Therefore, to elucidate possible modulation by GLP-1 receptors, the present study has evaluated the effects exendin-4 on enteroendocrine adaptation and reproductive function in high-fat fed mice.

Methods:
Female TO-mice (4-weeks-old) were fed high-fat-diet (HFD) for 12-weeks and appropriate normal-chow diet (ND) controls. Mice were then administered twice daily i.p injections for 21-days of saline or exendin-4 (25 nmol/kg body weight). Estrous cycling and reproductive hormones were measured. Excised pancreas and intestinal ileum were used for quantitative immunohistochemical analysis.

Results:
After 12-weeks of HFD, female TO-mice exhibited significantly ($p<0.05$) increased body weight (ND=33.67±1.402;HF=45.00±1.138) with hyperinsulinaemia, indicative of insulin resistance. Exendin-4 administration in HFD group reduced ($p<0.05$) food intake by day 14 compared to ND controls. 70% of HFD fed mice had irregular cycle lengths compared to 40% in the normal diet group ($p<0.001$). Percentage time spent in time in metestrus was significantly ($p<0.01$) higher by exendin-4 compared to HFD controls. Exendin-4 administration significantly ($p<0.05$) decreased time in diestrus stage. Circulating levels of FSH were significantly ($p<0.05$) 3-times higher in HFD group administered with exendin-4 compared to HFD controls. Immunochemical analysis of intestine revealed exendin-4 administration significantly ($p<0.05$) reduced PYY-cell population in ileal villi by 24% compared to HFD controls. Exendin-4 also significantly ($p<0.01$) increased ileal GLP-1 content by 86%. Interestingly, exendin-4 treatment in HFD group increased ($p<0.01$) islet co-expression of PYY in alpha-cells by 52% suggesting switching of proglucagon-gene product expression to PYY.

Conclusions:
Exendin-4 promotes enteroendocrine adaptation, boosts circulating levels of FSH and decreases time that HFD mice spend in diestrus which is a marker for disordered fertility and PCOS. These observations suggest that GLP-1R modulation could represent a novel non-invasive means for treating energy-related reproductive disorders and preserving female fertility.

PP8: Christopher Bannon

Exploring PYY Levels in Patients with Bile Acid Diarrhoea in Relation to Bile Acid Metabolism

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Introduction
Bile acids can stimulate GLP1 and PYY release from L cells, via basolateral TGR5 receptors. L cells in the distal gut are more likely to contain PYY, and proximal L cells GLP1. Bile acid diarrhoea (BAD) is a condition caused by excess bile acids in the colon, due to idiopathic overproduction of bile acids (primary) or terminal ileum loss (secondary). We explored the levels of GLP1 and PYY within these patient groups.
Methods
Serum samples from a previous study (Walters et Al., 2015) were measured for total GLP1 and PYY using immunoassays. Samples from patients with primary (n=9) and secondary BAD (n=10) had a meal study performed after 2 weeks of stopping bile acid sequestrants alongside control patients with irritable bowel syndrome with diarrhoea (IBS-D) in which BAD had been excluded (n=8). GLP-1 and PYY levels were measured at fasting, 1 hour and 3 hour post a standard mixed meal and were compared to previously measured fasting C4 (7a-OH-4-cholesten-3-one) levels, a marker of bile acid synthesis, and serum bile acids (BA).
Results
PYY levels differed between patient groups, with secondary BAD having higher PYY levels than IBS-D patients. Regression analysis showed PYY to be positively correlated with fasting C4 and serum BA levels and negatively correlated with BMI. GLP1 levels did not vary between patient groups, and were not correlated with fasting C4 or serum BA levels.
Discussion
PYY levels, but not GLP1 levels were shown to be predicted by bile acid synthesis and serum bile acid levels. Further exploration of the metabolic sequelae of bile acid diarrhoea is required, alongside the effects of excess colonic bile acids on gut hormone metabolism.
Reference:
Samples obtained from: Walters, J.R.F., Johnston, I.M., Nolan, J.D., Vassie, C., Pruzanski, M.E. and Shapiro, D.A. (2015), The response of patients with bile acid diarrhoea to the farnesoid X receptor agonist obeticholic acid. Aliment Pharmacol Ther, 41: 54-64. https://doi.org/10.1111/apt.12999

PP9: Darren Robertson

Dual Glucagon Like Peptide-1 and Glucagon Receptor Agonism with Cotadutide Promotes Differential Beneficial Effects in the Liver

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Introduction: Cotadutide is a dual GLP1-glucagon receptor agonist in development for NASH and CKD with T2DM.

Methods: In this Ph2a study we evaluated the effect of cotadutide on liver glycogen and fat. Obese participants with T2DM received either cotadutide 300 µg (n = 9), liraglutide 1.8 mg (n=10) or placebo (n = 11) for 35 days and underwent MRS and MRI-PDFF scans to measure liver glycogen and fat.

Results: There was a significant reduction from baseline in fasting hepatic glycogen levels versus placebo with cotadutide (-83.18 mmol/L [27.0%], 90% CI, -111.76, -54.60 p=0.004). A significant reduction in fasting hepatic glycogen was observed versus liraglutide (-63.44 mmol/L [-27.3%], 90% CI -102.30, -24.59, p =0.012) and there was a significantly greater relative reduction in hepatic fat of -11.72% vs liraglutide 90% CI -20.13, -3.30, p=0.030 despite comparable body weight loss. In addition, significant differences in post-prandial glucose, amino acids, triglycerides and indices of liver health were observed between cotadutide and liraglutide.

Conclusions: The results suggest that cotadutide promotes glycogenolysis and achieves target engagement of the glucagon receptor in the liver. Combining glucagon with GLP-1 receptor agonism promotes superior off-loading of excess energy substrates in the liver than GLP1 receptor agonism alone and underscores the potential of cotadutide as a differentiated and beneficial therapy for NASH.

PP10: Dawood Khan

Hunger hormone ghrelin regulates female reproductive health in normal and diet-induced obese mice.

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Background/Aims:
Expression of ghrelin receptor in reproductive organs in rodents and humans suggests possible role in the reproductive health. Therefore, the present study has evaluated the effects ghrelin on reproductive function and altered reproductive hormone status in healthy and high-fat-fed mice.
Methods:
Effects of ghrelin on metabolic parameters and estrous cycling were evaluated in 8-10 weeks old female TO-mice (n=9) administered twice daily i.p injections for 16-days of saline or ghrelin (25 nmol/kg body weight). In a second series, female TO-mice (4-weeks-old) were fed with high-fat-diet (HFD) for 12-weeks. Mice were administered twice daily i.p injections for 21-days of saline or ghrelin (25 nmol/kg body weight). Estrous cycling and reproductive hormones were measured.
Results:
Treatment for 16-days with ghrelin significantly ($p<0.05$-$p<0.01$) reduced food intake by 18% and body weight by 7%. Percentage of the total time spent in the various stages of estrous cycle showed reduced ($p<0.05$) time in metestrus (10±2%) and proestrus (10±2%) stages with ghrelin treatment compared to 20±3% and 18±2% for controls. After 12-weeks of HFD, female TO-mice exhibited significantly ($p<0.001$) 1.5-fold increased body weight with hyperinsulinaemia, indicative of insulin resistance. 70% of HFD-mice had irregular cycle lengths compared to 40% in the normal diet control group ($p<0.001$). Ghrelin administration in HFD-fed mice reduced ($p<0.05$) food intake by 18% compared to saline controls. Percentage time spent in time in metestrus was significantly ($p<0.05$) increased by 9% by ghrelin compared to HFD-controls. Circulating levels of LH were significantly ($p<0.05$-$p<0.01$) 3-times higher in HFD group irrespective of ghrelin administration and progesterone levels in plasma were decreased ($p<0.01$) by 5-times compared to normal-diet controls.
Conclusions:
These data highlight the importance of ghrelin on gut-reproductive axis in both health and high fat diet-induced obesity. Taken together, these data suggest that modulation of satiety regulating hormone in reproductive axis could represent novel therapies for reproductive dysfunction associated with metabolic disturbances.

PP11: Elisabeth O'Flaherty Rottenberger

Exploring non-SGLT1 mediated mechanisms of glucose stimulated GLP-1 release in vivo

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SGLT1 facilitates postprandial intestinal glucose absorption and mediates *ex vivo* glucose stimulated GLP-1 and GIP secretion from L and K type EECs respectively. *In vivo*, SGLT1 inhibition or KO, improves postprandial glucose tolerance and leads to sustained GLP-1, not GIP secretion. We investigated non-SGLT1 mechanisms of glucose stimulated GLP-1 secretion.
SGLT1-WT and -KO ileal organoids were used to study *in vitro* GLP-1 responses to 10mM D-glucose or L-glucose (an enantiomer not transported by SGLT1). For *in vivo* incretin investigations, two cohorts of mice were used: Cohort 1: SGLT1-WT and -KO mice; Cohort 2: SGLT1-WT mice pretreated with antibiotics. Mice were fasted overnight and cohort 2 gavaged with an SGLT1 inhibitor LX4211 (60mg/kg) or vehicle at -30mins. Both cohorts were gavaged with D-glucose or L-glucose (5g/kg) and tail vein sampled at minutes 0,5,60 for tGLP-1/tGIP and 0,5,15,30,60 for glucose analysis.
In vitro, SGLT1-WT organoids secreted GLP-1 in response to D-glucose ($p<0.05$), not L-glucose. No GLP-1 response was seen in SGLT1-KO organoids. SGLT1-WT mice increased plasma tGIP in response to D-glucose from 5mins ($p<0.01$), -KO mice did not. SGLT1-WT mice increased plasma tGLP-1 at 5mins in response to D-glucose ($p<0.01$), returning to baseline at 60mins. SGLT1-KO mice dosed with D-glucose increased plasma tGLP-1 up to 60mins ($p<0.01$). L-glucose elevated 60mins plasma tGLP-1 in SGLT1-WT and -KO mice ($p<0.01$), but not tGIP. Antibiotic treated mice dosed with D-glucose had normal tGIP secretion profiles but lacked the prolonged plasma tGLP-1 elevation observed in control mice after LX4211. L-glucose stimulated GLP-1 secretion up to 60mins was unaffected by microbiota depletion in these mice ($p<0.01$).
SGLT1 is needed *in vitro,* not *in vivo,* for D-glucose stimulated GLP-1 secretion. Our results suggest that non-SGLT1 mediated sustained increased plasma GLP-1 in SGLT1-KO mice may be via fermentation of D-glucose by resident microbiota to GLP-1 secretagogues, warranting further investigation.

PP12: Emily Miedzybrodzka

Regulation of 5-HT secretion from human duodenal enterochromaffin cells

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The majority of the body's 5-HT (serotonin) is produced from enterochromaffin cells (ECs) of the intestinal epithelium. 5-HT has important roles within the gastrointestinal tract in the modulation of motility, secretion and inflammation, while also signalling satiety and discomfort to the central nervous system. The factors regulating release of 5-HT from human small intestinal ECs have not been clearly elucidated: although circulating 5-HT levels typically increase after a meal and EC-derived 5-HT is a critical postprandial satiety signal, the importance of direct nutrient stimulation of ECs versus paracrine regulation by other gut hormones – including GLP-1 –remains debated.
To investigate the mechanisms of 5-HT release from ECs, organoids established from human duodenum, ileum and rectum were CRISPR-Cas9-modified to fluorescently label tryptophan hydroxylase 1 (TPH1) expressing ECs. Bulk RNA sequencing was performed on human duodenal ECs purified by fluorescence-activated cell sorting and this dataset was considered in parallel with single cell RNA sequencing of human small intestinal enteroendocrine cells, isolated based on chromogranin A expression.
The long-chain fatty acid receptor *FFAR1* was notably absent in duodenal ECs but most other nutrient-sensing receptors were significantly enriched, including the amino acid responsive *GPR142*, the short-chain fatty acid

receptor *FFAR2*, the monoacylglycerol receptor *GPR119* and the bile acid receptor *GPBAR1*. Receptors for several gut hormones (including GIP, insulin-like 5 and somatostatin) were also significantly enriched in duodenal ECs, with *GLP1R* detectable at lower levels. Consistent with transcriptomic data, single cell calcium and cyclic AMP imaging suggests a stimulatory role for several short-chain fatty acids, the aromatic amino acid tryptophan and adrenergic agonists in duodenal EC subpopulations.
Ongoing work aims to measure 5-HT secretion from human duodenal organoids in response to these potential regulators of EC function. Collectively this data will provide novel insights into the physiological control of 5-HT release from human small intestine.

PP13: Francois Briand

Semaglutide improves cardiometabolic parameters in diet-induced obese NASH hamsters

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Background/Aim - Cardiovascular disease is the leading cause of deaths in nonalcoholic steatohepatitis (NASH) patients. Mouse models, while widely used for drug development, do not fully replicate human NASH nor integrate the associated cardiac dysfunction, i.e. heart failure with preserved ejection fraction (HFpEF). To overcome these limitations, we established a nutritional hamster model developing obesity, NASH and HFpEF. Here we evaluated the effects of the GLP-1 receptor agonist semaglutide (SEMA).
Methods –Hamsters were fed with a free choice diet, which presents hamsters with a choice between control chow (CC) or high fat/cholesterol (HFC) diet, and normal water (NW) or 10% fructose water (FW). After 20 weeks of diet, obese hamsters were treated s.c. QD for 5 weeks with vehicle or SEMA.
Results –Compared with vehicle, SEMA induced a lower HFC/FW and higher CC/NW intake, leading to a 17% body weight loss ($p<0.01$) and a 48% lower visceral fat mass ($p<0.001$). SEMA significantly reduced fasting glycemia, hyperinsulinemia and HOMA-IR index (-77%, $p<0.0001$). SEMA decreased plasma total cholesterol levels (-24%, $p<0.001$) and hypertriglyceridemia (-50%, $p<0.001$). Although SEMA did not improve NAFLD activity scoring and fibrosis score significantly, significant improvement in liver steatosis was observed with lower liver weight (-28%, $p<0.0001$ vs. vehicle) and liver triglycerides levels (-25%, $p<0.01$). SEMA showed substantial benefits on HFpEF with significantly improved E/A, E'/A' and E/E' ratios measured by echocardiography.
Conclusion –SEMA improves cardiometabolic parameters in the obese hamster. This preclinical model will be useful for validating novel drugs or combination therapies for the treatment of NASH and associated HFpEF.

PP14: Florian Merkle

Rational drug targeting of obesity-associated genes in human POMC neurons

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Melanocortin neurons located in the hypothalamus, including those expressing pro-opiomelanocortin (POMC), have been identified as critical regulators of energy homeostasis. However, it has been difficult to connect which genes associated with obesity might exert their functions via POMC neurons. To address this question we generated human hypothalamic POMC neurons derived from human Pluripotent Stem Cells (hPSCs), analyzed them by single-cell RNA sequencing, and integrated those data with genetic association results from large-scale population studies of adult and childhood obesity to identify druggable obesity-associated genes that are enriched in human POMC neurons. This analysis revealed a number of obesity-associated genes, including the glucagon-like peptide (GLP-1) receptor whose activation in POMC neurons by has been shown to decrease food intake in

mice. We are now using a combination of high-content automated confocal imaging and calcium imaging to study the responses of human POMC neurons to GLP-1 receptor agonists and other candidate compounds. Identifying targets that increase the activity of POMC neurons alone or in combination may provide a foundation for the development of efficacious anti-obesity drugs.

PP15: Giuseppina Biondi

Incretin-based drugs counteract the deleterious effects of pasireotide on pancreatic beta-cell survival and function

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Pasireotide is a new generation somatostatin analogue used for the treatment of acromegaly, Cushing disease and neuroendocrine tumors. Due to its high affinity for somatostatin receptors 5 and 2 (SSTR5/2), which are highly expressed in pancreatic beta-cells and enteroendocrine cells, pasireotide may reduce the secretion of insulin, GLP-1 and GIP, causing hyperglycemia in some patients. It has been already demonstrated that the diabetogenic effects of pasireotide are reduced in vivo by co-administration of liraglutide or vildagliptin. We explored the mechanisms by which pasireotide interferes with beta-cell survival and function, and the ability of incretin-based drugs to counteract these effects.
INS-1E cells and human pancreatic islets were stimulated with pasireotide (1- 1000 nmol/l, 2 or 24 h) or DMSO as control; in another set of experiments, INS-1E cells were pretreated with an incretin-based drug (10 nmol/l exendin-4, lixisenatide, liraglutide or 100 nmol/l saxagliptin) for 24 h, prior to stimulation with pasireotide. Glucose-induced insulin secretion (GSIS), insulin content, and apoptosis levels were assessed through specific ELISA assays, while signaling proteins and calcium levels were evaluated by immunoblotting and fluorimetric assay, respectively.
10 nmol/l pasireotide induced apoptosis and reduced GSIS, without altering the insulin content in INS-1E cells and human islets. Incretin-based drugs were able to increase the phosphorylation of AKT, which was reduced by pasireotide, and prevent pasireotide-induced apoptosis in INS-1E cells. Furthermore, exendin-4 and liraglutide prevented the alteration of the PKA and intracellular calcium pathways and restore the secretory dysfunction induced by pasireotide.
In conclusion, pasireotide can induce hyperglycemia and diabetes through the reduction of both beta-cell survival and function. Incretin-based drugs can prevent these effects by restoring the AKT, PKA and intracellular calcium pathways.
Supported by Novartis

PP16: Hamza Olleik

Endoc-βH5 human beta cells: a unique thaw and go model for accelerating incretin discovery and development

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In 2021, 537 million adults were living with diabetes worldwide (90% T2D), a number that is predicted to rise to 643 million by 2030. The need for physiologically relevant human cellular models to study human beta cell function, insulin secretion and diabetes is thus greater than ever. To date, human pancreatic islets present many

drawbacks related to lack of availability and reproducibility that limit their use. While animal models remain far from human reality. Different assays were used for the validation of Endoc-βH5. For example, microscopy and FACS analysis were performed to examine the morphology of the cells and detect the beta cell identity markers. Furthermore, GSIS and incretin pharmacology analysis were performed in 96 well plates to study the functional capacity of Endoc-βH5. Also siRNA, using lipofectamine, was used to knock down genes of interest for disease modeling. Specifically, Endoc-βH5 is a pure population of insulin expressing cells (100% INS/92.3% PDX1/90% NKX6.1). Insulin content is close to that of native pancreatic beta cells (> 5µg/million cells). Endoc-βH5 cells dose dependently respond to physiological concentrations of glucose (highest potentiation of insulin secretion between 2.8 and 8 mM glucose) with elevated absolute insulin secretion values (hundreds of ng/hr/10^6 cells) similar to native islets. Furthermore, they dose dependently respond to GLP1R agonists, GIP agonist D-ALA-2-GIP, and dual agonist tirzepatide with reproducible EC_{50} and increased insulin secretion >3-fold over high glucose. In addition, Endoc-βH5 cells can be assayed in 384-well plates paving the way for HTS. Finally, expression of diabetes related genes can be modified to produce relevant disease models. Overall, Endoc-βH5 represent a novel human pancreatic beta cell solution with very high potential for developing human diabetes models, unraveling diabetes mechanisms in human cells and incretin discovery and development.

PP17: Ida Modvig

Characterization of L-valine induced GLP-1 secretion from the perfused rat small intestine

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Previously, we have described that L-valine is distinctively the most powerful luminal stimulator of GLP-1 release by the upper part of the rat small intestine, making L-valine a target for nutritional based modulation of GLP-1 secretion. However, the molecular mechanism of L-valine-induced secretion remains unknown. Therefore, in this study we aim at identifying the molecular details of L-valine stimulated GLP-1 release using the isolated perfused rat small intestine.
Luminal L-valine (50 mM) powerfully stimulated GLP-1 release from the perfused rat small intestine ($p<0.0001$, $n=8$). Inhibition of voltage-gated Ca^{2+}-channels with nifedipine (10 uM) blocked the GLP-1 response to luminal infused L-valine ($p<0.001$, $n=7$). In line with this, depletion of luminal Na+ decreased L-valine-induced GLP-1 secretion ($p<0.05$, $n=6$), however, the GLP-1 secretion was not blocked, suggesting that co-transport of L-valine and Na+ is not crucial for the depolarization necessary to activate the voltage-gated Ca^{2+}-channels. As an attempt to block the uptake of L-valine, the Peptide Transporter 1 inhibitor, 4-aminomethyl benzoic acid (4-AMBA; 20 mM), was used, as 4-AMBA was previously demonstrated to block the absorption of a solution of mixed amino acids (Vamin®). 4-AMBA completely blocked L-valine induced GLP-1 secretion ($p<0.01$, $n=6$), however, interestingly the amino acid absorption was only slightly reduced, indicating that 4-AMBA must block essential transporters for L-valine-stimulated GLP-1 secretion, whereas transporters not involved in GLP-1 stimulation remain unblocked.
This study demonstrated that v-gated Ca^{2+}-channels are involved L-valine induced GLP-1 secretion, and that Na+-coupled uptake of L-valine and subsequent cellular depolarization may be partly involved, although it cannot be the only mechanism by which L-valine stimulates GLP-1 secretion. The downstream pathways leading to the opening of voltage-gated Ca^{2+}- channels remain to be fully established.

PP18: Joachim Holt

Impaired meal-induced neurotensin response is associated with weight regain after bodyweight loss

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Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen[1], Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen Dr Winning Lehmann has been employed by Novo Nordisk since January 2022. . She has no conflicts of interest during preparation of this abstract[2], Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen and NNF Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen[3], Department of Endocrinology, Hvidovre University Hospital, Copenhagen[4]

Background: Diet-induced weight loss is often followed by weight regain. The gut derived hormone neurotensin (NT) is upregulated after bariatric surgery and may contribute to weight loss. We investigated whether diet-induced weight loss impacts on fasting and meal-induced levels of circulating NT and analyzed association with early postprandial NT response and weight regain after weight loss.
Methods: Study 1: Plasma samples from 42 people with obesity completing an 8-week low-calorie diet in a randomized controlled trial were analyzed. Total NT was measured in plasma samples during fasting and during a meal test before and after diet-induced weight loss and after one year of intended weight maintenance. Study 2: Obese mice were fed ad-libitum or a restricted diet to obtain similar weight loss as observed in the human study. At termination, plasma and intestinal segments were collected for analysis.
Results: The participants lost 13% body weight ($p<0.0001$), which was associated with 40 % reduction in fasting plasma NT levels ($p<0.001$). NT concentration increased more 30 minutes postprandially in participants who lost additional weight compared to the participants who regained weight during the 1-year weight maintenance period ($p<0.05$). As in the human study, food restriction in obese mice, which reduced body weight by 14% ($p<0.0001$) was associated with reduction in fasting plasma NT by 64% ($p<0.0001$), without causing enteroendocrine cell atrophy.
Conclusion: Diet-induced weight loss decreased fasting plasma NT levels in both humans and mice with obesity. Early meal-induced NT response was impaired in participants who regained weight compared to participants who lost additional weight after one year. Our results indicate that early meal induced increase in levels of NT after weight loss may contribute to maintain body weight after diet-induced weight loss.

PP19: Julia Kenkre

PYY3-36 and PYY1-36 levels in Good and Poor Responders to Bariatric Surgery

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Background: Bariatric surgery is a very efficacious treatment for sustainable weight loss and diabetes remission. However, some patients achieve suboptimal weight loss or fail to maintain the initial weight loss. Gut hormones have been implicated in the response to bariatric surgery both in terms of weight loss and diabetes remission. PYY3-36 has been known for its anorectic effects and increases markedly post-bariatric surgery. The role of PYY1-36 is less clear in humans. It has been reported that PYY levels differ between good and poor responders post-Roux-en-Y Gastric Bypass surgery (RYGB). The aim of our study was to investigate whether there are any differences in PYY secretion post-operatively between good and poor responders to Sleeve Gastrectomy (SG).
Methods: Six good responders and eight poor responders as defined by % weight loss of more or less than 20% respectively were studied more than 2 years post-Sleeve Gastrectomy with a Mixed Meal Tolerance Test

(MMT). Glucose, Insulin and C-peptide levels were measured at fasting state and during the MMT. PYY3-36 and PYY1-36 were also measured using LC-MS/MS.
Results: Good responders differed significantly in terms of weight compared to the poor responders (p=0.0207). There was no significant difference in fasting or post-prandial levels of glucose, insulin or c-peptide between the two groups. PYY3-36 and PYY 1-36 secretion did not differ between good and poor responders.
Conclusions: Differences in PYY3-36 and PYY1-36 secretion do not seem to explain the difference in outcomes between good and poor responders post-SG, unlike after RYGB.

*L Cardillo and J Kenkre contributed equally to this work

PP20: Kleopatra Alexiadou

The effect of PYY (3-36) on metabolic and bone turnover markers in obese patient with type 2 diabetes

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Background: Gut hormones have emerged as new promising therapies for the treatment of obesity and type 2 diabetes and viable alternatives to bariatric surgery. PYY has mainly been studied for its effect on appetite suppression and lately for its role in diabetes remission post-bariatric surgery. Animal data suggest that PYY may act on the bone remodelling process by increasing bone resorption. The aim of our study was to assess the effect of chronic PYY infusion on weight, glycaemia and bone turnover markers in obese patients with type 2 diabetes.
Methods: Ten obese patients with type 2 diabetes on diet or one hypoglycaemic agent were enrolled in the study. An infusion of PYY (3-36) was administered daily for up to 16 hours for a duration of 4 weeks. Metabolic profiling at fasting state and during a Mixed Meal Test (MMT) was performed prior to the initiation of the infusion and at the end of 4 weeks. Bone markers (plasma P1NP, CTX, osteocalcin) were measured at the beginning and the end of the 4 weeks both at fasting state and during the MMT. Urinary NTX was measured at fasting state only.
Results: After 28 days of daily infusion with $PYY_{3-36,}$ participants lost a mean of 1.73 kg in weight which was associated with a significant reduction in food intake (p=0.02). There was a decrease in iAUC for glucose during MMT (p=0.0255). There was no significant difference in bone turnover markers before and after the intervention.
Conclusions: Subcutaneous administration of PYY_{3-36} for 28 days resulted in significant reduction in food intake and favourable glycaemic profiles with no evidence of tachyphylaxis or adverse effects on bone turnover markers.

PP21: Liva Krogh

Extrapancreatic effects of the incretin hormones

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The peptide hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are secreted from specific cells in the gut in response to food intake. Both hormones potentiate glucose-induced insulin release from pancreatic beta cells, helping regulate postprandial glucose excursions, and both

hormones have several other physiological effects –also outside the pancreas, for example in bone and adipose tissue, the gastrointestinal tract and the brain but GIP receptors (GIPRs) and GLP-1 receptors GLP-1Rs) are found in other tissues as well.
The insulinotropic effects of GIP and GLP-1 have led to investigations regarding their potential anti-diabetic effects and several anti-diabetic drugs have been developed based on GLP-1. GIP does not seem to reduce plasma glucose levels in patients with type 2 diabetes but, recently, a drug called tirzepatide activating both the GLP-1R *and* the GIPR has shown promising anti-diabetic effects in these patients –even more than the potent GLP-1 analogue semaglutide.
The mechanisms behind the superior effects of this novel GIPR-GLP-1R co-agonist are unknown. As insulin sensitivity, and possibly other extrapancreatic targets of tirzepatide may contribute to its effects, we aim to better understand separate and combined extrapancreatic effects of GIP and GLP-1. In pancreatectomised persons (persons without a pancreas), we will intravenously infuse the selective GIPR and GLP-1R antagonists GIP(3-30)NH_2 and exendin(9-39)NH_2, separately and combined, during a liquid mixed meal test. By frequent blood sampling, we will be able to measure changes in markers of glucose, fat, and bone metabolism (and other extrapancreatic markers such as gastric emptying, appetite, and food intake) between the interventions.
The study is designed as an exploratory, randomised, double-blinded, placebo-controlled crossover study and we will recruit 12 pancreatectomised patients to participate in four experimental days each. The intervention is a 5 hour intravenous infusion of either the GIPR antagonist GIP(3-30)NH_2, the GLP-1R antagonist exendin(9-39)NH_2, GIP(3-30)NH_2 + exendin(9-39)NH_2 or saline (placebo), respectively, during which a 4 hour liquid mixed meal test and a subsequent *ad libitum* meal test is performed. During the experimental day, the participants will fill out standardised questionnaires (VASs) once per hour about their appetite, satiety, nausea, fatigue, displeasure, and thirst. Blood samples for analysis of abovementioned markers with be drawn every 15th minutes.
With a unique combination of GIPR and GLP-1R antagonist infusions in persons without insulin and glucagon secreting pancreatic cells, we will be able to contribute with important mechanistic knowledge about extrapancreatic incretin hormone actions with a view to the mode of action of a novel anti-diabetes drug class.

PP22: Mads Helsted

Safety of native glucose-dependent insulinotropic polypeptide (GIP) in humans

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BACKGROUND AND AIM: Glucose-dependent insulinotropic polypeptide (GIP)(1-42) has been administered to humans with and without metabolic diseases in numerous clinical studies investigating physiology and pathophysiology, but the safety and possible adverse effects (AE) of GIP in humans have, to our knowledge, never been systematically reviewed.
MATERIALS AND METHODS: The PubMed database was searched using the MESH term "gastric inhibitory polypeptide" and the search terms "gastric inhibitory polypeptide", "glucose-dependent insulinotropic polypeptide", "glucose dependent insulinotropic polypeptide", and "GIP". All clinical studies administering synthetic human GIP(1-42) to humans were included and reports were scrutinised for AEs and other safety measures.
RESULTS: Of the included studies (65), most were acute (hours). Only 1 study was longer (6-day infusion). Of the 65 studies, 80% did not mention AEs, 6.2% of the studies reported that no AEs were observed, and 13.8% of the studies reported AEs in relation to GIP infusion. The most frequently reported AE was an increase in heart rate. Mild gastrointestinal AEs, hypoglycaemia, and decrease in blood pressure were also reported. Infusion rates ranged from 0.8 to 16 pmol/kg/min. A positive correlation between infusion rate and maximum concentration of intact GIP(1-42) was found and seemed independent of participant type (healthy individuals, patients with

impaired glucose tolerance, and patients with type 2 diabetes). There was no correlation between achieved maximum concentration of GIP(1-42) and reported AEs.
CONCLUSIONS: In 20% (13) of the included studies, we found data on the safety of exogenous GIP(1-42) in humans mainly from acute studies and a lack of longer-term studies. The available data suggest that the safety profile of exogenous GIP(1-42) (both physiological and supraphysiological infusion rates) when used for short-term infusion studies is benign. The long-term safety of exogenous GIP(1-42) is unknown.

PP23: Malory Couchot

Development of dual GIP/GLP-2 analogues for the treatment of bone fragility

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Aside from the gut microbiome, evidence has been provided that several intestinal peptides were important for controlling bone strength. Among all gut hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-2 (GLP-2) exhibited potential effects in increasing bone material properties and strength in preclinical models. Recent studies suggest that GIP and GLP-2 exert separate effects on bone turnover and that combined administration may represent a viable alternative to treat bone fragility. The aim of the present study was to develop a series of dual GIP/GLP-2 analogues and to validate their action and potency in vitro and in vivo.
An in-silico program has been developed and led to the generation of several dual GIP/GLP-2 analogues. In vitro validation has been conducted to assess receptor binding, reduction of osteoclast formation and enhancement of bone material properties. Two best candidates, GL-0001 and GL-0007, were further tested in a mice model of ovariectomy-induced bone loss and compared with 100µg/kg zoledronic acid. Three-point bending, microCT and Fourier-transform infrared microspectroscopy were performed to evaluate bone strength, microarchitecture and bone material properties. Analyses of variance have been performed and considered significant at $p<0.05$.
In vitro, dual GIP/GLP-2 analogues demonstrated different potencies in binding to hGIPr or hGLP-2r, reducing osteoclast formation and increasing collagen maturity. GL-0001 but not GL-0007 increased bone strength in OVX mice by 37% ($p=0.003$) and more importantly, the magnitude of effect was 27% higher than with zoledronic acid. Interestingly, GL-0001 improved bone strength by enhancing significantly bone material properties and collagen maturity and limiting bone loss following ovariectomy.
This study highlights the potential of dual GIP/GLP-2 analogues for the treatment of bone fragility. Further studies are required to assess the potency of these molecules in humans.

PP24: Maria Ebbesen

Glucagon-like peptide-1 is associated with systemic inflammation in pediatric patients treated with hematopoietic stem cell transplantation

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Background: Patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) are challenged with severe side effects, propagated by mucosal barrier disruption, and the related microbial translocation and systemic inflammation. Glucagon-like peptide-1 (GLP-1) possesses anti-inflammatory properties and promotes regeneration of damaged intestinal epithelium in animal studies.
Objective: We hypothesized that the immense inter-individual variation in the degree of mucosal damage and systemic inflammation, seen after HSCT is influenced by endogenous GLP-1 and could be related to acute post-transplant complications.
Study Design: In this prospective study we measured serial fasting plasma GLP-1, C-reactive protein (CRP), and citrulline in 82 pediatric patients during HSCT together with a fasting plasma GLP-1 in healthy controls.
Results: Overall, GLP-1 levels were increased in the patients during the course of HSCT compared with the controls, but tended to decrease post-transplant, most pronounced in patients receiving high-intensity conditioning regimen.
The increase in CRP seen in the early post-transplant phase was lower day +8 to +13 in patients with GLP-1 above the upper quartile (>10 pmol/L) at day 0 ($P \leq 0.03$). Similar findings were seen for peak CRP levels after adjusting for type of conditioning (-47.0%; 95% CI, -8.1 –-69.4%, $P = 0.02$). Citrulline declined post-transplant illustrating a decrease in viable enterocytes. GLP-1 levels at day 0 associated with the recovery rate of citrulline from day 0 to +21 (34 percentage points (pp)/GLP-1 doubling; 95% CI, 10 –58pp; $P = 0.008$), also after adjustment for type of conditioning. This translated into a reduced risk of acute graft-versus-host disease (aGvHD) in patients with GLP-1 levels >10 pmol/L (cause-specific HR: 0.3; 95% CI, 0.2 –0.9, $P = 0.02$).
Conclusion: This study strongly suggests that GLP-1 influences regeneration of injured epithelial barriers and ameliorates inflammatory responses in the early post-transplant phase.

PP25: Mariana Monteiro

GLP-1 produces different shifts in visceral adipose tissue metabolic profile depending on status

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Visceral adipose tissue (VAT) metabolic fingerprints differ according to the individual's BMI and glycaemic status. Glucagon-like peptide 1 (GLP-1) acts through different mechanisms to improve not only the glycaemic status but also promote weight loss. Our purpose was to assess how GLP-1 influences VAT metabolic fingerprints and leptin secretion profiles according to the individual's glycaemic status.
Subjects (n=19) undergoing elective abdominal surgeries were included in this study. Subjects were allocated into 4 experimental groups according to BMI and glycaemic status, namely: obesity and euglycemia (Ob+NGT, n=5); obesity and pre-diabetes (Ob+Pre-T2D, n=5); obesity and T2D (Ob+T2D, n=5). Subjects without obesity or dysglycemia were used as controls (Non-Ob, n=4). VAT harvested during the surgical procedure was kept in culture media supplemented with insulin (100 nM) and exposed for 48 h to GLP-1 at different concentrations

(1, 10 or 100 nM). Metabolite analysis of collected cultured media was done by proton nuclear magnetic resonance and leptin was quantified by ELISA.
GLP-1 decreased leptin secretion on euglycemic VAT (-18.4%, n=7, $p<0.05$), producing no significant effects on dysglycemic VAT. GLP-1 induced changes in the metabolic profile of Ob+Pre-T2D VAT, increasing alanine (32.6%, $p<0.05$) and lactate production (43.8%, $p<0.01$), whilst decreasing valine consumption (-25.2%, $p<0.05$) and increasing pyroglutamate production (6.8%, $p< 0.05$). GLP-1 was also able to decrease acetate production in Non-Ob VAT (-25.2%, $p<0.05$), while no significant effect was observed on Ob+NGT and Ob+T2D VAT.
GLP-1 modified the metabolic profile of Ob+Pre-T2D VAT without influencing leptin secretion. The shifts induced by GLP-1 on VAT metabolite profile suggests a decrease in both gluconeogenesis and lipogenesis. Overall, out data suggests that GLP-1 could promote positive metabolic changes in VAT of individuals with pre-diabetes.
Funding: Fundação para a Ciência e Tecnologia (FCT), Portugal (PTDC/MECMET/32151/2017, UIDB/00215/2020 e UIDP/00215/2020). T.Morais is funded by FCT (SFRH/BD/123437/2016).

PP26: Mariwan Sayda

Differential levels of C-terminal amidation of bioactive peptides in tissue and organoids systems.

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Many bioactive gut hormones require post translational modifications (PTMs) to activate their cognate receptor. C-terminal amidation, a common form of PTM, is an end-product achieved by removal of a C-terminal glycine from a precursor peptide hormone by the enzyme peptidyl-glycine alpha-amidating monooxygenase (PAM). Several gut hormones are modified by PAM, including the incretin peptide GLP-I, which is converted from GLP-1 7-37 to GLP-1 7-36 amide during the L-cell vesicle maturation process. Levels of GLP-1 in plasma, tissue and organoid supernatants are routinely measured by immunoassay, using kits such as the MSD active GLP-I assay, which is highly specific for the 7-36 amide (cross reactivity with GLP-1 7-37 is 16%).
To study GLP-1 biosynthesis and secretion in humans, intestinal organoids have proved a robust model for generation new L-cells *in vitro*. A caveat of *in-vitro* models, however, is that they do not perfectly reproduce the characteristics of endogenous L-cells. The application of LC-MS techniques to study the peptidome of freshly collected human gut tissues showed that endogenous processing of proglucagon generates higher levels of GLP-1 7-36 amide compared with the unfinished GLP-1 7-37 form. However, peptidomics analysis of lysed intestinal organoid cultures revealed the opposite, with very low production of GLP-1 7-36 amide. Whist the LC-MS system is capable of monitoring both forms of GLP-1, immunoassays cannot simultaneously measure and distinguish them.
Our results suggest that care needs to be taken in the choice of GLP-1 immunoassay when working with intestinal organoids. The explanation for the apparent lack of PAM activity in human organoids remains unknown, and further tissue culture optimisation will be required to improve the activity of the PAM enzyme, which could also affect measurements of other C-terminally amidated peptides, such as PYY or PPY.

PP27: Marta Santos-Hernandez

Revisiting amino acid stimulated incretin secretion

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The incretin hormones glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released from intestinal L- and K-cells in response to drugs and nutrients. Although several studies have reported that amino acids, including phenylalanine (L-Phe) and tryptophan (L-Trp), stimulate incretin release, the roles of different transporters and G-protein coupled receptors (GPCR) involved in the sensing of amino acids remain incompletely elucidated.
The objective of this study is to identify the aromatic amino acid-sensing mechanisms underlying incretin release. For that purpose, aromatic amino acids were tested in vivo in wild-type and Slc6a19 knock-out mice and in mouse ileal organoids. To identify the GPCR involved in GLP-1 signaling, calcium sensing receptor (CASR) and GPR142 knock-out mouse ileal organoids were generated by CRISPR/Cas9 in a Cas9-GFP line.
Oral gavage of 0.5 g/kg L-Phe stimulated robust elevation of plasma GIP (~1.5-fold) and GLP-1 (~1.6-fold) in wild type mice at 5 min, but failed to elicit a response in mice lacking the brush border amino acid transporter Slc6a19. In contrast, conditional knock-out of Slc6a19 in either K- or L-cells did not affect L-Phe-stimulated hormone release in vivo. In vitro, L-Phe and L-Trp stimulated GLP-1 secretion by ~1.6-fold and 2.8-fold, respectively, and the secretagogue effect of the aromatic amino acids was reduced in presence of the CASR inhibitor Calhex231, suggesting its involvement in GLP-1 secretion. Whilst these results suggest that aromatic amino acids need to be transported across the epithelial brush boarder to reach CASR located at the basolateral surface of enteroendocrine cells, the questionable specificity of Calhex231 inspired us to validate the findings by knocking out CASR and GPR142 in organoids. At the time of abstract submission initial knock-out organoids have been established and we expect to present data clarifying the role of these two receptors in aromatic amino acid stimulated incretin release.

PP28: Matteo Fiorenza

Regulation of the adipokine-ceramide axis in response to liraglutide, exercise or both combined: Implications for cardiometabolic health in obesity

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Dyslipidemia plays a major role in the pathogenesis of obesity-related cardiometabolic disorders. Sphingolipids such as ceramides are amongst the most deleterious lipids for cardiovascular and metabolic health. Pre-clinical data indicate a strong link between a number of adipokines (i.e. leptin, adiponectin, FGF21) and ceramide metabolism, leading to the concept of an adipokine-ceramide axis governing cardiometabolic risk factors in obesity. Although weight loss appears to ameliorate the adipokine and ceramide profile, effective weight-loss maintenance may be key to preventing resurgence of obesity-related alterations within the adipokine-ceramide axis.
Herein, we conducted a randomized, parallel-group, placebo-controlled trial in adults with obesity. After an 8-week low-calorie diet-induced weight loss, participants were randomly assigned for 1 year to one of four weight-loss maintenance strategies: treatment with GLP-1 analogue liraglutide (n=35); an exercise program (n=35); GLP-1 analogue therapy plus exercise program (n=37); placebo plus usual activity (n=37).
By quantifying circulating levels of leptin, adiponectin, FGF21 and GDF15 in conjunction with targeted mass spectrometry-based analysis of plasma sphingolipids, we aim to characterize changes in the adipokine and ceramide profile in response to the different weight-loss maintenance strategies. Through comparative analyses of weight maintainers (WMs; <10% weight regain) and weight re-gainers (WRs; >50% weight regain), we seek to identify potential hormonal and/or sphingolipid biomarkers of weight re-gain susceptibility.
Preliminary analyses indicate that free plasma leptin decreased following the low-calorie diet and increased from randomization to the end of the treatment period, with the increment being attenuated by GLP-1 analogue therapy and exercise and almost ablated in WMs compared to WRs. By contrast, plasma levels of C16:0 and C18:0 ceramide increased following the low-calorie diet and decreased throughout the weight-loss maintenance phase independent of the treatment strategy, with no differences between WMs and WRs.

Ongoing analyses of adiponectin, FGF21, and GDF15 along with a panel of sphingolipid species will elucidate how the adipokine-ceramide axis is regulated during healthy weight-loss maintenance and how this relates to improvements in cardiometabolic health.

PP29: Michael Nauck

A novel experimental paradigm to compare effects of sitagliptin vs. placebo treatment on the incidence and symptoms of, and counter-regulatory response associated with hypoglycaemic episodes in type 2 diabetic patients treated with tightly titrated insulin glargine

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Background/aims. DPP-4 inhibitors like sitagliptin may have favorable effects when added to basal insulin treatment in the treatment of type 2 diabetes, e.g. a lower risk for hypoglycaemia. We aimed at testing for such effects with a novel experimental paradigm simulating slight insulin excess.
Patients/methods. 20 patients with type 2 diabetes previously treated with basal insulin, metformin, and additional oral glucose-lowering agents were recruited for a two-way crossover study comparing placebo or sitagliptin treatment in addition to insulin g*largine* and metformin. 17 completed both periods (3 women, 14 men; age 57 ±7 yrs.; body-mass-index 28.8 ±3.9 kg/m^2; HbA_{1c} 7.4 ±0.7 %). After tightly titrating insulin *glargine* on metformin plus the randomized treatment, patients were invited to a three-day inpatient observation period. Insulin *glargine* doses administered the night before days 1-3 were 100, 110, and 120 % of the dose resulting from the titration process. Hypoglycemic episodes were identified through the measurement of venous plasma glucose or by symptoms, and prompted the measurement of counter-regulatory hormones at the plasma glucose nadir and 30 and 60 min later.
Results. Hypoglycemic episodes (plasma glucose <70 mg/dl or <3.9 mmol/l) mainly occurred during the nocturnal period (10 p.m. to 8 a.m.). Their incidence was not significantly different (2.04 ±0.35 vs. 2.04 ±0.39 episodes/patient per 24 h with placebo and sitagliptin treatment, respectively, p >0.99). The incidence of symptomatic episodes during daytime (8 a.m. to 10 p.m.) was higher with sitagliptin (0.35 ±0.12 vs. 0.06 ±0.06, p = 0.034). Counter-regulatory responses were suppressed with sitagliptin treatment in the case of cortisol and glucagon, unchanged for growth hormone and prolactin, and somewhat (not significantly) accentuated for adrenaline and noradrenaline.
Conclusions. Our innovative protocol has reliably provoked hypoglycaemic episodes in type 2-diabetic patients by prompting a slight insulin excess. This novel experimental protocol may be helpful in mimicking the pathogenesis of hypoglycaemic episodes in clinical practice. Sitagliptin did not significantly change the risk for hypoglycaemic episodes.

PP30: Natalie Figueredo Burgos

Investigating the mechanism by which protein improves glucose tolerance

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Glucose homeostasis is regulated by interactions between peripheral organs and the central nervous system (CNS). Hormones released from the gut, together with absorbed nutrients, can act directly on the brain and

pancreas, and can influence the activity of sensory vagal afferent fibres which signal to glucoregulatory CNS regions to modulate glucose homeostasis. Protein is the macronutrient shown to most improve glucose tolerance when given as a preload, and sodium-glucose cotransporters (SGLT1 and SGLT2) seem to be implicated. The project aimed to investigate the mechanism underlying these effects. To achieve this, expression of the neuronal activation marker C-Fos was mapped in the brainstem and hypothalamus of mice orally administered whey protein with or without the SGLT1 and SGLT2 antagonist phloridzin. *In vivo* glucose tolerance tests were performed using an SGLT1 antagonist (KGA-2727) to determine its involvement. Oral administration of whey and intraperitoneal (I.P) administration of phloridzin (1g/kg) significantly increased neuronal activation in the nucleus tractus solitarius (Control: Median:26.5, IQR:16-36.25; Vs Phz + Whey: Median:148.5 , IQR:136.5-177, adjusted to $p<0.05$) and dorsal motor nucleus of the vagus (Control: Median:6, IQR:3.5-7; Vs Phz + Control: Median:22 IQR:21-23.5, adjusted to $p<0.05$). Furthermore, I.P. or oral administration of the specific SGLT1 antagonist (KGA-2727) did not block protein-mediated improvements in glucose tolerance. Our results suggest that the NTS and DVM of the brainstem are implicated in protein sensing and SGLT activity. Furthermore, we confirmed that SGLT1 does not influence the mechanism by which protein improves glucose tolerance. In summary, these data suggest that nutrient sensing in the gut and periphery are important contributors to the mechanism by which protein improves glucose tolerance.

PP31: Nicola Marrano

Irisin as potential mediator of glp-1 receptor agonists action

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Irisin is a hormone secreted by skeletal muscle following physical activity or excess of saturated fatty acids, able to promote energy expenditure and improve metabolic homeostasis. Serum irisin levels are reduced in type 2 diabetes (T2D), while exogenous irisin administration improves glycemic control in diabetic mice. Interestingly, irisin and GLP-1 share comparable pleiotropic effects and activate similar intracellular pathways, both at pancreatic and extra-pancreatic levels. This study investigated the effects of GLP-1-based therapies on irisin levels in T2D patients and irisin secretion in the culture media of human skeletal muscle cells.
195 T2D patients were enrolled and stratified by anti-diabetes therapy: diet only (24); metformin only (met, 37); met plus GLP-1 receptor agonists (GLP-1RAs, 39); met plus DPP-4 inhibitors (DPP-4i, 24); met plus SGLT2 inhibitors (SGLT2i, 20); other therapies (15). The control group included 36 sex-, and BMI-matched subjects without diabetes. Human skeletal muscle cells were exposed *in vitro* to 1-100 nM of different GLP-1RAs (exendin-4, dulaglutide, semaglutide, and liraglutide) for 24 h.
T2D patients showed lower irisin levels than controls (19.2 [4.2-50.8] vs 29.1 [14.1-43.5] ng/mL, $P<0.01$). Patients treated with met plus GLP-1RAs showed increased serum irisin levels (25.6 [11.0-50.8] ng/mL) compared to patients treated with diet or met (18.4 [9.9- 40.5] e 16,9 [10.7-28.8] ng/ml, respectively; $P<0.05$). Interestingly, *in vitro* treatment of human skeletal muscle cells with different GLP-1RAs resulted in enhanced irisin release in the culture medium compared to control cells.
In conclusion, in T2D, GLP-1-based therapies significantly increased serum irisin to levels comparable to those of non-diabetic subjects, possibly through GLP-1 receptor-mediated stimulation of irisin release by skeletal muscle cells. This suggests that irisin may represent a potential mediator of the effects of GLP-1-based therapies.

PP32: Patrik Rorsman

GLP-1 metabolite GLP-1(9-36) is a systemic inhibitor of pancreatic islet glucagon secretion

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Diabetes mellitus is associated with impaired insulin secretion, often combined with oversecretion of glucagon. Therapeutic interventions should correct both defects. Glucagon-like peptide 1 (GLP-1) has this profile but exactly how it exerts its glucagonostatic effect remains obscure. Here we show that the degradation product GLP-1(9-36) shares the capacity of GLP-1(7-36) to inhibit glucagon secretion evoked by low glucose (IC_{50} 3 pM). GLP-1(9-36) also potently inhibits glucagon secretion stimulated by β-adrenergic stimulation and amino acids. Although circulating GLP-1(9-36) levels (≥30 pM) are sufficient to strongly (70%) lower plasma glucagon *in vivo,* high exogenous GLP-1(9-36) results in a small additional suppression during insulin-induced hypoglycemia. GLP-1(9-36) retains its glucagonostatic effects after genetic/pharmacological inactivation of the GLP-1 receptor. In HEK293T cells expressing GCGRs and GTP-binding proteins, GLP-1(9-36) specifically leads to the dissociation of G_o. In islet α-cells, GLP-1(9-36) leads to pertussis toxin-sensitive inhibition of PKA and depletion of the docked pool of secretory granules, effects that are prevented by the GCRR antagonist REMD2.59, explaining the 280% increase in circulating glucagon produced *in vivo* and reversal of the GLP-1(9-36)'s inhibitory effect on glucagon secretion *in vitro*. We conclude that the GLP-1 metabolite GLP-1(9-36), via its glucagonostatic effect, plays a previously unrecognized role in systemic metabolism.

PP33: Pierre Larraufie

Metabolic regulations of enteroendocrine cells by hydrogen sulfide

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Enteroendocrine cells (EECs) play an important role in the sensing of intestinal content and, through the secretion of gut hormones, participate in the response of the host to food intake and microbiota perturbations. Distal EECs are in contact with various microbial compounds that can modulate EEC production or secretion of gut hormones. Most of the mechanisms described so far are based on the study of the receptors expressed by these cells that directly regulate secretion or gene expression. However, some metabolites can also impact cell energy metabolism and could potentially regulate EECs through new and unconventional pathways.
The aim of this project was to characterize the metabolic response of EECs to microbial metabolites and the consequences on secretion, using hydrogen sulfide (H2S) as a model to increase or inhibit cell respiration due to its dual effects on respiration depending on its concentration.
We used cell line models for enteroendocrine cells, mainly NCI-h716 to compare their capacity to metabolize H2S at low concentration, by measuring their oxygen consumption. We confirmed that like other intestinal cell lines (HT-29, Caco2), NCI-h716 cells could quickly metabolize H2S at low concentration, resulting in transient increased respiration. Higher H2S concentrations inhibited their respiration, the threshold of inhibition depending on cell density. We then assessed the effect of these conditions of increased or inhibition cell respiration on GLP-1 secretion, but observed little effects in both cases.
The role of H2S on EECs, which can be produced at high concentration in the distal intestine, has been poorly described. Here, we confirmed that EECs can metabolize and detoxify H2S at low concentration, but the changes on mitochondrial respiration had little effects on cell secretory activity. In our conditions, we therefore showed that modulating EEC respiration was not a direct modulator of secretion.

PP34: Rachel Foreman

Development of a novel LC-MS/MS method for the detection and analysis of CCK in human samples

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Cholecystokinin (CCK) is an important peptide hormone for satiety and stimulation of fat and protein digestion. The active peptide is suspected to exist in many forms which may all be biologically active, due to a shared sulphated tyrosine residue. However, measuring CCK in patient samples has proven difficult and historic studies of detecting CCK by radioimmunoassay have resulted in inconsistent results and unreliable data. Current ELISAs are still unable to distinguish between active and total concentrations so a more specific method is important to determine the bioavailability and activity of this hormone in metabolic diseases.
A novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the detection of multiple CCK peptides in biological matrices was developed and optimized using design of experiment (DoE) technique. By performing screening experiments, and the application of a D-orbital statistical model, different ionisation modes, collision energies, and mobile phase compositions were assessed. The DoE software was also applied to highlight some optimal extraction conditions for both the sulphated and non-sulphated forms of CCK8, as well as a propeptide fragment that may have undiscovered biological activity.
Optimization of the method by DoE has meant that CCK peptides can be quantified in human plasma and organoid lysate samples at picomolar concentrations. Secretion experiments were performed on duodenal organoid cultures and a consistent ratio of active CCK8 to the propeptide fragment, ”CCK21-44”, was confirmed. Further targeted analysis of CCK21-44 in human plasma samples following a mixed meal tolerance test confirmed the postprandial fluctuation of this proCCK fragment in healthy individuals. Due to its chemical properties, CCK21-44 is a better candidate for LC-MS/MS analysis and can be detected even when CCK8 levels fall below the detection limit, for example in fasted plasma samples. Based on the secretion correlation data with active CCK it could be a viable surrogate for monitoring CCK secretion.

PP35: Richard Kay

Characterisation of the plasma peptidome in gastrectomised patients

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The analysis of peptides in plasma using liquid chromatography linked to tandem mass spectrometry (LC-MS/MS) techniques is becoming more commonplace. Whilst the analytical sensitivity of LC-MS/MS approaches compared to immunoassay is generally lower, it can be highly multiplexed and is significantly more selective in its detection of peptides with similar amino acid sequences. In this study, samples were retrieved from a previously-published cohort of healthy subjects and lean patients with elective gastrectomy who received a 50g oral glucose tolerance test. Plasma samples were extracted and analysed using nano LC-MS/MS on an Orbitrap Q-Exactive plus.
Peptidomics analysis of the samples indicated positive identification of many gut hormone peptides, including GLP-I, oxyntomodulin, PYY, insulin, GIP and Neurotensin. A number of these peptides were significantly raised following glucose ingestion in the gastrectomy patients but not detected in the healthy subjects.
The LC-MS/MS derived levels of several gut hormones were compared against existing immunoassay data on the same samples, and showed good correlation in cases where the peptides were detected at all in the LC-

MS/MS system (PYY r^2= 0.93, insulin 0.98, GIP 0.81, GLP-I 0.66). Manual analysis of the raw LC-MS/MS data for peptides from the proglucagon gene was performed and additional peptides identified included GRPP and glicentin. The source of proglucagon peptides was believed to be intestinal L-cells, as the increased levels of GLP-1 following glucose ingestion in the gastrectomy cohort were accompanied by elevations in oxyntomodulin and glicentin.
These data show that LC-MS/MS is well suited for the analysis of bioactive gut hormone peptides from patients with gastric bypass surgery. However, this is only possible due to the supra-physiological plasma levels experienced during acute glucose ingestion, and improvements in the sensitivity of mass spectrometry methods are still required for the measurement of low abundance gut hormones in healthy subjects.

PP36: Rula Bany Bakar

Labelling and characterisation of Somatostatin Secreting D-Cells in Primary Human Duodenal Organoid Culture

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Backgrounds and aims: Enteroendocrine cells (EECs) are hormone-secreting cells within the intestinal epithelium that play an important role in regulating food absorption, insulin secretion and appetite. The somatostatin (SST)-producing D-cell is an EEC of particular interest due to the profound inhibition exerted by SST over other EECs, highlighting D-cells as critical regulator of the enteroendocrine axis. The aim of this study was to profile the transcriptome of human intestinal D-cells from organoids culture and to identify the key signalling pathways involved in the regulation of SST secretion.
Materials and methods: To label somatostatin secreting D-cells in human duodenal organoids CRISPR-Cas9 followed by homology-directed repair was used to insert Internal Ribosome Entry Site sequence, followed by the fluorescent protein tdTomato sequence and puromycin resistant cassette under control of the endogenous somatostatin promoter. Fluorescence-activated cell sorting (FACS) was used to purify organoids-derived D-cells. Bulk RNA sequencing of FACS-purified SST-tdTomato positive and negative cells was performed.
Results: The transcriptional profiles of FACS purified D-cells and control populations were analysed. The principal component analysis exhibited a wide separation between these two populations on the first component (87% of variance), and narrow separation on the second component (8 % of variance). tdTomato-positive cells were strongly enriched for SST gene, which was found at ~1000-fold-higher levels in fluorescent compared to non-fluorescent cells ($p < 0.001$). RNA sequencing identified enriched expression of several G-protein coupled receptors in D-cells including short-chain fatty acids receptor (FFAR2), bile acids receptor (GPBAR1), amino acids receptor (GPR142), trace amines receptor (TAAR1) and the vasopressin receptor (AVPR1B).
Conclusion: This study provides the first in-depth transcriptomic analysis of human intestinal D-cells which provide an important foundation to guide future studies for functional characterisation of this cell type.

PP37: Sarah Byberg

Plasma levels of liver-expressed antimicrobial peptide 2 (LEAP2) during meal challenge before and after weight loss in individuals with obesity

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Background: Liver-expressed antimicrobial peptide 2 (LEAP2) is the only known endogenous antagonist to the orexigenic hormone ghrelin. LEAP2 might play a role in the regulation of food intake, as infusion with LEAP2 substantially decrease caloric intake in healthy lean individuals. This study explores if endogenous LEAP2 increase in the postprandial state. We applied an assay directed against the N-terminus of LEAP2, which is the site that inhibits the ghrelin receptor. We further investigate if the meal response differs before and after diet induced weight loss in twenty participants with obesity.
Method: This is an exploratory analysis of samples from the S-LiTE study (ClinicalTrials.gov number, NCT04122716). In brief eligible participants were adults (>18 years) with obesity (BMI 32-43 kg/m^2) and without diabetes mellitus. The participants lost at least five percent of their body weight during an eight week hypocaloric diet (800 kcal/day). Samples from twenty randomly selected individuals were measured. A liquid meal challenge (Nutricia Nutridrink, 600 kcal, 49.0 E% from carbohydrates, 35.2 E% from fat and 15.8 E% from protein) was performed at baseline and after the weight loss. The meal was ingested from zero to 15 minutes and blood samples were collected at the following time points: 0, 15, 30, 45, 60, 90, 120, 150 and 180 minutes. Plasma samples were analyzed using our in-house developed radioimmunoassay.
Statistical analysis: To analyze changes in plasma LEAP2 during the meal challenge we applied a linear mixed model including the time points (categorical) as a fixed effect. To account for the correlation in the repeated measurements as well as possible variance heterogeneity over time, we assumed an unstructured covariance pattern. Data were logarithmically transformed to fit model assumptions. Fasting and mean postprandial LEAP2 were compared between visits by paired t-test. A p-value <0.05 and a 95% confidence interval not including zero were interpreted as statistically significant.
Results: Eight men and twelve women were included in the analysis. LEAP2 plasma levels increased by 29% (95%CI: 8 to 54%, p=0.008) at 120 minutes during the meal challenge at baseline. However, following weight loss a 28% (95%CI: 8 to 51%, p=0.007) increase in plasma levels were detected at 15 minutes. No difference were detected in fasting or postprandial LEAP2 levels between visits.
Conclusion: These preliminary findings points to a postprandial increase in plasma LEAP2, which might support the hypothesis that LEAP2 signaling is involved in meal termination. We noted a delayed increase in plasma LEAP2 in response to meal intake before diet induced weight loss. No difference was detected in fasting or postprandial levels before and after weight loss. These finding need to be tested in a larger sample size.

PP38: Sheyma Kizilkaya

The molecular- and physiological impact of naturally occurring glucose-dependent insulinotropic polypeptide receptor (GIPR) variants detected in the Danish population

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Introduction and aim: The gut hormone, glucose-dependent insulinotropic polypeptide (GIP) and its receptor (GIPR), plays an important role in several physiological systems related to glucose homeostasis, bone metabolism, fat deposition, and the cardiovascular system. Recent data suggests that genetic variants of the *GIPR* are associated with phenotypical changes such as lower bone mineral density, higher fracture risk, lower body weight and BMI, and altered blood pressure. The aim of the present study is to characterize the molecular pharmacological properties of 47 *GIPR* variants detected in ~5000/12,000 individuals by targeted sequencing in Danish diabetes cohorts. Moreover, 32 of the variants were also detected in the UK biobank, which is a large-scale databank containing genomic/phenotypic information from half a million individuals.
Material and methods: All variants were investigated through *in vitro* studies by examination of signaling properties through measurement of cAMP production and β-arrestin recruitment in comparison to wild-type GIPR. Additionally, competitive binding experiments were performed to determine affinity of the native GIP

hormone to all variants and estimate receptor surface expression. All *in vitro* studies were done in human embryonic kidney 293 cells (HEK293).
Results: 29 out of the 47 *GIPR* variants showed less than 50% receptor activation via $G\alpha_s$-activation within the physiological doses of GIP at 100pM efficacy. Correspondingly, a lower binding capacity (B_{max}) of the hormone was observed for the variants with impaired receptor activity, despite wild-type like affinities (K_d-values). In addition, eight out of the 29 variants with impaired $G\alpha_s$-activation showed a preference for increased β-arrestin recruitment.
Conclusion and perspectives: We show that carriers of 29 out of 47 *GIPR* variants detected in the Danish population have impaired GIPR activity (loss-of-function), which might be associated with various phenotypical consequences related to the physiological function of GIP. Future studies will determine if this is the case.

PP39: Shiqian Chen

Tissue-specific cAMP signalling with biased GLP-1R agonists

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The GLP-1R is expressed in several metabolic tissues, including pancreatic islet cells and anorectic neurons. Here we aimed to uncover tissue-specific patterns of cAMP signalling with biased GLP-1R agonists that display differential effects on G protein *versus* β-arrestin recruitment.
Two N-terminally modified forms of exendin-4 were used in this study: exendin-phe1 (ExF1) and exendin-asp3 (ExD3). Agonist pharmacology was characterised using nanoBiT complementation in HEK293 cells. Real-time monitoring of cAMP signalling was performed in tissues and cells isolated from mice encoding the cAMP FRET sensor $^{T}Epac^{VV}$ expressed under the control of relevant promoters. Hormone secretion and RNAseq were used to assess downstream responses.
ExF1 showed moderately attenuated mini-Gs recruitment (75% reduction), and markedly attenuated β-arrestin recruitment (95% reduction), compared to ExD3. In *Glp1r*-expressing nodose ganglion (NG) neurons and hypothalamic brain slices, as well as pancreatic alpha and delta cells, ExF1 was a partial agonist for cAMP when measured acutely. However, ExF1 was a full agonist acutely in pancreatic beta cells. Follow-up receptor titration studies suggested that the full agonist response to ExF1 in beta cells depends on adequate levels of receptor surface expression. In contrast to the acute effects, sustained stimulation of beta cells led to paradoxically higher steady-state cAMP levels with ExF1 compared with ExD3, along with greater insulin secretion and larger transcriptional changes. This ExF1-favouring pattern was not seen with NG neurons under the same conditions. These findings demonstrate that biased GLP-1R agonists can display tissue-specific signalling profiles, which could underlie the observation that their effects on glucose homeostasis are more marked than those on appetite regulation.

PP40: Signe Torekov

Sleep quality is improved by weight loss and maintained with exercise in adults with obesity

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Study objectives: Weight loss may improve sleep, but impaired sleep in individuals with obesity may affect weight regain after weight loss. We investigated sleep duration and quality during weight loss followed by weight maintenance treatment with exercise and/or the GLP-1-receptor agonist liraglutide.

Methods: In this randomized controlled, two-by-two factorial study, 195 adults with obesity successfully completed an 8-week low-calorie diet and were then randomly assigned to 52 weeks weight maintenance treatment with or without exercise and liraglutide 3.0 mg/day or placebo. Sleep duration was measured using accelerometers before and after the low-calorie diet, and during weight maintenance. Sleep quality was estimated using the Pittsburgh Sleep Quality Index (PSQI). To test associations between insufficient sleep and weight regain, participants were stratified into subgroups according to sleep duration (more or less than 6 hours/night) or sleep quality (below/above a PSQI score of 5) at randomization.
Results: After the diet-induced weight loss (12 kg), sleep duration increased by 17 min/night (P <.0001) and sleep quality improved (P <.0001). After one year, the sleep quality improvements from the initial weight loss were maintained in the exercise groups compared with non-exercise groups (P=.02). Liraglutide groups had increased sleep duration compared with placebo after 26 weeks (5 vs. -15 min/night) but similar sleep duration and quality as placebo after 52 weeks. During 1-year weight maintenance, participants with short sleep duration at randomization regained 4.4 kg (P=.008) compared with those with normal sleep duration. Poor quality sleepers regained 2.9 kg (P=.04) compared with good sleepers.
Conclusions: Weight loss improved sleep quality, which was maintained by exercise. Adults with short sleep duration or poor sleep quality at the initiation of weight maintenance may be less successful in maintaining weight loss than those with adequate sleep.
ClinicalTrials.gov: NCT04122716, EudraCT number: 2015-005585-32.

PP41: Simon Birk Kjær Jensen

The combination of exercise and glucagon-like peptide-1 receptor agonist improves glucose tolerance and beta cell function and decreases glucagon response after diet-induced weight loss in adults with obesity

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Aim: Sustained weight loss seems to reduce the incidence of impaired glucose tolerance and type 2 diabetes. However, the best treatment strategy for maintaining healthy weight-associated improvements in glucose tolerance, glucagon response and beta cell function is unknown. We therefore aimed to investigate whether exercise, the glucagon-like peptide-1 receptor agonist liraglutide, or the combination improves post-prandial glucose and glucagon response, and beta-cell function after a diet-induced weight loss in people with obesity.
Methods: In this randomised, double-blinded, placebo-controlled trial, adults with obesity (BMI 32–43 kg/m^2) without diabetes underwent an 8-week low-calorie diet (800 kcal/day) and were randomly assigned (1:1:1:1) to 52 weeks of moderate-to-vigorous intensity aerobic exercise, liraglutide 3.0 mg/day, exercise and liraglutide combined, or placebo. Change in glucose and glucagon response to a 3-h mixed meal test, and disposition index, as a measure of beta cell function, were measured from randomization to end-of-treatment.
Results: A total of 195 participants completed the low-calorie diet and were randomised. After 52 weeks of treatment, the placebo group had regained weight, the exercise and liraglutide groups maintained weight loss, and body weight was further reduced with the combination of both treatments. After one year of treatment, the combination group had decreased postprandial glucose response by -9% (95% CI, -14 to -3; P=0.002), improved beta cell function by 49% (95%CI, 16 to 93; P=0.02), and decreased glucagon response by -18% (95%CI, -34 to -3) compared with placebo, while liraglutide alone only improved postprandial glucose response by -7% (95%CI, -12 to -1; P=0.02), but not beta cell function or glucagon. Exercise alone had similar postprandial glucose response, beta cell function and glucagon compared with placebo.
Conclusions: In adults with obesity, the combination of exercise and liraglutide after diet-induced weight loss improved glucose tolerance, beta cell function, and glucagon response, which were not observed with either treatment alone.
Trial registration: EudraCT, 2015-005585-32; ClinicalTrials.gov, NCT04122716.

PP42: Simone Mazzaferro

Using Pluripotent Stem Cells as a model to determining expression, function and pharmacology of GLP-1 receptor in human hypothalamic POMC neurons.

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Glucagon-like peptide (GLP-1) and other incretins play an important biological role in regulating metabolism, in part by acting on neuron populations in the hypothalamus. This circuitry has been well described in mice, where activation of hypothalamic pro-opiomelanocortin (POMC) neurons by GLP-1 receptor agonists promote weight loss by reducing food intake. However, the molecular mechanisms that lead to POMC neuron activation are not fully understood. To address this issue, we generated a knock-in induced Pluripotent Stem Cell (iPSC) line where green fluorescent protein (GFP) is expressed under control of the endogenous POMC promoter, and differentiated this cell line into hypothalamic neurons. We found that GFP was appropriately expressed in human POMC neurons, enabling these cells to be isolated for bulk RNA sequencing and functional studies. This analysis revealed that human POMC neurons are enriched in GLP-1 receptor and express downstream effectors and channels that may mediate its action. We are now profiling the transcriptional responses of these POMC neurons to GLP-1 receptor agonists, and using single-cell patch clamp and calcium Imaging quantify the effect of GLP-1 receptor agonists on the excitability of human POMC neuron and to determine the molecular mechanisms underlying this effect. These studies may reveal molecular targets for further enhancing the appetite-reducing action of GLP-1 receptor agonists.

PP43: Vera Penshovska-Nikolova

Safety effect of combinate Therapy with liraglutide, metformin and statins on HbA1c and lipid markers (ApoA1 and ApoB) in patients with T2DM.

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Background; Main hormones involved in regulation of glucose are glucagon like peptid GLP-1 and glucose dependent insulotropic polypeptide GIP. Both of them are inactivated by the enzyme dipeptil peptidasa -4 (DPP-4). Incretin based therapies include GLP -1 receptor agonist and DPP-4 inhibitors which main actions are to increase secretion of insulin and inhibit secretion of glucagon. Apolipoproteins ApoA1 and Apo B are proteins that have role in prediction of cardiovascular disease.
Objectives; review of clinical trials show that liraglutide can cause decrease of HbA1c, but the object of our study is that liraglutide combined with statins and metformin can cause safety decreased levels of three examined markers (HbA1C; ApoA1 and ApoB).
The aim of study was to examine whether combination of statins with liraglutide and metformin can cause significant decrease in ApoA1, Apo B and Hba1C in patients with DMtip2 and hyperlipidemia.
Research and methods; 44 T2DM patients (age=57+-1 year; 47% female; BMI=34,6+-0,9; diabetes duration =7,8+-0,9 year; FPG=185+-8;HbA1C=8,6+-0,1%) were randomized to receive for 16 weeks (i)metformin 2g ; (ii) liraglutide1,8 mg and (iii) statin 20 mg. Statins were given to patients at first visit, while they were on treatment with metformin and GLP before that. ApoA1, Apo B and HbA1c plasma concentration were measured each 4 weeks during the period of 16 weeks.
Results; In patients after 4 weeks of treatment with statins, liraglutide and metformin significant reduction in ApoA1, Apo B and HbA1c was registered (p=0,00013, p=0,016 and p<0,0001), compared with basic plasma concentration, measured before beginning of combined treatment. (p=0,02, p=0,00074 and p<0,0001).

ApoA1 concentration was increased in 43,6 %patients,16.01% patients, 14.89 % patients and 11,76% patients appropriate in analyzed time spots. After 4 months treatment with statins, Liraglutide and metformin was registered significant decreasing of Apo A1 compared before the treatment. (p=0,00221)
Apo B concentration was increased in 62,6 % patients,54.01% patients, 33.89 % patients and 21,76% patients appropriate in analyzed time spots. After 4 months of treatment with statins, liraglutide and metformin was registered significant decreasing of Apo B compared before the treatment (p=0,003).
HbA1c concentration was increased in 88,6 %patients,16.01 %patients, 8.89 % patients and 7,76% patients appropriate in analyzed time spots. After 4 months of treatment with statins, liraglutide and metformin was registered significant decreasing of HbA1C concentration compared before the treatment(p<0.0001).
Conclusion; These results support a possible role for decreased HbA1C, ApoA1 and ApoB levels in the long term, caused by combined treatment of GLP-1 agonist, metformin and statins which suggest that can be used for prevention of possible cardiovascular future events.

PP44: Victor Gault

ΔTRTX-Ac1: a peptide derived from the venom of the tarantula Aphonopelma chalcodes is non-toxic, exhibits positive effects on beta-cell function and augments GLP-1-induced appetite reduction

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Aims: GLP-1 mimetics are clinically approved for the treatment of diabetes and obesity. This is based on the ability of GLP-1 receptor activation to augment glucose-induced insulin secretion and suppress appetite leading to weight loss. We recently discovered a 28-aa peptide, ΔTRTX-Ac1, from the venom of the *Aphonopelma chalcodes* tarantula. In the current study, we have examined its effects on pancreatic beta-cell function, insulin secretion, and ability to augment GLP-1-induced actions on glucose homeostasis and appetite suppression in mice. **Methods:** Insulin secretion studies (10^{-12} –10^{-6} M; 20 min) were performed in BRIN-BD11 beta-cells. Effects on beta-cell proliferation and apoptosis were assessed by Ki-67 and TUNEL staining, respectively, with MTT assay used to determine impact on cell viability. Acute *in vivo* gluco-regulatory and satiety actions of ΔTRTX-Ac1 alone (25-250 nmol/kg bw), or in combination with the GLP-1 mimetic exenatide (0.25-25 nmol/kg bw), were investigated in 12-week-old C57BL/6 mice. **Results:** ΔTRTX-Ac1 (10^{-6} M) significantly increased (p<0.05-p<0.001) insulin secretion from BRIN-BD11 cells at 5.6, 11.1 and 16.7 mM glucose. ΔTRTX-Ac1 did not negatively affect BRIN-BD11 cell viability and augmented (10^{-10} –10^{-6} M; p<0.01 and p<0.001, respectively) beta-cell proliferation. Moreover, ΔTRTX-Ac1 significantly (p<0.001) protected against cytokine-induced beta-cell apoptosis. When injected co-jointly with glucose in mice at a dose of 250 nmol/kg, ΔTRTX-Ac1 decreased (p<0.001) blood glucose concentrations during a 60-min experimental period. Conversely, ΔTRTX-Ac1 was unable to augment the glucose-lowering actions of exenatide. However, at doses of 25 or 250 nmol/kg, ΔTRTX-Ac1 reduced (p<0.05-p<0.001) food intake in overnight fasted mice and significantly (p<0.05-p<0.001) augmented the appetite suppressive actions of exenatide. **Conclusion:** ΔTRTX-Ac1 is a tarantula venom-derived peptide with a biological action profile of therapeutic interest for diabetes and obesity, both alone and in combination with GLP-1R signaling.

PP45: William Roell

Tirzepatide improved beta cell function and insulin sensitivity in in a 28 week randomized double-blind study in type 2 diabetes

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Tirzepatide, a glucose-dependent insulinotropic polypeptide (GIP)/GLP-1 receptor agonist, shows a remarkable ability to lower blood glucose, enabling many patients with long-standing type 2 diabetes to achieve normoglycaemia. To better understand mechanisms contributing to glycemic control, meal tolerance tests and glucose clamp studies were conducted at baseline and 28 weeks in a randomized double-blind mechanism of action study in participants with T2D treated with TZP, semaglutide, or placebo. With tirzepatide, the clamp disposition index increased from a least squares mean of 0·3 pmol m-2 L min-2 kg-1 (SE 0·03) at baseline by 1·9 pmol m-2 L min-2 kg-1 (0·16) to total 2·3 pmol m-2 L min-2 kg-1 (SE 0·16) at week 28 and, with placebo, the clamp disposition index did not change much from baseline (least squares mean at baseline 0·4 pmol m-2 L min-2 kg-1 [SE 0·04]; change from baseline 0·0 pmol m-2 L min-2 kg-1 [0·03]; least squares mean at week 28 0·3 [SE 0·03]; estimated treatment difference [ETD] tirzepatide vs placebo 1·92 [95% CI 1·59-2·24]; p<0·0001). The improvement with tirzepatide in clamp disposition index was significantly greater than with semaglutide (ETD 0·84 pmol m-2 L min-2 kg-1 [95% CI 0·46-1·21]). This result reflected significant improvements in total insulin secretion rate (ETD 102·09 pmol min-1 m-2 [51·84-152·33]) and insulin sensitivity (ETD 1·52 mg min-1 kg-1 [0·53-2·52]) for tirzepatide versus semaglutide. On meal tolerance testing, tirzepatide significantly reduced glucose excursions (lower insulin and glucagon concentrations) compared with placebo, with effects on these variables being greater than with semaglutide. These findings demonstrate the glycaemic efficacy of GIP/GLP-1 receptor agonist tirzepatide in type 2 diabetes results from concurrent improvements in key components of diabetes pathophysiology, namely β-cell function, insulin sensitivity, and glucagon secretion and help to explain the remarkable glucose-lowering ability of tirzepatide.
