(AFM) based force spectroscopy on model human proximal tubule cells (HK2) ± TGF-β1 (10ng/ml) at 37°C. Fitted with a spherical bead, AFM cantilevers functioning under constant force mode measured mechanical forces over time and viscoelastic properties were calculated using the Maxwell model of viscoelasticity.

Results: Data suggest that both control and TGF-β1-treated cells exhibit transient (5s) force relaxation of 0.7 and 0.3nN, respectively, in response to physical deformation. The initial phase was followed by stabilisation in force over a subsequent 30-s period. TGF-β1-treated cells demonstrated a reduction in relaxation characteristics, suggesting that complex viscoelastic components are strongly affected by reorganisation of the actin cytoskeleton. Data indicate that viscosities after treatment vary up to 35% (12 cells, $n = 3$, $p < 0.001$). Treated cells showed a threefold reduction in experimental decay of the force time curve that leads to significant changes in the adhesive deformation behaviour.

Conclusion: TGF-β1 (10ng/ml) triggered complex nanomechanical changes in the viscous-elastic behaviour of single cells. Our research suggests that the progression of the disease instigates intricate physical changes that may, in part, mediate altered cell–extracellular matrix (ECM) interactions linked to altered cell phenotype in tubular injury.

P14

Abstract withdrawn

Basic and clinical science posters: Beta cells, islets and stem cells

P15 BS D

Identification of pro-apoptotic signalling through the GPRC5B receptor in beta cells by CRISPR-Cas9-mediated genome editing

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Background/Aims: GPRC5B is an orphan receptor that is highly expressed in human and mouse islets, and we have previously reported that its down-regulation in islets protects against apoptosis. As there are no agonists identified for GPRC5B, we investigated its role in MIN6 beta cells by stably deleting it using the CRISPR-Cas9 technology, then re-introducing it in a graded fashion.

Methods: Efficiency of GFP plasmid delivery into MIN6 beta cells was determined by cell sorting following transfection with eight reagents. CRISPR-Cas9 was used to stably delete the *Gprc5b* gene in MIN6 beta cells, and 0.01% to 100% *Gprc5b* plasmid was introduced by transient transfection to restore *Gprc5b* expression. Standard techniques were used to quantify *Gprc5b* mRNA expression, apoptosis, and signalling pathways downstream of GPRC5B were identified using Cignal 45-Pathway Reporter Arrays and western blotting.

Results: JetPRIME® provided the best MIN6 beta cell transfection efficiency (29 ± 0.5% GFP-positive cells). Isolation of *Gprc5b* KO cell clones using CRISPR-Cas9 was established, and DNA sequencing confirmed deletion of *Gprc5b* nucleotides by this strategy. Re-expression of GPRC5B in *Gprc5b* KO MIN6 beta cells increased apoptosis (119.5 ± 4% of maximum cytokine response, $p < 0.01$) and induced up-regulation of transforming growth factor (TGF)-β1 and Interferon gamma (IFNγ) pro-apoptotic pathways (3.4 ± 0.9% and 2.9 ± 0.3 log2 fold change, respectively). Activation of pro-apoptotic signalling was confirmed by western blotting, which indicated significant ($p < 0.001$) GPRC5B-induced phosphorylation of AKT (+125 ± 7.6%) and STAT1 (+185 ± 14.0%).

Conclusion: We have developed an efficacious CRISPR-Cas9 gene editing protocol, which we used to delete the *Gprc5b* gene in MIN6 beta cells. Re-introduction of GPRC5B expression allowed us to identify signalling pathways linking GPRC5B with beta cell apoptosis.

P16 BS D

Roles for the Type 2 diabetes-associated genes *C2CD4A* and *C2CD4B* in the control of insulin secretion

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Background: Single-nucleotide polymorphisms near *C2CD4A* and *C2CD4B* are associated with altered pro-insulin levels and Type 2 diabetes risk at genome-wide significance. Altered expression of both C2CD4A and C2CD4B has been reported in islets in association with risk variants. Both genes encode Ca^{2+} binding proteins thought to be localised to the nucleus. Here, we address their potential role in glucose homeostasis *in vivo* and *in vitro*.

Methods: *C2CD4B* null mice were generated by the International Mouse Phenotyping Consortium using CRISPR/Cas9-mediated genome editing. Intraperitoneal glucose tolerance tests (IPGTTs) were performed using standard protocols. Subcellular analysis of flag-tagged constructs was performed by immunocytochemistry and confocal fluorescence microscopy.

Results: Animals deleted globally for *C2cd4b* showed mild dysglycaemia (females at 12 weeks: 15min IPGTT: wild type (WT), 11.5 ± 0.85 mmol/l; knockout (KO) 14.78 ± 1.05 mmol/l, $n = 7$ to 11, $p = 0.0129$; males at 16 weeks, WT, 13.38 ± 0.67 mmol/l; KO, 16.00 ± 0.56 mmol/l, $n = 8$, $p = 0.045$). In human-derived EndoC BH1 beta cells, C2CD4A and C2CD4B were localised to the cytoplasm and nucleus in 92.31 ± 3.3% and 79.22 ± 10.12% of cells, respectively ($n = 100$ and 80 cells). Additional localisation to the plasma membrane was observed in 3.64 ± 3.6% and C2CD4B

6.06 ± 3.07% of cells. Exclusive localisation to the nucleus was observed in only 0.37 ± 0.37% and 5.7 ± 2.08% of cells.

Conclusions: Our data suggest that altered C2CD4B expression may contribute to disease risk conferred by variants at this locus by altering glucose homeostasis, and support an extra-nuclear role for this factor, and C2CD4A, in the beta cells.

P17 D

The selective serotonin reuptake inhibitor fluoxetine improves glucose homeostasis *in vivo* by promoting insulin secretion and maintaining functional beta cell mass

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Refer to Oral number A51

P18

Administration of PD153035 improves glucose tolerance and alters beta cell mass in C57BL/6 mice

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Refer to Oral number A52

P19

A role for Free Fatty Acid Receptor 2 (FFAR2) in the beneficial effects of diet-derived short-chain fatty acids on glucose homeostasis

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Aims: The role of free fatty acid receptor 2 (FFAR2) was investigated in the effects of the short-chain fatty acids sodium acetate (SA) and propionate (SP) on insulin secretion and protection from apoptosis in both mouse (MI) and human islets (HI).

Methods: Insulin secretion and $[Ca^{2+}]_i$ were profiled by perifusion and microfluorimetry, respectively; protein kinase C (PKC) was down-regulated by 20-h exposure to 200nM PMA, and PLC was inhibited by 10mM U73122; caspase levels were quantified by chemiluminescence.

Results: SA- and SP-induced potentiation of glucose-induced insulin secretion was dependent on PLC (20mM glucose + SA: 67.3 ± 28.7 pg islet^{-1} min^{-1}, +U73122: 34.3 ± 3.3, $p < 0.05$) and PKC in HI (20mM glucose + SA: 44.5 ± 12.6 pg islet^{-1} min^{-1}; PKC-depleted: 19.7 ± 1.1, $p < 0.01$; 20mM glucose+SP: 18.7 ± 0.8; PKC-depleted: 5.6 ± 0.4, $p < 0.01$), and it was inhibited by FFAR2 deletion in MI (wild type (WT), 20G+SA: 11.2 ± 2.2 pg islet^{-1} min^{-1}; FFAR2 knockout (FFAR2KO) 6.5 ± 0.7, $p < 0.05$; WT, 20G+SP: 14.8 ± 1.2; FFAR2KO 6.6 ± 0.9, $p < 0.01$). SA-induced elevation in $[Ca^{2+}]_i$ was lost in FFAR2KO MI as was the ability of SA and SP to protect against apoptosis (WT, +cytokines: 2.21 ± 0.12; +SA: 1.72 ± 0.09; +SP: 1.51 ± 0.13, $p < 0.01$; FFAR2KO, +cytokines: 2.43 ± 0.19, +SA: 2.29 ± 0.19; +SP: 2.74 ± 0.20, $p > 0.2$). FFAR2 was also required for SA and SP protection of islets against apoptosis induced by 0.5mM palmitic acid (PA) (WT, +PA: 1.61 ± 0.10; +SA: 1.24 ± 0.09; +SP: 1.41 ± 0.16, $p < 0.05$; FFAR2KO, +PA: 1.45 ± 0.16, +SA: 1.38 ± 0.09; +SP: 1.64 ± 0.13, $p > 0.2$).

Results: SA and SP increase insulin secretion through FFAR2-dependent signalling via PLC and PKC and protect islets from both cytotoxic and lipotoxic insults in a FFAR2-dependent manner. These effects are all beneficial for the treatment of Type 2 diabetes and position FFAR2 as a potential pharmacological target.

P20 D

Use of Raman spectroscopy as a novel means to study lipid deposition and composition in cultured beta cells following exposure to exogenous fatty acids

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Aims: Aberrant lipid handling and deposition have been proposed to contribute to the dysfunction and demise of pancreatic beta cells in Type 2 diabetes. Spontaneous Raman spectroscopy was employed as a novel means to compare the deposition of palmitate and its methyl-ester into neutral lipid droplets (NLDs) in cultured beta cells.

Methods: INS-1 cells were exposed to palmitate (PA) or its methyl-ester (MePA) at 0.125mM for 24h. Raman maps were acquired between intensity 2,835 and 2,855 cm^{-1}, corresponding to the CH2 symmetric stretch vibration in lipid. Lipid content was determined as a fraction of total cell area. Spectra of the lipid-corresponding pixels were averaged using cluster analysis and compared with that of pure PA to verify its presence in NLDs.

Results: Exposure to PA did not increase the mean area of beta cell NLDs, but the values were more variable (2.5% to 32.5%) than in controls (16.2% to 26.9%) indicating that the cell population may respond to PA in a non-homogeneous manner. A major peak appeared at 2,880cm^{-1} in cells exposed to PA, consistent with incorporation of this fatty acid into NLDs. No equivalent peak was seen in control cells or in those treated with MePA. This confirms that, unlike its parent fatty acid, MePA is not a substrate for fatty acid incorporation into beta cell NLDs.

Summary: We conclude that Raman spectroscopy provides a novel, non-invasive, label-less means to monitor the deposition of long-chain fatty acids into NLDs in beta cells.

P21

Pancreatic beta cell-selective deletion of the mitochondrial calcium uniporter (MCU) impairs glucose-stimulated insulin secretion *in vitro* but not *in vivo*

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Background and aims: In response to stimulation of beta cells with elevated glucose concentrations, uptake of Ca^{2+} into mitochondria has been proposed to enhance oxidative metabolism and consequently insulin secretion. To test this hypothesis, we deleted the mitochondrial uniporter (MCUa, *Ccdc109a*) selectively in the beta cell in mice.

Methods: Mice deleted for all MCUa splice variants in the beta cell knockout (KO) were generated by crossing C57BL6 mice bearing MCUa alleles with *Flox*P sites flanking exons 11 and 12 to animals carrying *Cre* recombinase at the Ins1 locus ($Ins1^{Cre}$). Intraperitoneal glucose and insulin tolerance along with insulin secretion *in vivo* were quantified using standard protocols, and insulin secretion from islets measured during perifusion. Beta cell mass was determined by optical projection tomography. Mitochondrial and cytosolic free Ca^{2+} were monitored using the recombinant fluorescent probes R-GECO and Fura-Red, respectively.

Results: Glucose (17 vs 3 mM)-stimulated mitochondrial Ca^{2+} uptake and first-phase insulin secretion were both significantly impaired in KO vs wild type (WT) mouse islets ($p < 0.05$, $n = 3$ to 5, three mice per genotype). Nevertheless, examined at eight weeks, KO mice displayed improved glucose tolerance vs WT ($p < 0.01$, $n = 11$ to 15), despite lowered beta cell mass ($30.6 \pm 1.4\%$, $n = 5$, $p \leq 0.05$). No differences in glucose tolerance were apparent from 12 weeks of age.

Conclusions: Mitochondrial Ca^{2+} uptake is required in pancreatic beta cells for glucose-stimulated insulin secretion *in vitro* and for the achievement of a normal beta cell mass. Presently undefined compensatory mechanisms reduce the importance of this process *in vivo*.

P22 D

Harnessing the mesenchymal stromal cell secretome to improve the efficiency of islet transplantation

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Aims: We have previously shown that mesenchymal stromal cells (MSCs) enhance islet function *in vitro* and *in vivo*. We identified annexin A1 (ANXA1) and an array of other MSC biotherapeutics in mouse MSCs. ANXA1 preculture mimics the beneficial effects of MSCs *in vitro*, translating to improvements in graft function *in vivo*, albeit not to the extent seen with MSCs. This study aimed to determine whether preculturing mouse islets with a "cocktail" of MSC biotherapeutics produces superior islet transplantation outcomes to those observed when using ANXA1 alone.

Methods: Islets were precultured with ANXA1, stromal-cell-derived factor-1 (SDF-1), complement component C3a or combinations of these MSC biotherapeutics for 48h. Cytokine-induced apoptosis and glucose-stimulated insulin secretion (GSIS) were measured 24 to 72 h following removal of MSC biotherapeutics from the culture media. Streptozotocin-diabetic C57Bl/6 mice were implanted under the kidney capsule with a minimal mass of 150 C57Bl/6 mouse islets precultured alone, with ANXA1 alone, or with a cocktail of ANXA1/SDF-1/C3a. Blood glucose concentrations were monitored for one-month post-transplantation.

Results: GSIS was higher in islets precultured with ANXA1/SDF-1/C3a than in islets precultured with ANXA1 alone, or precultured alone, at 24 and 72 h following removal of MSC biotherapeutics from the media (24h: 2.9 ± 0.3 vs 1.7 ± 0.2 vs 1.4 ± 0.3 ng/islet/h; 72h: 1.6 ± 0.2 vs 1.1 ± 0.3 vs 0.9 ± 0.2 ng/islet/h, $p < 0.01$, $n = 15$). The average blood glucose of mice transplanted with ANXA1/SDF-1/C3a precultured islets was lower than mice transplanted with islets precultured with ANXA1-alone, or without MSC biotherapeutics (one-month post-transplantation:18.7 ± 2.6 vs 24.5 ± 3.0 vs 28.8 ± 1.5 mmol/l, $p < 0.05$, RM-ANOVA, $n = 7$ to 9).

Conclusion: Preculturing islets with a "cocktail" of MSC biotherapeutics represents a defined, rapidly translatable cell-free modification to improve clinical islet transplantation outcomes.

P23

The effects of metabolic conditions relevant to Type 2 diabetes on alternative splicing

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Aim: Type 2 diabetes is a metabolic disorder associated with chronic hyperglycaemia and hyperlipidaemia that results in insulin resistance and a progressive deterioration of beta cell mass and function. Alternative splicing is a post-transcriptional process that allows a single gene to encode multiple transcripts and is a crucial mechanism for generating proteomic diversity. Errors in alternative splicing occur in many disorders. However, little is known about the role of alternative splicing in diabetes. The aim of this project was to test whether metabolic conditions relevant to Type 2 diabetes affect alternative splicing in beta cells.

Methods: The expression of a panel of genes which regulate alternative splicing was determined using SYBR green-based quantitative reverse-transcriptase polymerase chain reaction in beta cells exposed to high glucose and free fatty acid conditions. Cell survival and expression of the stress marker *DP5* were also examined.

Results: Decreased expression of genes encoding splicing-regulatory RNA-binding proteins was observed at 46h following exposure to high glucose and free fatty acid conditions, including *Elavl4* ($p = 0.02$), *Nova2* ($p = 0.03$) and *Ptbp1* ($p = 0.03$, $n = 3$ independent experiments). These changes preceded any effect on beta cell survival and correlated with an increase in the stress marker *DP5*. Interestingly, no significant changes were observed in the expression of serine/arginine family genes, though some variation was evident.

Conclusion: Our data demonstrate that metabolic factors can affect the regulation of alternative splicing genes and suggest that the metabolic conditions existing in many people with Type 2 diabetes may affect alternative splicing in beta cells.

P24

Developing a decellularised pancreas matrix for islet bioengineering

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Aims: Type 1 diabetes results from the autoimmune destruction of insulin producing islet beta cells of the pancreas. Stem cells could theoretically be used to recreate multicellular islets and to cure diabetes. The tissue-specific composition of the extracellular matrix provides specific cues to direct stem cell differentiation. Organ decellularisation can be used to produce a physiological matrix scaffold on which to differentiate stem cells, but to date has not been used to differentiate beta cells. The aim of this project is to optimise and validate a protocol to successfully decellularise mouse pancreas, with the future goal of testing whether this can be used to support the differentiation of fully functional beta cells.

Methods: Ligation of mesenteric veins and cannulation of the hepatic portal vein were used to perfuse a 1% sodium dodecyl sulfate (SDS) solution through the pancreas over a period of 5h. Immunohistochemistry was used to validate the extent of decellularisation and confirm the presence of an intact matrix.

Results: The success of the optimised protocol for decellularising the pancreas was evident by the absence of cellular structures and hormone and amylase staining. The vasculature remained intact as determined by injection of trypan blue dye into the cannulated portal vein. The intact pancreatic matrix scaffold was visualised by staining with antibodies to collagen I, collagen IV and fibronectin.

Conclusions: We have successfully optimised and validated a protocol for decellularisation of mouse pancreas, which will form the basis for future work testing the effect of this scaffold on stem cell differentiation.

P25

Donor HbA1c is inversely related to islet yield but does not impact on islet function at one year following islet transplantation in subjects with Type 1 diabetes

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Background: Islet transplantation is of proven efficacy in Type 1 diabetes. It is not understood how donor HbA1c independently impacts on islet yield and graft function following islet transplantation.

Aim: To assess the effect of donor HbA1c on islet yield and graft function at one year post islet transplantation.

Methods: Retrospective analyses of 46 islet isolations transplanted into 32 subjects with Type 1 diabetes. The relationships between donor HbA1c and (1) islet yield adjusted for donor weight and (2) recipient graft function at one year (beta scores) adjusted for islet yield and recipient and donor body weight were examined using multiple linear regression.

Results: Forty-six donors, age median (interquartile range) 45 (39 to 51) years, weight 85 (79 to 92) kg, body mass index 30.2 (27.0 to 33.3) kg/m^2 had HbA1c of 35 (33 to 37) mmol/mol; five had raised HbA1c (>41mmol/mol). Islet equivalents (IEQs) were 335,500 (292,000 to 406,000), purity 85 (73 to 90)% and viability 92 (88 to 95)%. Recipients with Type 1 diabetes received 9,205 (7,717 to 11,005) IEQ/kg recipient body weight (n = 5, ×1 transplant; n = 27 ×2 transplants). At one–year, beta scores were 3 (2 to 4). Univariate analyses showed a positive association between donor weight and IEQs isolated ($p = 0.03$), but a negative non-significant relationship between donor HbA1c and IEQs isolated ($p = 0.06$). When adjusted for donor weight, higher donor HbA1c was significantly associated with lower islet yields (IEQs) ($p = 0.002$). HbA1c did not significantly impact 12-month beta scores on univariate analyses or multivariate analyses adjusting for islet numbers and recipient and donor body weight ($p > 0.05$).

Conclusion: Higher HbA1c negatively impacts on islet yield but was not associated with reduced islet function at one year post transplant. Larger studies are needed to confirm these observations.

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P26

The SIV isoform of coxsackie–adenovirus receptor (CXADR) has an unexpected subcellular distribution in human pancreatic beta cells

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Aims and objectives: Human pancreatic beta cells are susceptible to infection by enteroviruses, especially Coxsackie B viruses, and such infections could contribute to the development of Type 1 diabetes. Enteroviruses gain entry via receptors, one of which, the coxsackie -adenovirus receptor (CXADR), is thought to be localised principally at the cell surface. CXADR exists as five isoforms, and we have studied their expression and distribution in the human pancreas.

Methods: Formalin-fixed paraffin-embedded pancreatic sections from 17 controls without diabetes and 6 patients with Type 1 diabetes were studied together with a human tissue microarray. RNA and protein analysis were used to examine the expression and cellular localisation of CXADR isoforms in human pancreas.

Results: One specific isoform of CXADR (SIV) was expressed preferentially in human beta cells at both the RNA and protein level. This isoform contains a unique C-terminal PDZ-binding domain and, surprisingly, it was distributed in a punctate manner mainly within the cytoplasm of the cells, rather than at the cell surface. Within the cytoplasm, it co-localised with ZnT8, PC1/3 and insulin (but less so with proinsulin), suggesting a distribution within secretory granules. This was confirmed directly by cryo-immunogold electron microscopy, which revealed that SIV is displayed principally on dense core granules in beta cells.

Conclusion: Human beta cells express mainly the SIV isoform of CXADR, but, unexpectedly, this is localised to insulin secretory granules rather than at the cell surface. We propose that beta cells

may become sensitive to enteroviral infection following translocation of CXADR to the surface, during exocytosis.

P27

Expression and function of the adhesion G protein-coupled receptors (GPCR), GPR56, in the developing pancreas

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Aims: It has been reported that G protein-coupled receptor 56 (GPR56) is highly enriched in Neurogenin-3 ($NGN3^+$) endocrine progenitors and we have shown that it regulates beta cell proliferation. Here, we used GPR56 knockout (KO) mice to investigate whether GPR56 is crucial for islet development.

Methods: The effect of GPR56 deletion on islet development was investigated using fluorescence immunohistochemical analysis of pancreas sections. Images were quantified by Image J.

Results: Immunostaining revealed that GPR56 was expressed by insulin promoter factor 1 ($PDX1^+$), $NGN3^+$ and $SOX9^+$ pancreatic endocrine progenitor cells. It was strongly expressed early in pancreas development and became down-regulated as the cells differentiated (% area $GPR56^+$ cells; E11 0.87 ± 0.04, E13 0.85 ± 0.06, E15 0.36 ± 0.08, E16 0.15 ± 0.05, $n = 10$ sections, $p < 0.05$). It was up-regulated postnatally, at the stage of beta cell replication (% area $GPR56^+$ cells; E18 0.15 ± 0.05, P9 0.47 ± 0.07, $n = 10$ sections, $p < 0.05$). At E16, there was an increased number of $NGN3^+$ progenitors in GPR56 KO pancreas ($NGN3^+$ cells/mm^2; wild type (WT) $3{,}012 \pm 211$, KO $3{,}880 \pm 266$, $n = 6$ to 12 replicates, $p < 0.05$), suggesting GPR56 deletion reduced differentiation. However, there was no change in proliferation of progenitor cells at E16 bromodeoxyuridine ($BrdU^+$ cells/mm^2; WT 475.8 ± 44.6, KO 448.2 ± 18.2, $n = 6$ to 12 replicates, $p > 0.2$). The number of cells proliferating and still in the cell cycle was significantly lower in KO islets at P9 ($BrdU^+Ki67^+$cells/μm^2; WT 115.9 ± 18.2, KO 50.9 ± 6.3, $n = 3$ mice/genotype, $p < 0.05$), leading to fewer beta cells (%beta cells/islet; WT 68.5 ± 0.8, KO 54.8 ± 3.0, $n = 3$ mice/genotype, $p < 0.05$), but higher numbers of alpha cells in GPR56 KO islets (% alpha cells/islet; WT 17.7 ± 0.9, KO 33.7 ± 2.8, $n = 3$ mice/genotype, $p < 0.01$).

Summary: Our data suggest that GPR56 may play an important role in islet development by modulating islet cell growth and differentiation.

P28 D

The calcium sensor sorcin maintains activating transcription factor 6 (ATF6) transcriptional activity while lowering ER stress

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Aims: Islet levels of the activating transcription factor 6 (ATF6), a key mediator of the unfolded protein response (UPR), are markedly reduced in Type 2 diabetes and in rodent models of the disease including *ob/ob* and high fat-fed mice. Sorcin is a calcium-binding protein involved in maintaining endoplasmic reticulum (ER) Ca^{2+} homeostasis. We have previously shown that sorcin overexpression protects beta cells from lipotoxicity. Here, we investigate 1) changes in sorcin expression during ER and lipotoxic stress and 2) ATF6 signalling after sorcin overexpression or inactivation.

Methods: Sorcin mRNA and protein levels were measured by quantitive reverse transcription-polymerase chain reaction (qRT-PCR) and Western blotting, respectively, in human islets, MIN6 and HEK293 cells. ATF6 activation was assessed in $Sri^{-/-}$and $Sri^{+/+}$ MEFs and in *SRI*-null HEK293 cells generated by CRISPR/Cas9-mediated genome editing. Three different luciferase reporter systems were used to assess ATF6 activity: endogenous ATF6 on p5xATF6-GL3 reporter, overexpressed GAL4-ATF6 fusion protein on GAL4 reporter (p5xGAL4-Elb-GL3) and ATF6 luminal domain fused to *Cypridina noctiluca* (ATF6LD-Cluc), a secreted luciferase.

Results: Sorcin mRNA levels were significantly increased in response to the ER stress-inducing agents thapsigargin and tunicamycin, but not by palmitate. Indeed, palmitate caused a significant decrease in sorcin expression as assessed by both qRT-PCR and Western blotting ($p < 0.005$). ATF6 activation was consistently significantly decreased in cells where sorcin was absent, despite an increase in ER stress, expected to stimulate ATF6.

Conclusion: These data suggest that sorcin down-regulation during lipotoxicity may prevent ATF6 activation and a normal UPR response in beta cells during the progression of obesity and insulin resistance.

P29

Olanzapine disrupts normal beta cell function in MIN6 cells

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Aims: There is a link between antipsychotic medication and Type 2 diabetes with approximately 40% of treated patients developing diabetes during treatment. Recent work aiming to elucidate this link has revealed some antipsychotic targets which promote hyperglycaemia. This work aimed to establish if there was an accompanying defect in the normal insulin response to induced hyperglycaemia to fully understand how these drugs cause Type 2 diabetes.

Methods: MIN6 beta cells were treated with different concentrations of olanzapine for up to 72h in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 2% Fetal Bovine Serum (FBS). Cell viability was established using 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) metabolism assay and Hoechst propidium iodide (HPI) staining. Low glucose (5.5mM) and glucose-stimulated (25mM) responses were measured using high range insulin enzyme-linked immunoabsorbent assay. Interaction with the mammalian target of rapamycin (mTOR) pathway was analysed using a combination of rapamycin and olanzapine in various functional assays.

Results: 72h incubation with 100μM olanzapine reduces MTT metabolism to approximately 36% (±4.8) of control ($p < 0.0001$) ($n = 4$). 48h incubation with 10μM olanzapine reduces glucose-stimulated insulin secretion from 11.39% (±0.7) of total insulin content to 6.84% (±0.5) ($p < 0.005$, $n = 3$)

Summary: High concentration olanzapine affects the function of beta cells by reducing MTT metabolism, indicating that there is a drug target which reduces viability or proliferation of beta cells and may promote diabetes. Our rapamycin data suggest that there

may be an interaction with the mTOR pathway, a key regulator of cell growth and metabolism. Glucose-stimulated insulin secretion was reduced by subtoxic concentrations of olanzapine; further work is now underway to investigate real-time insulin secretion with carbon fibre microelectrodes.

P30

CRISPR/Cas9-mediated engineering of insulin-secreting cells to study Type 2 diabetes risk variants of the gene *SLC30A8*/ ZnT8 the Zn2+ transporter

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Objectives: In recent years, genome-wide association studies have identified more than 90 genomic loci associated with higher Type 2 diabetes risk. Among the genes identified, *SLC30A8* encodes the Zn^{2+} transporter ZnT8, which resides on the secretory granule membrane in pancreatic beta cells, and is responsible for Zn^{2+} accumulation and insulin crystallisation. Importantly, a non-synonymous variant (rs13266634) at R325 influences Type 2 diabetes risk and Zn^{2+} transport activity. However, rare loss-of-function *SLC30A8* variants unexpectedly lower disease risk. Here, we use genome editing to explore the role of ZnT8 in controlling Zn^{2+} levels in beta cells.

Methods: CRISPR/Cas9-mediated genome editing technology was used to create ZnT8 null INS-1 (832/13) beta cell line, and mutation was confirmed by automated DNA sequencing. To assess the impact of gene deletion on intracellular Zn^{2+} zinc homeostasis, we deployed recently-developed Förster resonance energy transfer–based Zn^{2+} sensors, including cytosolic eCALWY-4 and secretory granule-targeted Vamp2-eZinCh-2 sensor.

Results: Cytosolic free Zn^{2+} concentrations were significantly decreased in INS-1-knockout vs control cells (1,010 $\pm$ 340 pM, 1,708 $\pm$ 223 pM, $p < 0.02$), in line with previous observations in primary beta cells from ZnT8 null mice. Furthermore, a significantly lower free Zn^{2+} content was apparent in insulin secreting granules, as reflected by a strong decrease in fluorescence intensity ratio (0.071 $\pm$ 0.005, 0.113 $\pm$ 0.007, $p < 0.0001$).

Conclusions: These findings confirm the importance of *SLC30A8*/ ZnT8 for normal Zn^{2+} homeostasis in beta cells. Engineered cells ZnT8 null cells will provide a substrate to assess the effects of re-expression of *SLC30A8* variants on Zn^{2+} metabolism, insulin secretion and survival of beta cells.

P31

Short-term inflammation improves glucose tolerance in mice by increasing the beta cell functional mass

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Aims: Chronic inflammation induced by metabolic stress has been implicated in the development of insulin resistance and glucose intolerance, leading to the development of Type 2 diabetes when beta cell compensatory mechanisms can no longer maintain glucose homeostasis. We have assessed the early beta cell compensatory responses to experimentally induced inflammation in mice.

Methods: Systemic inflammation was induced in male Balb/cJ mice by weekly injection of escalating doses (0.33 to 0.83 mg/kg) of lipopolysaccharide (LPS) for six weeks. Glucose homeostasis was assessed by intraperitoneal glucose and insulin tolerance tests, and beta cell proliferation and beta cell area were assessed by immunostaining for Ki67 and insulin.

Results: LPS treatment significantly increased beta cell proliferation over six weeks ($Ki67^+$/$insulin^+$: saline controls, 0.010 $\pm$ 0.001; LPS, 0.014 $\pm$ 0.002, $n = 3$, $p < 0.05$), causing an increase in the islet beta cell area (saline control 13,000 $\pm$ 900 μm^2; LPS, 15,000 $\pm$ 700). The increased beta cell mass was associated with improved glucose tolerance, with significantly ($p < 0.01$) reduced plasma glucose levels in LPS-treated mice at 30, 60 and 90 min after glucose administration. In contrast, insulin tolerance was unaffected, with insulin administration inducing similar reductions in plasma glucose in LPS- or saline-treated mice at all times measured (0 to 60 min).

Summary: Short-term LPS-induced inflammation stimulated beta cell proliferation and increased the beta cell mass, resulting in improved glucose tolerance without having any detectable effect on insulin sensitivity. Therefore, systemic inflammation is alone sufficient to influence the functional beta cell mass in the absence of systemic insulin resistance.

P32

Glycaemic variability induces beta cell dysfunction: The role of oscillations in glucose concentration

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Aims: It has long been established that tight glycaemic control ensures fewer diabetes-related complications. Hyperglycaemia is known to be damaging to pancreatic cell function, but the exact effects of glycaemic variability remain unclear. The aim of this study was to determine if persistent high glucose concentrations, or persistent periods of glycaemic variability (oscillating 'low to high' glucose concentrations) were more damaging to beta cell viability and function.

Methods: Min6 beta cells were cultured in traditional 2D monolayers or in 3D pseudo islets and maintained in low glucose (0.5mM), high glucose (25mM), or in oscillating glucose concentrations for 48h. A time course of oscillation periods were examined, initially utilising 12h oscillations. Cell viability was measured using the 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) metabolic activity assay, confocal microscopy and immunocytochemistry using a range of viability and beta cell functional markers.

Results: Beta cells cultured in oscillating glucose concentrations were shown to have significantly lower cell viability than cells cultured in static glucose concentrations ($p < 0.05$). Over a range of oscillation intervals, beta cells maintained in static 25mM glucose concentrations were significantly more viable than either cells maintained in static 0.5mM glucose ($p < 0.05$), or in oscillating glucose concentrations ($p < 0.05$).

Conclusion: Our study has shown that Min6 beta cells are significantly more damaged by oscillating glucose concentrations than by constant low or constant high glucose. Increased understanding of the potentially damaging role of glycaemic variability both *in vitro* and *in vivo* is fundamentally important in maintaining beta cell viability and function.

P33 D

Characterising the differential effects on viability of palmitic acid between a human and rodent-derived pancreatic beta cell line

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Aim: Long-chain saturated fatty acids (LC-SFAs), such as palmitate (C16:0), cause toxicity in rodent beta cells, but there have been suggestions that human beta cells may be more resistant to the toxic effects of LC-SFA. Therefore, we have compared the effects of palmitate in rat-derived INS-1 beta cells with those in the human beta cell line, EndoC-βH1.

Methods: EndoC-βH1 and rodent INS-1 cells were exposed to palmitate for increasing periods and viability assessed using vital dye staining. The early uptake and disposition of palmitate was studied with a fluorescent palmitate analogue, BODIPY FL C_{16}. The oxygen consumption rate of cells was measured using a Seahorse XF96^e analyser.

Results: Exposure of INS-1 cells to palmitate caused a dose-dependent (50 to 500 μM) loss of viability over 48h, whereas EndoC-βH1 cells were resistant to palmitate at concentrations up to 1mM (dead cells: control 7.0 ± 0.1%; C16:0 −9.6 ± 1.5%) and during exposure periods extending to at least 72h. The initial rate of BODIPY FL C_{16} accumulation was rapid in both cell types, reaching saturation within 5min. In INS-1 cells, minimal changes in cell respiration were measured during acute exposure and the labelled FA became concentrated in the Golgi apparatus. By contrast, within 5min of exposure, BODIPY FL C_{16} fluorescence was distributed in a punctate manner throughout the cytosol in EndoC-βH1 cells, a feature not seen in INS-1 cells.

Summary: The results imply that rodent and human beta cells may handle LC-SFA differently during acute exposure. This may account for their differential sensitivity to these molecules.

P34

The Type 2 diabetes genome-wide association study (GWAS) gene *STARD10* controls beta cell granule morphogenesis and proinsulin release

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Aims: Risk alleles in the *STARD10* locus, associated with Type 2 diabetes, impair glucose-induced insulin secretion and are associated with decreased proinsulin:insulin ratios. We have recently shown that the Type 2 diabetes risk associated with variation at this locus was most probably mediated by lowered *STARD10* expression in the beta cell. Here, we investigate the mechanisms by which STARD10, a lipid transfer protein, controls insulin processing and secretion.

Methods: Islets were isolated from StarD10$^{fl/fl}$:Ins1Cre mice (β*StarD10*KO) and littermate controls. RNA sequencing used an Illumina HiSeq 4000 machine. Transmission electron microscopy (EM) was performed after chemical fixation and ultramicrotome sectioning. For pulse-chase experiments, islets were labelled for 20min with ^{35}S-cysteine and methionine before chase at 5.5mM glucose for 1.5 or 4 h or after stimulation in 20mM glucose for 20min.

Results: RNA-Sequencing identified 88 regulated genes ($p < 0.05$, $n = 6$) in β*StarD10*KO islets, including genes implicated in exocytosis and calcium fluxes. EM analysis revealed markedly altered dense core granule appearance in β*StarD10*KO beta cells, with a >4-fold increase in granules containing "rod-like" structures (wild type (WT) 2.78 vs knockout (KO) 12.05% total granules; $p < 0.05$, $n = 3$). Pulse chase studies showed an increase in the secretion of newly synthesised proinsulin in the presence of low glucose after 4h of chase in KO vs WT islets ($p < 0.05$, $n = 4$).

Conclusion: These data imply roles for STARD10 in secretory granule morphology, trafficking and fusion with the plasma membrane. Constitutive release of newly formed proinsulin, enriching the regulated granule pool with fully processed insulin, may explain the apparent increase in prohormone processing observed in risk variant carriers.

P35

Corticotropin-releasing hormone: A role in regulating insulin secretion from male and female islets

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Aims: It has previously been shown that corticotropin-releasing hormone (CRH) can have effects on glucose homeostasis through both direct and indirect effects on the pancreatic islets. The presence of CRH and CRH-related peptide receptors on pancreatic islets suggests that CRH may have direct effects on islets, and CRH treatment has been shown to improve islet transplantation; however, these effects are currently poorly understood.

Methods: Expression of CRH and CRH receptors was confirmed in male and female CD1 mouse islets via quantitative polymerase chain reaction (qPCR). Effects of CRH on the dynamic insulin secretion were assessed using a temperature controlled perifusion system and insulin release was measured by radioimmunoassay.

Results: Islets of male and female CD1 mice express mRNA for CRH and both CRH receptor subtypes (CRHR1 and CRHR2), with a greater relative expression shown for CRHR1 (female CRHR1: 0.007 ± 0.003 vs CRHR2: 0.001 ± 0.001, male CRHR1: 0.003 ± 0.0004 vs CRHR2: 0.0008 ± 0.0004 mRNA expression ratio relative to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)). In a dynamic perifusion system, acute exposure to 50nM CRH at 20mM glucose potentiated glucose stimulated insulin secretion in both male and female islets, though this effect was more pronounced in male islets (264 ± 79% and 75 ± 22% increase compared with 20mM glucose alone in male and female islets respectively, $p < 0.001$).

Conclusions: Our data confirm that CRH directly potentiates insulin secretion from isolated mouse islets. The physiological purpose of this signal is unclear, and a possible role in the islet response to physiological and psychological stressors remains to be investigated.

Acknowledgement: Diabetes Research Group

P36 D

CB1 antagonists SR141617A and AM251 stimulate insulin secretion from mouse and human islets

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Aims: Cannabinoid receptor type 1 (CB1) and G protein-coupled receptor 55 (GPR55) are G protein-coupled receptors (GPCRs) expressed by islet beta cells that are coupled to regulation of insulin secretion. In this study, we evaluated the requirement for GPR55 in the effects of the CB1 antagonists/GPR55 agonists SR141617A and AM251 in mouse and human islets.

Methods: Islets from wild type (WT) C57BL6/J and GPR55 knockout (GPR55KO) mice and human donors were perifused in the absence and presence of SR141617A or AM251, and insulin secretion was determined by radioimmunoassay. Both compounds were also tested for G_s and G_i activities by quantifying cyclic Adenosine Monophosphate (cAMP) in mouse islets using a homogeneous time-resolved fluorescence immunoassay.

Results: A 10μM SR141617A potentiated insulin secretion from WT mouse islets (2.74 ± 0.3 pg/islet/min maximum increase above 20mM glucose response), and it also had stimulatory effects in GPR55KO islets: 6.42 ± 0.3 pg/islet/min maximum increase, n = 5 independent experiments. Similar effects were obtained with 10μM AM251 (WT 4.43 ± 0.6 pg/islet/min maximum increase above 20mM glucose response; KO 2.33 ± 0.8, n = 5 independent experiments). Stimulation of glucose-induced insulin secretion from human islets was also observed in response to 10μM SR141617A (control area under the curve (AUC) 671.0pg insulin/20 min; SR141617A 1,391, $p < 0.001$, n = 4) and AM251 (control AUC 524.4; AM251 1,124, $p < 0.01$, n = 4). 10μM SR141617A and AM251 did not cause cAMP accumulation (basal 1.99 ± 0.2nM cAMP; SR141617A 2.27 ± 0.1; AM251 2.89 ± 0.3, $p > 0.2$, n = 6) nor cAMP reduction (1μM forskolin 36.1 ± 2.1nM; SR141617A 36.4 ± 2.6; AM251 32.7 ± 1.94, $p > 0.2$, n = 6), indicating that they did not signal through G_s or G_i in mouse islets.

Conclusion: SR141617A and AM251 potentiate insulin secretion in a GPR55-independent manner, and they do not couple to G_s or G_i pathways in mouse islets.

Acknowledgement: Diabetes Research Group

P37 D

Development of multiplex methylation-sensitive strategy to detect beta cell death in Type 1 diabetes

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Aims: In Type 1 diabetes, beta cells destroyed by the immune system release cell-free DNA (cfDNA) into the circulation. Some cfDNA fragments derived from the beta cell epigenome exhibit unique methylation patterns. Detection of methylation-specific cfDNA offers the potential to monitor the rate of beta cell death but requires improved sensitivity. The aim of this study was to identify novel targets to facilitate a multiplex assay to quantify beta cell death.

Methods: DNA methylation patterns in islets (n = 5) and peripheral blood mononuclear cell (PBMC) (n = 3) samples were tested using the genome-wide Illumina EPIC methylation array. Data were analysed using Linear Models for Microarray (LIMMA) analysis in R software. Taqman assays were developed by multiplexing combinations of multiple beta cell specific, methylation-sensitive markers and tested using droplet digital PCR (dPCR, Bio-Rad). Tissue specificity was tested in DNA extracted from multiple tissues.

Results: Genome-wide comparison of methylation patterns between islets and PBMC identified 3,263 individual CpG sites and 49 regions that reached epigenome-wide significance ($p < 10E-10$). Of these, 425 unique hypomethylated and 228 hypermethylated sites in human islets were identified. Combinations of targets were tested to identify three tissue-specific markers which could be analysed simultaneously without cross-reactivity. Compared with singleplex amplification, triplex ddPCR yielded improved discrimination between islet/beta cells and non-islet tissues ($p < 0.01$).

Conclusions: Genome-wide methylation array has provided multiple novel targets to increase sensitivity to directly measure the rate of beta cell death. These targets require further validation in peripheral samples from individuals with and "at risk" of Type 1 diabetes.

P38

miR-125b is a new regulator of pancreatic beta cell function

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Aims: miRNAs are small non-coding RNAs that repress gene expression post-transcriptionally. Hundreds of miRNAs are expressed in beta cells and their depletion alters beta function, identity and survival resulting in hyperglycaemia. Nevertheless, the specific function of most beta cell miRNAs remains unknown. MiR-125b is up-regulated in islets from mice with beta cell-specific ablation of Adenosine Monophosphate (AMP)-activated protein kinase (AMPK; Martinez-Sanchez et al, unpublished) an enzyme involved in energy homeostasis and a regulator of beta cell function and survival. Here, we explore the impact of miR-125b over-expression on these cells.

Methods: Expression, content and secretion of insulin, proliferation and apoptosis were measured in beta cell lines transfected with miR-125b or control mimics (Exiqon) using standard protocols. RNA-seq was performed on an Illumina platform (HiSeq 4000). EnrichR was used for gene ontology (GO) analysis.

Results: Over-expression of miR-125 altered MIN6 and EndoC-betaH1 morphology and reduced insulin content (0.6-fold, $p < 0.0001$ in MIN6, 0.9-fold, $p = 0.08$ in EndoCbetaH1) and apoptosis (0.7-fold, $p < 0.05$ in MIN6). MiR-125b overexpression in MIN6 cells resulted in down- and up-regulation of 298 and 309 genes, respectively. GO analysis suggest that miR-125b regulates pathways important for beta cell function and survival, such as mitogen activated activated protein kinase (MAPK) signalling, oxidative stress, metabolism and endocytosis.

Conclusions/perspectives: Our findings show that miR-125b is an important regulator of beta cell function and survival. Future experiments will explore the role of this miRNA in human islets and in mouse models of inducible beta cell specific miR-125b overexpression or inhibition.

P39 D

Monomeric and dimeric forms of extracellular nicotinamide phosphoribosyltransferase (eNAMPT) have contrasting roles in the regulation of insulin secretion

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Background and aims: Serum levels of extracellular nicotinamide phosphoribosyltransferase (eNAMPT) (visfatin/PBEF) are elevated in Type 2 diabetes, suggesting a role in pathophysiology. However, other studies report that eNAMPT exerts beta cell protective effects. eNAMPT predominantly exists in dimeric form; however, we have reported elevation of a monomeric form in mouse models of Type 2 diabetes, suggesting a role for eNAMPT monomer in Type 2 diabetes pathophysiology. Here, we examined the effects of physiological (>1ng/ml) and pathophysiological (<5ng/ml) levels of eNAMPT dimer and eNAMPT monomer on islets.

Methods: Isolated mouse and human islets were treated with monomeric or dimeric eNAMPT (24 to 72 h; 0.5 to 10 ng/ml). We assessed islet glucose-stimulated insulin secretion (GSIS; static and dynamic); calcium flux ($[Ca^{2+}]_{cyt}$; Fura-2 confocal imaging); apoptosis (Caspase-Glo 3/7 activity); beta cell proliferation (BrdU incorporation) and gene expression (qPCR).

Results: Glucose-stimulated insulin secretion (GSIS) and intracellular $[Ca^{2+}]_{cyt}$ were significantly increased in islets treated with 1ng/ml eNAMPT dimer ($p < 0.05$, 0.01, respectively). In contrast, treatment with higher concentrations of eNAMPT dimer (<5ng/ml) impaired GSIS ($p < 0.05$) and $[Ca^{2+}]_{cyt}$. Correspondingly, apoptosis ($p < 0.05$) and inflammatory gene expression monocyte chemoattractant protein-1 (MCP1: $p < 0.01$, Il1β: $p < 0.05$) were also enhanced following exposure to <5ng/ml eNAMPT dimer. In contrast, islets treated with eNAMPT monomer displayed reduced GSIS and increased inflammation ($p < 0.001$).

Conclusions: The contrasting effects of eNAMPT on beta cell function may be explained by the presence of structurally and functionally distinct monomeric and dimeric forms. eNAMPT monomer predominates in Type 2 diabetes and mediates beta cell failure, while eNAMPT dimer predominates during normal physiology and maintains normal beta cell function. We also hypothesise that at high concentrations, eNAMPT dimer breaks apart leading to elevated levels of pathophysiological eNAMPT monomer.

P40

Adenosine Monophosphate (AMP)-activated protein kinase (AMPK) regulates the expression of miR-184 and other miRNAs important for beta cell function

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Aims: AMP-activated protein kinase (AMPK) is an energy sensor suggested to mediate the effects of certain anti-diabetic agents. In beta cells, AMPK is reduced by elevated glucose concentrations, and beta cell-selective AMPK-inactivation in mice (AMPKdKO) impairs beta cell identity and insulin secretion. miRNAs are small non-coding RNAs that silence gene expression, are essential for beta cell function and altered in Type 2 diabetes. Here, we aim to identify miRNAs involved in AMPK signalling in human and mouse beta cells.

Methods: miRNAs were profiled using real time quantitative polymerase chain reaction (RT-qPCR)-based arrays (Exiqon) in islets of mice with floxed AMPKα1 and α2 alleles expressing Ins1-Cre (AMPKdKO) or not (Control). miRNA-target prediction and gene ontology (GO) enrichment analysis were performed with TargetScan and EnrichR.

Results: Fourteen miRNAs were down-regulated and nine up-regulated in AMPKdKO islets (FDR < 0.15). GO analysis of predicted targets revealed enrichment in pathways important for beta cell function and identity. The most down-regulated miRNA, miR-184, is an important regulator of compensatory expansion that is down-regulated by glucose and reduced in Type 2 diabetes. Forced AMPK activation with the pharmacological agents C-199 and C-13 in human islets increased miR-184 expression, indicating conserved AMPK-dependent regulation in man. Finally, we demonstrate that AMPK is required for glucose-dependent down-regulation of miR-184 *in vitro* and *in vivo* and report sexual dimorphism in miR-184 expression in mouse and human islets.

Conclusions: AMPK is a critical mediator of the negative effects of glucose on miR-184 levels and regulates the expression of miRNAs predicted to control beta cell function and identity. A glucose-AMPK-miR184 pathway may contribute to beta cell dysfunction in Type 2 diabetes.

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P41

Islet transplantation improves islet function in a monogenic mouse model of diabetes

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Aims: Heterozygous male *Ins2*$^{+/G32S}$ mutant mice develop severe hyperglycaemia by five weeks of age, most likely due to misfolded insulin causing aberrations in islet function. This mutation causes neonatal diabetes in humans and therefore presents as a potentially translatable mouse model of diabetes. The aim of this study was to investigate whether endogenous islet function can be improved in these animals by restoring normoglycaemia.

Methods: Islets were isolated at 20 weeks and insulin secretion assessed at basal 2-mM glucose and stimulatory 20-mM glucose; 500 islets were transplanted under the kidney capsule of diabetic *Ins2*$^{+/G32S}$ males at 12 weeks. Eight weeks later, the endogenous pancreatic islets were isolated from the transplanted animals and secretion studies were performed.

Results: Heterozygous males at 20 weeks had a 91% reduction in glucose stimulated insulin secretion (0.138 ± 0.03 ng/islet/h vs wild-type: 1.47 ± 0.41 ng/islet/h; $p < 0.001$, n = 5 to 7) and a 96% reduction in insulin content (2.11 ± 0.8 ng vs wild-type: 48.4 ± 10.8 ng; $p < 0.001$, n = 5 to 7). Age-matched transplanted *Ins2*$^{+/G32S}$ mice achieved normoglycaemia for at least four weeks prior to isolating their endogenous islets for functional studies. Pancreatic islets from these mice showed a fourfold improvement of glucose stimulated insulin secretion (0.40 ± 0.09 ng/islet/h, n = 6) and partial recovery of insulin content (15.65 ± 7.69 ng, n = 6) compared with the non-transplanted heterozygous males.

Conclusion: The *Ins2*$^{+/G32S}$ mutation causes a severe impairment of islet function in male mice which is partially recovered following islet transplantation and restored normoglycaemia. This indicates that the impaired islet function in these mice is exacerbated by hyperglycaemia.

P42 D

Measuring real-time islet blood vessel responses to hormonal challenges using the platform of transplanted islets in the anterior chamber of the murine eye

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Aim: Transplantation into the anterior chamber of the eye (ACE) serves as a novel platform for longitudinal *in vivo* imaging of islet biology. Here, we use this approach to assess the time course effects of acute hormonal challenges on islet blood vessel dynamics *in vivo*.

Methods: C57BL6 mice received syngeneic donor islets (n = 12, 2 to 5 islets per animal) into the ACE. Following full implantation (≥4 weeks), mice were anaesthetised (isoflurane) in a stereotaxic frame and the implanted islets visualised with confocal microscopy (CrestOptics spinning disk, 20× water dipping 1.0 NA objective; Nikon eclipse Ti microscope). Islet vasculature was visualised following tail vein injection of TexasRed-conjugated dextran. At different imaging sessions, mice randomly received 0.2-ml intraperitoneal injections of saline, noradrenaline (1mg/kg) or glucagon (1mg/kg). Z-stacks (ex.:561nm, 1-µm increments) were acquired at 5-min intervals post injection up to 30min. Images were analysed with ImageJ and Huygens software.

Results: Noradrenaline did not produce a global change in islet blood vessel calibre during the imaging period; however, subanalysis of individual vessels may produce evidence of shunting which will be further explored. Glucagon injection resulted in a sustained increase in islet blood vessel surface area from 5 to 30 min post injection.

Conclusion: We demonstrate that the ACE is a useful platform for studying real-time islet vascular responses, with great potential. Even though glucagon is also considered a "stress hormone", we provide evidence for divergent effects on islet blood vessel responses with noradrenaline.

P43

Glucose regulates miR-125b expression via AMP-activated protein kinase (AMPK)

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Aims: miRNAs are ~22 nucleotide RNAs that decrease the expression of target mRNAs and are critical regulators of beta cell function. AMPK acts as a cellular energy rheostat that is inactivated at high-glucose concentrations. A recent unbiased screen (Martinez-Sanchez et al, unpublished) indicated that specific inactivation of AMPK in mouse beta cells increases miR-125b expression. Here, we aimed to confirm the regulation of miR-125b by AMPK in mouse and human islets and to determine whether the expression of this miRNA is regulated by glucose through AMPK.

Methods: MiR-125b was measured by real time quantitative polymerase chain reaction (RT-qPCR) in islets from wild-type or mutant mice or human donors without diabetes. Target prediction was performed with TargetScan. MiR-125b overexpression was achieved by transfection of miR-125b mimics (Exiqon).

Results: MiR-125b expression increased significantly (1.2- to 1.3-fold, $p < 0.05$) in mouse and human islets cultured for 48h at high (11 to 25 mM vs 3 mM) glucose concentrations. These effects were abolished in mouse islets with beta cell-specific deletion of AMPK (AMPKdKO), indicating a central role of AMPK in mediating the effects of glucose. Conversely, miR-125b expression was reduced in human islets treated with the pharmacological AMPK activators C-13 and C-991 and in islets of mice fed a ketogenic (low sugar) diet. Forced overexpression of miR-125b in MIN6 and EndoCβH1 cells reduced the levels of predicted targets *Bmf* and *Hnf4g* involved in beta cell apoptosis and differentiation, respectively.

Conclusions: High glucose increases the expression of miR-125b by inhibiting AMPK. miR-125b may, therefore, contribute to the deleterious effects of hyperglycaemia on beta cell survival and differentiation.

P44

Characterising the role of ghrelin and the growth hormone secretagogue receptor Type 1a (GHSR1a) antagonist, YIL781, in the regulation of beta cell survival

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Aims: Ghrelin reportedly acts via GHSR1a to influence beta cell function. However, the receptor expression in beta cells remains controversial. The aims of this study were to evaluate the effect of the acyl-ghrelin (AG) on beta cell survival and to determine the expression of ghrelin and GHSR1a in MIN6 beta cells and islets.

Methods: Islet and MIN6 cells were pretreated with AG or the GHSR1a antagonist, YIL781 (5μM), for 48h prior to 20-h exposure to palmitate (0.5mM) or tumour necrosis factor-α (1,000U/ml) and interleukin-1β (50U/ml). Apoptosis was assessed by measurement of caspase 3/7 activity. mRNA expression was determined using qPCR.

Results: Ten or 100nM AG did not affect palmitate-induced caspase activity in MIN6 cells (20h, $n = 6$, $p > 0.2$ vs control) nor cytokine-induced apoptosis in islets (20h, $n = 6$; $p > 0.1$ vs control). However, YIL781 ± AG significantly reduced apoptosis in palmitate-treated MIN6 cells compared with control ($n = 6$; $p < 0.05$ YIL781 only; $p < 0.01$ YIL781+10nM AG; $p < 0.01$ YIL781+100nM AG) and in cytokine-treated islets compared with 100nM AG only ($n = 6$; $p < 0.05$, YIL781+100nM AG). GHSR1a mRNA was expressed in islets and traceable in MIN6 cells. Ghrelin mRNA was detected in both tissues. GHSR1a and ghrelin mRNA expression was down-regulated in islets following 48-h treatment with 100nM AG (GHSR1a: 53% reduction compared to controls, ghrelin: 95%), whereas GHSR1a (61% compared with control) but not ghrelin mRNA expression (108%) was down-regulated by 5μM YIL781.

Conclusion: Although GHSR1a is expressed in the islet, exogenous AG does not affect beta cell survival. The effect of the GHSR1a antagonist suggests a potential novel role for YIL781 in apoptosis.

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P45 D

The role of a long non-coding RNA at the *Pax6* locus in controlling beta cell identity and function

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Aims: *Pax6* is an essential transcription factor (TF) for endocrine development of both beta and alpha cells and is preferentially expressed in the latter. Long non-coding RNAs (lncRNA) regulate expression of several beta cell TFs (*Pdx1*, *Nkx2.2*). Here, we examine the role of a lncRNA (*Pax6os1*) expressed from the *Pax6* locus in beta cell function.

Methods: *Pax6os1* expression was silenced in MIN6 beta cells using small-interfering RNAs (siRNA). RNA sequencing was performed on an Illumina platform (HiSeq 2000) and differentially expressed genes identified with DESeq2. Cell proliferation was assessed using a Cyquant kit. Subcellular fractionation was performed by differential centrifugation.

Results: Silencing of*Pax6os1* led to a 25 ± 0.07% ($n = 9$ experiments, $p = 0.0015$) increase in *Pax6* expression. RNA-seq revealed differential expression of genes involved in beta cell identity, including 'disallowed' beta cell genes. Cellular proliferation was significantly reduced ($p < 0.05$) 72h post transfection. *Pax6os1* was enriched 2.5-fold ($p = 0.0001$, $n = 3$) within the cytoplasm, while localisation of *Pax6* mRNA also tended to be preferentially (1.6-fold) enriched in the nuclear fraction. mRNA encoding other islet TFs (*Pdx1*, *Nkx6.1*) showed no preferential retention in the nucleus. Cytoplasmic localisation of *Pax6os1* and *Pax6* were enhanced by 6h incubation at elevated (5 vs 25 mmol/l) glucose concentrations.

Conclusions: Our findings indicate that *Pax6os1* is a negative regulator of *Pax6* expression. Interestingly, *Pax6* mRNA sequestration in the nucleus, and *Pax6os1* action, thus appears to attenuate *Pax6* expression and function in the beta cells. Enhanced *Pax6* function during hyperglycaemia may facilitate a drift in beta cell identity towards an alpha-cell fate.

P46

Transcriptomic analysis of dedifferentiated pancreatic beta cells

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Aim: Pancreatic beta cell dedifferentiation has been revealed as a novel mechanism of insulin insufficiency in Type 2 diabetes. Previous work from our group revealed increased microRNA-7 (miR-7) expression in islets of mouse models of Type 2 diabetes. Mice with beta cell-specific overexpression of miR-7 (Tg7) develop hyperglycaemia and display decreased expression of beta cell identity markers, revealing miR-7 induction as a novel mechanism triggering beta cell dedifferentiation. Here, we perform RNA sequencing on Tg7 islets to gain further insights into the molecular mechanisms driving beta cell dedifferentiation in Type 2 diabetes.

Methods: High throughput RNA sequencing was performed on pancreatic islets from 2 (non-diabetic) and 12 (diabetic) week old Tg7 mice. Gene set enrichment analysis (GSEA) was performed to identify over-represented signalling pathways. Quantitative PCR and immunofluorescence were used to validate gene expression *in vitro* and *in vivo*.

Results: GSEA analyses revealed a down-regulation of genes involved in protein secretion and pancreatic beta cell identity in Tg7 islets. Interestingly, Tg7 islets exhibited up-regulation of genes involved in epithelial to mesenchymal transition (EMT) and stemness. A subset of mesenchymal and progenitor cell genes were also reactivated in Tg7 islets pointing to increased islet cell plasticity in mutant mice. In agreement with this, diabetic Tg7 mice displayed an increased number of bihormonal cells co-expressing insulin and glucagon (Ins+/Gcg+). Ongoing genetic lineage tracing experiments will disclose the origin of bihormonal cells.

Conclusion: Our data indicate that beta cell dedifferentiation in Type 2 diabetes is associated with an EMT process, which could provide increased plasticity to pancreatic islet cells.

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P47

Anti-inflammatory effects of epoxyeicosatrienoic acids (EETs) and their corresponding diols against pro-inflammatory cytokine toxicity in BRIN-BD11 cells

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Introduction: CYP450-derived EETs display anti-inflammatory activity in cardiac models of inflammation, in part through activation of Peroxisome proliferator-activated receptor gamma (PPARγ) and inhibition of Nuclear Factor kappa B (NF-kB) activation. Therefore, we aimed to investigate the cytoprotective effects of EET regioisomers and their corresponding vicinal diols in a beta cell model of pro-inflammatory cytokine toxicity.

Methods: BRIN-BD11 cells were treated with 100U/ml interleukin-1β, 20U/ml Interferon gamma (IFNγ) and 500U/ml tumour necrosis factor-α in co-incubation with either 10μM 8 (9)-EET, 11 (12)-EET, 14 (15)-EET, or their dihydroxyeicosatrienoic acid derivatives (DHETs) for 24h. Cell viability was assessed by vital dye exclusion (Trypan Blue; expressed as viable cells/ml) or multicaspase-activity assay and NF-kB activity was measured using a NanoLuc® luciferase reporter assay.

Results: All EETs protected against cytokine-induced cell death, such that cytokines decreased viable cell number from 0.79×10^6 to 0.33×10^6 and in co-incubation with 8 (9)-EET, 11 (12)-EET and 14 (15)-EET, this increased to 0.64×10^6, 0.61×10^6 and 0.59×10^6, respectively ($p < 0.05$). Similarly, cytokine treatment increased caspase activity to 35% (±5.7), decreasing to 18% (±1.9), 19% (±2) and 19% (±1.9) in the presence of these EETs ($p < 0.05$), accompanied by a 32% decrease in NF-kB activation. Of the corresponding diols only, 8 (9)-DHET attenuated cytokine toxicity, reducing caspase activity from 41% (±4.5) to 21% (±2.4) ($p < 0.05$).

Conclusion: EETs protected against cytokine toxicity in BRIN-BD11 cells, in part, via reduced activation of NF-kB. We also consider the novel observation that 8 (9)-DHET, unlike other EET-derived DHETs, similarly protected against cytokine-induced apoptosis. These data highlight a potential role of EETs in attenuating cytokine toxicity in Type 1 diabetes, and our ongoing work is exploring the production of endogenous EETs by CYP450 isoforms and actions of EET analogues.

P48 D

Identification of potential hub beta cells using single-cell RNA-Seq

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Aims: Imaging of calcium dynamics in intact islets combined with network analysis has identified highly connected 'hub' (pacemaker) beta cells which appear to have a role in coordinating beta cell activation. The advent of single-cell RNA-Seq (scRNA-Seq) provides the opportunity to identify the transcriptome of potential hub cells and thus infer specialised features of these cells.

Methods: Some characteristics of hub cells had previously been identified: high glucokinase expression and low PDX1 and INS expression. These criteria were used to identify potential hub cells in scRNA-Seq data from both mouse (GSE77980) and human islets, and their transcriptomes were compared with other beta cells.

Results: Despite the limited dynamic range of scRNA-Seq technologies, potential hub cells were identified in both datasets. Depending on the thresholds used, up to 16% of mouse beta cells (51/313) were identified as potential hubs, whereas only 5% were identified in the human dataset (18/339). Among genes identified as differentially expressed in hub cells, that encoding a mitochondrial pyruvate carrier (*MPC2*) was up-regulated in human hub cells, with a similar but non-significant trend being observed in the mouse data. This is potentially indicative of the higher mitochondrial activity observed in hub cells.

Conclusions: These data demonstrate the potential of using scRNA-Seq data to identify sub-populations of beta cells with different functional properties.