Effects of first-line diabetes therapy with biguanides, sulphonylurea and thiazolidinediones on the differentiation, proliferation and apoptosis of islet cell populations

Dipak Sarnobat, R Charlotte Moffett, Peter R Flatt and Andrei I Tarasov*

School of Biomedical Sciences, Ulster University, Cromore Road, Coleraine, Northern Ireland, BT52 1SA, UK

*corresponding author, email: a.tarasov@ulster.ac.uk

ORCID IDs

DS: https://orcid.org/0000-0003-0416-5083

CRM: https://orcid.org/0000-0002-7196-7863

PRF: https://orcid.org/0000-0001-8548-7943

AIT: https://orcid.org/0000-0002-8883-176X

Running title: β-cell depletion rescued by oral hypoglycaemic agents

Key words: oral hypoglycaemic agents, beta-cell proliferation, transdifferentiation

Word count: 3,133 (main body); 245 (Abstract)

With 47 references, 3 Figures, 1 Suppl figure, 2 Suppl tables

Abstract

Aims. Metformin, rosiglitazone and sulfonylureas enhance either insulin action or secretion and thus have been used extensively as early-stage antidiabetic medication, independently of the aetiology of the disease. When administered to newly diagnosed diabetes patients, these drugs produce variable results. Here, we examined the effects of the three early-stage oral hypoglycaemic agents in mice with diabetes induced by multiple low doses of streptozotocin, focusing specifically on the developmental biology of pancreatic islets.

Methods. Streptozotocin-treated diabetic mice expressing a fluorescent reporter specifically in pancreatic islet α-cells were administered the biguanide metformin (100 mg/kg), thiazolidinedione rosiglitazone (10 mg/kg) or sulfonylurea tolbutamide (20 mg/kg) for 10 days. We assessed the impact of the treatment on metabolic status of the animals as well as on the morphology, proliferative potential and transdifferentiation of pancreatic islet cells, using immunofluorescence.

Results. The effect of the therapy on the islet cells varied depending on the drug and included enhanced pancreatic islet β-cell proliferation, in case of metformin and rosiglitazone; de-differentiation of α-cells and β-cell apoptosis with tolbutamide; increased relative number of β-cells and bihormonal insulin+glucagon+ cells with metformin. These effects were accompanied by normalisation of food and fluid intake with only minor effects on glycaemia at the low doses of the agents employed.

Conclusions. Our data suggests that metformin and rosiglitazone attenuate the depletion of the β-cell pool in the streptozotocin-induced diabetes, whereas tolbutamide exacerbates the β-cell apoptosis but is likely to protect β-cells from chronic hyperglycaemia by directly elevating insulin secretion.

Key words

Oral hypoglycaemic agents, streptozotocin-induced diabetes, α-cells,

Abbreviations

T1D (T2D) type 1 (2) diabetes mellitus; LADA, latent autoimmune diabetes of adults; YFP, yellow fluorescent protein; OHA, oral hypoglycaemic agent; STZ, streptozotocin; PPARγ, peroxisome proliferator-activated receptor type γ; K_{ATP} channel, ATP-sensitive potassium channel; AMPK, AMP-activated protein kinase.

Introduction

Type 2 diabetes (T2D) is a metabolic disease of increasing incidence fuelled by obesity and ageing demographics [1]. Corresponding to >80% of the less common type 1 diabetes (T1D) cases [2], latent autoimmune diabetes of adults (LADA) bears close clinical similarity with type 2 diabetes [3,4]. Thus, T2D and LADA patients receive an initial treatment with oral hypoglycaemic agents (OHA), which leads to variable results ranging from the attenuation to the progression of the phenotype [4].

The onset of severe diabetes in LADA and T2DM is associated with increased impairment of pancreatic islet hormone secretion, which directly impacts body's glucose homeostasis [5]. The latter is controlled by a concert of two islet antagonising hormones, insulin (secreted by β-cells) and glucagon (α-cells), that ensure glucose clearance from or recruitment into the systemic circulation, respectively. Loss of β-cells, typical to early stages of LADA [6] or later stages of T2DM, is believed to intensify the work of the surviving β-cell population [3], which enhances the expression of autoantibodies by β-cells, in LADA [7]. The depletion of β-cells has been also reported to induce transdifferentiation of other cell types into β-cells [8-10]. An unidentified signal triggering the compensatory mechanism [8] may involve changes in expression of transcription factors such as the increase in *Pdx1* [11], *Pax4* [12], *Ngn3*, *MafA* [13] or loss of *Arx* [14], *Men1* [15], *Dnmt1* [16]. The plasticity of highly committed pancreatic cells, especially the second-largest population of α-cells, is viewed as a tool for regeneration of the β-cell mass [8,17], an expectation strengthened by reports of therapeutically induced α-cell/β-cell transdifferentiation [15,18].

An OHA of the thiazolidinedione family with a proven effect on T2DM and LADA progression, rosiglitazone inhibits the activity of PPARγ, increasing insulin sensitivity

[19] and glucose uptake by adipose tissue and liver [20]. Thiazolidinediones have been also shown to impact various aspects of β-cell biology [21], such as mitochondrial metabolism [22]. Sulfonylurea tolbutamide targets pancreatic β-cells directly, by inhibiting the intracellular 'metabolic sensor' [23], ATP-sensitive K^+ channels, thereby triggering insulin secretion [24]. Historically the oldest OHA, biguanides are believed to impose their glucose-lowering effect by activating AMP-activated protein kinase (AMPK), which inhibits hepatic glucose production [25], possibly affecting the β-cell function [26].

In the current study, we examined the impact of three oral anti-diabetic agents used for early-stage treatment of both T2DM and LADA, rosiglitazone, tolbutamide and metformin, on proliferation and plasticity of pancreatic islet α-cell pool, under the conditions of severe β-cell loss. The latter was modelled in mice bearing an inducible fluorescent label in α-cells ($Glu^{CreERT2}$; ROSA26e-YFP) that were repeatedly treated with low doses of streptozotocin (STZ) to induce apoptosis in β-cells, which is expected to provide a critical signal to compensate for the β-cell loss.

Materials and methods

Animals

All experiments, carried out under the UK Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63EU, were approved by the University of Ulster Animal Welfare and Ethical Review Body. Animals were maintained in environmentally controlled rooms at 22±2°C with a 12h dark and light cycle and given *ad libitum* access to standard rodent diet (10% fat, 30% protein and 60% carbohydrate; Trouw Nutrition, Northwich, UK) and water.

$Glu^{CreERT2}$;ROSA26-eYFP mice

Nine-week old male $Glu^{CreERT2}$;ROSA26-eYFP transgenic mice were used to perform all studies. An original colony, developed on the C57Bl/6 background at the University of Cambridge [27], was subsequently transferred to the animal facility at Ulster University and genotyped to assess Cre-ERT2 and ROSA26eYFP gene expression (Table S1). Three days prior to STZ dosing, mice were injected with tamoxifen (i.p. 7 mg/mouse) to activate the tissue-specific expression of yellow fluorescent protein (YFP) in pancreatic islet α-cells (Figure 1A).

Diabetes model and antidiabetic medications

Our study was designed to evaluate direct effects of rosiglitazone, tolbutamide and metformin on islet morphology and cell transdifferentiation on background of sustained hyperglycaemia. To exclude the effects mediated by changes of insulin sensitivity or blood glucose (that affect islet composition and function [28,29]), we used mice with insulin-deficient diabetes [30] that was induced by a 5-day course of injections with STZ (Sigma-Aldrich, Dorset, UK; 50 mg/kg body weight daily, i.p.) (Figure 1A), dissolved in 0.1 M sodium citrate buffer (pH 4.5). The animals that underwent STZ injections and developed hyperglycaemia (non-fasting blood glucose>10 mM [31]) were then divided into 4 groups (n=6) and treated orally, once a day, with saline vehicle, rosiglitazone (TCI, Oxford, UK; 10 mg/kg), metformin (TCI, Oxford, UK; 100 mg/kg) or tolbutamide (Sigma-Aldrich, Poole, UK; 20 mg/kg) for 10 successive days (Figure 1A). The doses were selected on the basis of ameliorating milder genetic or high-fat-induced [32] but not STZ-induced [33] forms of diabetes, in order to elucidate the direct effects on islet cell plasticity. Food and fluid intake were assessed every 2 days, whereas blood glucose and body weight were assessed every 4 days. Non-

fasting plasma insulin and glucagon were determined at the termination of the study (day 10).

Blood glucose and hormone measurements

Blood samples were collected from the tail vein of animals into ice-chilled heparin-coated microcentrifuge tubes. Blood glucose was measured using a portable Ascencia meter (Bayer Healthcare, Newbury, Berkshire, UK). For plasma insulin and glucagon, blood was collected in chilled fluoride/heparin-coated tubes (Sarstedt, Numbrecht, Germany) and centrifuged using a Beckman microcentrifuge (Beckman Instruments, Galway, Ireland) for 10 minutes at 12,000 rpm. Plasma was then stored at -20ºC. For hormone determination from tissues, samples underwent acid-ethanol extraction (HCl: 1.5% v/v, ethanol: 75% v/v, H_2O:23.5% v/v). Insulin concentrations were subsequently assessed by an in-house radioimmunoassay [34]. Plasma glucagon and pancreatic glucagon content were measured using glucagon ELISA (EZGLU-30K, Merck Millipore), or RIA kit (250-tubes GL-32K, Millipore, USA), respectively.

Immunohistochemistry and imaging

Following the removal of pancreatic tissue, samples were cut longitudinally and fixed with 4% PFA for 48 hr at 4°C. Fixed tissues were embedded and processed for antibody staining as described [30]. Tissue sections (7 µm) were blocked with 2% BSA, incubated with respective primary antibodies overnight at 4°C, and, subsequently, with appropriate secondary antibodies (Table S2). To stain nuclei, a final incubation was carried out at 37°C with 300 nM DAPI (Sigma-Aldrich, D9542). To assess cell proliferation and/or apoptosis, co-staining of mouse anti-insulin (Abcam, Cambridge, UK; 1:1000; ab6995) or guinea pig anti-glucagon (PCA2/4, 1:200; raised in-house) with rabbit anti-Ki-67 (1:200; Abcam ab15580) or TUNEL reaction mixture

(Roche Diagnostics Ltd, UK) was used. YFP, indicating the α-cell lineage, was detected by with a rabbit anti-GFP antibody (1:1000; Abcam, ab6556) (Table S2), which is reactive against all variants of *Aequorea Victoria* GFP, including YFP. The slides were imaged on an Olympus BX51 microscope, equipped with a 40x/1.3 objective. We aimed to include all the islets visible on the slide in the morphometry analysis, independently of their localisation in relation to other pancreatic structures, with at least 50 cells analysed within each islet cross-section in the per-cell studies (Figure 2B, Figure 3). The multichannel fluorescence was recorded using DAPI (excitation 350 nm/emission 440 nm), FITC (488/515) and TRITC (594/610) filters and a DP70 camera controlled by Cell^F software (Olympus, UK). Images were analysed using ImageJ software. All counts were determined in a blinded manner with 60-150 islets analysed per treatment group, as indicated in the figure legends. The non-stained cells visible in the middle of the islet were not excluded from the computation of the islet area.

Data analysis and statistics

Statistical analysis was performed using PRISM 5.0 (GraphPad, U.S.A.) or R. Values are expressed as mean±SEM. Comparative analysis between experimental groups were carried out using independent-samples Student's t-test or (for >2 samples) a one-way ANOVA with Bonferroni's post-hoc. The difference between groups was considered significant for $p<0.05$.

Results

STZ-induced intake of food and fluid is partially rescued by the antidiabetic drugs

The treatment with STZ resulted in a progressive diabetic phenotype in the mice, which was reflected by the elevation of blood glucose concentration (Figure 1B). Non-fasting blood glucose increased in the STZ-treated mice from 8.2±0.4 mM (end of the STZ treatment) to 32.6±0.4 mM 14 days afterwards (7.6±0.7 and 8.4±0.6 mM, respectively, in the control group).

As designed, 10-day administration of rosiglitazone, tolbutamide or metformin had no significant impact on glycaemia (30.8±0.7, 31.3±0.4, 30.0±0.1 mM, respectively) (Figure 1B). The onset of hyperglycaemia coincided with the 9% decrease in the body weight from 19.2±0.4 g at the end of the STZ treatment (20.7±0.3 g in the control group, n.s.) to 17.4±0.4 g after 14 days (20.5±0.3 g in the control group, $p<0.05$) (Figure 1C). 10-day administration of rosiglitazone and tolbutamide had no statistically significant impact on body weight (17.9±0.6, 17.4±0.3 g, respectively, $p<0.05$ vs control), whereas metformin tended to exacerbate (16.7±0.6 g, $p<0.05$ vs control) the weight loss (Figure 1C).

The effects of STZ treatment on the intake of food or fluid by the experimental animals were palpable 4 days post its cessation (day 0, Figure 1D,E) and were progressively elevating from that point. Both food and fluid intake were significantly attenuated after four days of treatment with metformin (Figure 1D,E), coincident with the decrease in the body weight (Figure 1C). Neither of the remaining two OHA influenced fluid intake (Figure 1E), however rosiglitazone and, at one point, tolbutamide significantly attenuated the intake of food (Figure 1D).

As a result of the STZ treatment, the non-fasting terminal plasma insulin levels that were measured on day 10 were substantially decreased (0.16±0.06 vs 0.95±0.04 ng/mL in STZ-treated and control groups, P<0.01), whereas the differences between corresponding glucagon levels did not attain statistical significance (0.19±0.07 vs 0.32±0.11 ng/mL) (Figure 1F). Whilst none of the OHA elevated insulin levels (Figure 1F), metformin induced a significant decrease of plasma glucagon levels, on the STZ-treatment background (0.15±0.03 vs 0.32±0.11 ng/mL in the control group, p<0.05) (Figure 1F).

In line with the effect on plasma hormone levels (Figure 1F), STZ substantially decreased pancreatic content of insulin (27.5±9.9 vs 109.2±8.0 ng/mg of tissue in control, p<0.05), without any appreciable effect on the glucagon content (Figure 1G). Following subsequent rosiglitazone treatment, the glucagon content was substantially decreased (13.3±3.8 vs 22.7±4.2 ng/mg of tissue in control, p<0.05), whereas tolbutamide or metformin had no effect on this parameter (Figure 1G).

The alleviation of the diabetic phenotype is associated with a mild effect on the islet composition

We did not detect any significant alteration in the islet number, in response to any treatments (red in Figure 2A). At the same time, the observed decrease in plasma and pancreatic insulin (Figure 1F,G) coincided with the decrease in the average cross-section area of islets in the STZ-treated mice (black in Figure 2A). The OHA therapy that followed the STZ treatment resulted in a mild increase in this metric (black in Figure 2A).

The STZ treatment produced a significant reduction in the relative β-cell area (red/insulin+ in Figure 2B,C) and, respectively, an increase in the relative α-cell area

(black/glucagon+ in Figure 2B,C). Remarkably, a 10-day oral administration of metformin, but not rosiglitazone or tolbutamide, counter-acted the effects of the STZ treatment, resulting in small but significant differences in the percentage of β-cells (55±2% vs 48±2% in STZ mice, $p<0.05$) and α-cells (44±2% vs 51±2% in STZ mice, $p<0.05$) (Figure 2B,C). Interestingly, the islets from the STZ-treated animals contained a palpable fraction of cells that did not express insulin or glucagon (Figure 2C): we need to stress that, among other types, islets contain significant numbers of vascular endothelial cells [35,36], which are likely to contribute to this phenomenon.

Oral hypoglycaemic agents increase proliferation but have no effect on apoptosis of β-cells

In line with the report of STZ inducing β-cell apoptosis, when used in small repeated doses [37], we observed a six-fold (2.2±0.1 vs 0.4±0.1% in control mice, $p<0.05$) increase in the percentage of β-cells expressing an apoptosis marker, TUNEL, in STZ-treated mice (red, Figure 3A, Figure S1A). The metformin therapy tended to attenuate the β-cell apoptosis whereas tolbutamide further increased the expression of TUNEL by β-cells (3.2±0.3% vs 2.2±0.1 in the STZ-treated mice, $p<0.05$) (red, Figure 3A). Although the STZ treatment *per se* has not affected the apoptosis of α-cells (black, Figure 3A), metformin administered to the STZ-treated animals mildly increased this characteristic (0.5±0.1% vs 0.4±0.1% in the STZ-treated group, $p<0.05$) (black, Figure 3A, Figure S1A).

The pro-apoptotic effect of the STZ treatment did not affect the percentage of proliferating β-cells, as was assayed via Ki-67 staining (red, Figure 3B, Figure S1B). This metric was increased by subsequent treatment with rosiglitazone or metformin (4.0±0.2% and 2.5±0.4% respectively, vs 1.3±0.1% in the STZ-treated group, $p<0.05$).

The STZ treatment produced a 5-fold increase in the fraction of proliferating α-cells (black in Figure 3B, Figure S1B), which was not affected by any of the OHA (black in Figure 3B).

Long-term administration of oral hypoglycaemic agents does not affect α-/β-cell transdifferentiation

The key feature of the animal model used in this study, the $Glu^{CreERT2}$; ROSA26-eYFP mice, is the tissue-specific targeting (pancreatic α-cells) and the inducible nature of the expression of YFP. When co-detected with anti-glucagon antibodies, 20 days post induction of the targeted YFP expression, the islets from these mice had only a small fraction of YFP+ cells that did not express glucagon (0.3±0.1%) (black, Figure 3C, Figure S1C). The YFP+ cell percentage was increased almost 3-fold after the STZ treatment (0.8±0.4%, $p<0.05$ vs control) and further potentiated by tolbutamide (2.7±0.8%, $p<0.005$ vs control, $p<0.01$ vs STZ) but not rosiglitazone or metformin (0.9±0.4%, $p<0.05$ vs control and 1.2±0.4%, $p<0.01$ vs control, respectively) (black, Figure 3C, Figure S1C). Of note, we were unable to detect YFP in almost half of $glucagon^+$ cells, which we believe to reflect a technical feature of the anti-GFP antibody staining (red in Figure 3C).

The percentage of the $YFP^+insulin^+$ cells was low in the experimental animals with α-cell-specific targeting of YFP (0.6±0.1% in the control group). The treatment with STZ however triplicated this number (1.7±0.1%) (red, Figure 3D, Figure S1D). Neither of the OHA was able to further enhance the commitment of the YFP+ cells towards the insulin lineage (red, Figure 3D). At the same time, the administration of each of the OHA, following the STZ treatment, increased the percentage of bi-hormonal (insulin+glucagon+) cells (black, Figure 3D, Figure S1D). The size of this small cell

subpopulation was unaffected by the STZ treatment (0.27±0.01% vs 0.25±0.02% in the control group), whereas subsequent rosiglitazone (0.33±0.02%), tolbutamide (0.31±0.02%) and metformin (0.38±0.03%) administration substantially expanded it (black, Figure 3D).

Discussion

We probed the mechanisms whereby the oral hypoglycaemic agents may partially compensate for the selective apoptotic damage of β-cells. In our hands, pancreatic β-cell population was partially replenished via increased proliferation, in response to metformin or rosiglitazone, whereas tolbutamide exacerbated apoptosis, arguably by putting an extra demand on insulin production and secretion mediated by cytosolic Ca^{2+} [38].

Diabetic phenotype of the mice

The diabetes model and the OHA dosage were designed to resolve the *direct* effects of the OHA on pancreatic islet cell plasticity [39]. We have opted for repeated injections of small doses of STZ [9,40] over high-fat diet or leptin receptor deficiency animal models of diabetes to enable cell lineage tracing and rule out any indirect effects, mediated by changes in insulin sensitivity or blood glucose, that may impact the islet plasticity. The model animals displayed stably elevated glycaemia and reduced body weight (Figure 1B,C), whereas the three treatments, at the doses chosen, affected only food and fluid intake (Figure 1D,E). Notably, the doses used compare well with daily human recommended doses, given the differences in the pharmacokinetics of the three drugs in the mouse and human systems [41-43].

Islet mass, morphology, apoptosis and proliferation of β- and α-cells

A side effect of the OHA therapy, lowering of the systemic glucagon in response to metformin (Figure 1F) is unlikely to reflect the depletion of the α-cell population due to its transdifferentiation or apoptosis, as α-cells are well in excess, in rodent islets [35,44]. The phenomenon could have stemmed from the elevation of circulating GLP-1 levels, reported to be induced by metformin [45]. Another possible explanation for this effect is the activation of the intraislet GLP-1 secretion system [46-48], under the conditions of the STZ treatment [9,49]. The likely mechanism for that is the acquisition of the proconvertase PC1/3 activity by α-cells [46], with a subsequent shift in the α-cell secretory output from glucagon to GLP-1. In line with the reported cytostatic effect of metformin [50] that, in our hands, stimulated apoptosis in α-cells (but, surprisingly, given earlier reports [51], not in β-cells, Figure 3A), the elevation of intra-islet and circulating GLP-1 could explain partial recovery of the ratio of β- and α-cells (Figure 2B), presumably by upregulating β-cell proliferation [9,30]. Notably, rosiglitazone, reported to increase the β-cell mass by reversing the apoptosis [52], was not efficient in doing so in our model (Figure 3A). In our hands, it induced a 4-fold increase of the proliferating β-cells, in line with previous reports [52].

Effects of OHA on alpha cell transdifferentiation

The STZ-induced β-cell injury *per se* resulted in a detectable expression of insulin and a loss of expression of glucagon by YFP^+ cells (Figure 3C), reflecting the α-cell population before the STZ treatment. The fact that none of the OHA affected the co-expression of insulin and YFP on a per-cell basis, suggests the lack of a role in regulating α-/β-cell transdifferentiation (Figure 3D).

In the present study, a small but detectable number of bi-hormonal [8] cells was evident after the STZ treatment, followed by administration of metformin (Figure 3D). This effect can be explained by non-pancreatic signals [53] that may induce α-cell trans-differentiation. On the contrary, the dedifferentiation of α-cells by tolbutamide (Figure 3C), likely to result from a direct effect on the K_{ATP} channels, was not associated with any β-cell phenotype.

Relative merits of different OHAs

Since sulphonylureas are actively prescribed for diabetes, further elucidation of their global effects on islet function is highly relevant. No previous study has reported, to our knowledge, on the effects of this drug class on islet cell transdifferentiation. Our data with tolbutamide are important in revealing that not only does the sulphonylurea lack beneficial effects on islet plasticity (unlike the two other classes of OHA) but that it exerts adverse effects on β-cell health and apoptosis. This can be viewed as a significant limitation of first-line sulphonylurea therapy and would suggest that incretin mimetics which have recently shown to have positive effects on β-cell transdifferentiation, apoptosis and proliferation [9,29] would be a better therapeutic option for direct β-cell actions.

Conclusion

Alongside peptide hormones [9,40], small molecules have been shown to induce transdifferentiation of pancreatic α-cells into β-cells. Metformin and rosiglitazone but not tolbutamide promoted the restoration of the β-cell pool via proliferation, with none of the three oral antidiabetic drugs affecting the α-cell transdifferentiation induced by the loss of β-cells. In contrary, metformin decreased the islet α-cell population via apoptosis, whereas tolbutamide, in turn, enhanced apoptosis in β-cells. Whether these

drugs impose similar effects in humans, alongside the reported antioxidant [54] and insulinotropic [55] activity, remains the matter of future research.

Duality of Interest statement

Authors declare no conflicts of interest.

Acknowledgements

These studies were supported in part by Young Investigator Award from Diabetes UK to RCM and Ulster University Vice-Chancellor Research Studentship award to DS. We thank Fiona Gribble and Frank Reimann (Cambridge University) for the gift of $Glu^{CreERT2}$; ROSA26e-YFP mice.

References

[1] Chobot A, Górowska-Kowolik K, Sokołowska M, Jarosz-Chobot P (2018) Obesity and diabetes—not only a simple link between two epidemics. Diabetes/metabolism research and reviews 34: e3042

[2] Ahlqvist E, Storm P, Käräjämäki A, et al. (2018) Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. The lancet Diabetes & endocrinology 6: 361-369

[3] Cernea S, Buzzetti R, Pozzilli P (2009) β-cell protection and therapy for latent autoimmune diabetes in adults. Diabetes Care 32: S246-S252

[4] Liang H, Cheng Y, Tang W, et al. (2019) Clinical manifestation and islet β-cell function of a subtype of latent autoimmune diabetes in adults (LADA): positive for T cell responses in phenotypic type 2 diabetes. Acta Diabetologica 56: 1225-1230

[5] Moffett RC, Patterson S, Irwin N, Flatt PR (2015) Positive effects of GLP-1 receptor activation with liraglutide on pancreatic islet morphology and metabolic control in C57BL/KsJ db/db mice with degenerative diabetes. Diabetes/metabolism research and reviews 31: 248-255

[6] Jörns A, Wedekind D, Jähne J, Lenzen S (2020) Pancreas Pathology of Latent Autoimmune Diabetes in Adults (LADA) in Patients and in a LADA Rat Model Compared With Type 1 Diabetes. Diabetes 69: 624-633

[7] Björk E, Kämpe O, Andersson A, Karlsson F (1992) Expression of the 64 kDa/glutamic acid decarboxylase rat islet cell autoantigen is influenced by the rate of insulin secretion. Diabetologia 35: 490-493

[8] Thorel F, Népote V, Avril I, et al. (2010) Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss. Nature 464: 1149-1154

[9] Sarnobat D, Moffett CR, Tanday N, et al. (2020) Antidiabetic drug therapy alleviates type 1 diabetes in mice by promoting pancreatic α-cell transdifferentiation. Biochemical Pharmacology: 114216

[10] Chera S, Baronnier D, Ghila L, et al. (2014) Diabetes recovery by age-dependent conversion of pancreatic δ-cells into insulin producers. Nature 514: 503-507

[11] Yang Y-P, Thorel F, Boyer DF, Herrera PL, Wright CV (2011) Context-specific α-to-β-cell reprogramming by forced Pdx1 expression. Genes & development 25: 1680-1685

[12] Collombat P, Xu X, Ravassard P, et al. (2009) The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into α and subsequently β cells. Cell 138: 449-462

[13] Matsuoka T-a, Kawashima S, Miyatsuka T, et al. (2017) Mafa enables Pdx1 to effectively convert pancreatic islet progenitors and committed islet α-cells into β-cells in vivo. Diabetes 66: 1293-1300

[14] Wilcox CL, Terry NA, Walp ER, Lee RA, May CL (2013) Pancreatic α-cell specific deletion of mouse Arx leads to α-cell identity loss. PloS one 8

[15] Lu J, Herrera PL, Carreira C, et al. (2010) α Cell–Specific Men1 Ablation Triggers the Transdifferentiation of Glucagon-Expressing Cells and Insulinoma Development. Gastroenterology 138: 1954-1965. e1958

[16] Chakravarthy H, Gu X, Enge M, et al. (2017) Converting adult pancreatic islet α cells into β cells by targeting both Dnmt1 and Arx. Cell metabolism 25: 622-634

[17] Puri S, Folias AE, Hebrok M (2015) Plasticity and dedifferentiation within the pancreas: development, homeostasis, and disease. Cell stem cell 16: 18-31

[18] Ackermann AM, Moss NG, Kaestner KH (2018) GABA and artesunate do not induce pancreatic α-to-β cell transdifferentiation in vivo. Cell metabolism 28: 787-792. e783

[19] Boughton CK, Munro N, Whyte M (2017) Targeting beta-cell preservation in the management of type 2 diabetes. British Journal of Diabetes 17: 134-144

[20] Kavak S, Ayaz L, Emre M (2012) Effects of rosiglitazone with insulin combination therapy on oxidative stress and lipid profile in left ventricular muscles of diabetic rats. Experimental Diabetes Research 2012

[21] Gupta D, Kono T, Evans-Molina C (2010) The role of peroxisome proliferator-activated receptor γ in pancreatic β cell function and survival: therapeutic implications for the treatment of type 2 diabetes mellitus. Diabetes, Obesity and Metabolism 12: 1036-1047

[22] Jamwal S, Blackburn J, Elsworth JD (2020) PPARγ/PGC1α signaling as a potential therapeutic target for mitochondrial biogenesis in neurodegenerative disorders. Pharmacology & Therapeutics: 107705

[23] Dabrowski M, Tarasov A, Ashcroft FM (2004) Mapping the architecture of the ATP-binding site of the KATP channel subunit Kir6. 2. The Journal of physiology 557: 347-354

[24] Tarasov AI, Welters HJ, Senkel S, et al. (2006) A Kir6.2 mutation causing neonatal diabetes impairs electrical activity and insulin secretion from INS-1 beta-cells. Diabetes 55: 3075-3082

[25] Apostolova N, Iannantuoni F, Gruevska A, Muntane J, Rocha M, Victor VM (2020) Mechanisms of action of metformin in type 2 diabetes: Effects on mitochondria and leukocyte-endothelium interactions. Redox Biology: 101517

[26] Sun G, Tarasov A, McGinty J, et al. (2010) Ablation of AMP-activated protein kinase α1 and α2 from mouse pancreatic beta cells and RIP2. Cre neurons suppresses insulin release in vivo. Diabetologia 53: 924-936

[27] Campbell JR, Martchenko A, Sweeney ME, et al. Essential role of Munc18-1 in the regulation of glucagon-like peptide-1 secretion. https://tspacelibraryutorontoca/handle/1807/98201

[28] Brereton MF, Rohm M, Ashcroft FM (2016) β-Cell dysfunction in diabetes: a crisis of identity? Diabetes, obesity and metabolism 18: 102-109

[29] Tanday N, Flatt PR, Irwin N, Moffett RC (2020) Liraglutide and sitagliptin counter beta-to alpha-cell transdifferentiation in diabetes. Journal of Endocrinology 245: 53-64

[30] Vasu S, Moffett RC, Thorens B, Flatt PR (2014) Role of endogenous GLP-1 and GIP in beta cell compensatory responses to insulin resistance and cellular stress. PloS one 9

[31] Ando H, Ushijima K, Shimba S, Fujimura A (2016) Daily fasting blood glucose rhythm in male mice: A role of the circadian clock in the liver. Endocrinology 157: 463-469

[32] McKillop AM, Stevenson CL, Moran BM, Abdel-Wahab YH, Flatt PR (2018) Tissue expression of DPP-IV in obesity-diabetes and modulatory effects on peptide regulation of insulin secretion. Peptides 100: 165-172

[33] Tahara A, Matsuyama-Yokono A, Nakano R, Someya Y, Hayakawa M, Shibasaki M (2009) Effects of the combination of dipeptidyl peptidase-IV inhibitor ASP8497 and antidiabetic drugs in streptozotocin-nicotinamide-induced mildly diabetic mice. European journal of pharmacology 605: 170-176

[34] Flatt P, Bailey C (1981) Abnormal plasma glucose and insulin responses in heterozygous lean (ob/+) mice. Diabetologia 20: 573-577

[35] Tarasov AI, Galvanovskis J, Rorsman O, et al. (2018) Monitoring real-time hormone release kinetics via high-content 3-D imaging of compensatory endocytosis. Lab on a Chip 18: 2838-2848

[36] Adam J, Ramracheya R, Chibalina MV, et al. (2017) Fumarate Hydratase Deletion in Pancreatic beta Cells Leads to Progressive Diabetes. Cell Reports 20: 3135-3148

[37] O'BRIEN BA, Harmon BV, Cameron DP, Allan DJ (1996) Beta-cell apoptosis is responsible for the development of IDDM in the multiple low-dose streptozotocin model. The Journal of pathology 178: 176-181

[38] Efanova IB, Zaitsev SV, Zhivotovsky B, et al. (1998) Glucose and tolbutamide induce apoptosis in pancreatic β-Cells a process dependent on intracellular Ca2+ concentration. Journal of Biological Chemistry 273: 33501-33507

[39] Brereton MF, Iberl M, Shimomura K, et al. (2014) Reversible changes in pancreatic islet structure and function produced by elevated blood glucose. Nature communications 5: 1-11

[40] Sarnobat D, Moffett RC, Gault VA, et al. (2019) Effects of long-acting GIP, xenin and oxyntomodulin peptide analogues on alpha-cell transdifferentiation in insulin-deficient diabetic GluCreERT2; ROSA26-eYFP mice. Peptides: 170205

[41] Kulkarni NM, Malampati S, Mahat MYA, et al. (2016) Altered pharmacokinetics of rosiglitazone in a mouse model of non-alcoholic fatty liver disease. Drug metabolism and personalized therapy 31: 165-171

[42] Cox PJ, Ryan DA, Hollis FJ, et al. (2000) Absorption, disposition, and metabolism of rosiglitazone, a potent thiazolidinedione insulin sensitizer, in humans. Drug Metabolism and Disposition 28: 772-780

[43] Gross AS, Bridge S, Shenfield GM (1999) Pharmacokinetics of tolbutamide in ethnic Chinese. British journal of clinical pharmacology 47: 151-156

[44] Hamilton A, Zhang Q, Salehi A, et al. (2018) Adrenaline Stimulates Glucagon Secretion by Tpc2-Dependent Ca(2+) Mobilization From Acidic Stores in Pancreatic alpha-Cells. Diabetes 67: 1128-1139

[45] Bahne E, Sun EW, Young RL, et al. (2018) Metformin-induced glucagon-like peptide-1 secretion contributes to the actions of metformin in type 2 diabetes. JCI insight 3

[46] Marchetti P, Lupi R, Bugliani M, et al. (2012) A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets. Diabetologia 55: 3262-3272

[47] Taylor SW, Nikoulina SE, Andon NL, Lowe C (2013) Peptidomic profiling of secreted products from pancreatic islet culture results in a higher yield of full-length peptide hormones than found using cell lysis procedures. Journal of proteome research 12: 3610-3619

[48] Chambers AP, Sorrell JE, Haller A, et al. (2017) The role of pancreatic preproglucagon in glucose homeostasis in mice. Cell metabolism 25: 927-934. e923

[49] Campbell SA, Golec D, Hubert M, et al. (2020) Human islets contain a subpopulation of glucagon-like peptide-1 secreting α cells that is increased in type 2 diabetes. Molecular Metabolism: 101014

[50] Queiroz EA, Puukila S, Eichler R, et al. (2014) Metformin induces apoptosis and cell cycle arrest mediated by oxidative stress, AMPK and FOXO3a in MCF-7 breast cancer cells. PloS one 9: e98207

[51] Kefas BA, Cai Y, Kerckhofs K, et al. (2004) Metformin-induced stimulation of AMP-activated protein kinase in β-cells impairs their glucose responsiveness and can lead to apoptosis. Biochemical pharmacology 68: 409-416

[52] Holloway A, Petrik J, Bruin J, Gerstein H (2008) Rosiglitazone prevents diabetes by increasing beta-cell mass in an animal model of type 2 diabetes characterized by reduced beta-cell mass at birth. Diabetes, Obesity and Metabolism 10: 763-771

[53] Tarasov AI, Rorsman P (2016) Dramatis Personae in β-Cell Mass Regulation: Enter SerpinB1. Cell metabolism 23: 8-10

[54] Masini M, Anello M, Bugliani M, et al. (2014) Prevention by metformin of alterations induced by chronic exposure to high glucose in human islet beta cells is associated with preserved ATP/ADP ratio. Diabetes research and clinical practice 104: 163-170

[55] Lupi R, Marchetti P, Giannarelli R, et al. (1997) Effects of glibenclamide and metformin (alone or in combination) on insulin release from isolated human pancreatic islets. Acta diabetologica 34: 46-48

Figure legends

Figure 1 Rosiglitazone, tolbutamide and metformin partially rescue the diabetic phenotype of the streptozotocin-treated mice.

A: Experimental timeline. Antidiabetic treatment starts on day 0. Tamoxifen is fed to the animals 11 days prior to that to induce the tissue-specific expression of YFP in α-cells. STZ is administered to model type 1 diabetes for five successive days, 4 days before the start of the treatment. The ability of the latter to improve the diabetic phenotype is then assayed. *B, C, D, E:* Non-fasting blood glucose (*B*), body weight (*C*), food (*D*) and fluid (*E*) intake of $Glu^{CreERT2}$;ROSA26-eYFP mice, following STZ treatment and the administration of antidiabetic drugs, as indicated, for groups of n=6 mice each. 'STZ', streptozotocin; 'Rosi', rosiglitazone; 'Tolb', tolbutamide; 'Metf', metformin; 'Ctl', saline control. *F:* plasma insulin (red) and glucagon (black) *G:* pancreatic insulin (*red*) and glucagon (*black*) content. *F, G* measurements were done on day 10, in separate groups of mice. *p<0.05, **p<0.01 and ***p<0.001 compared to saline control group. Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 compared to the STZ group.

Figure 1

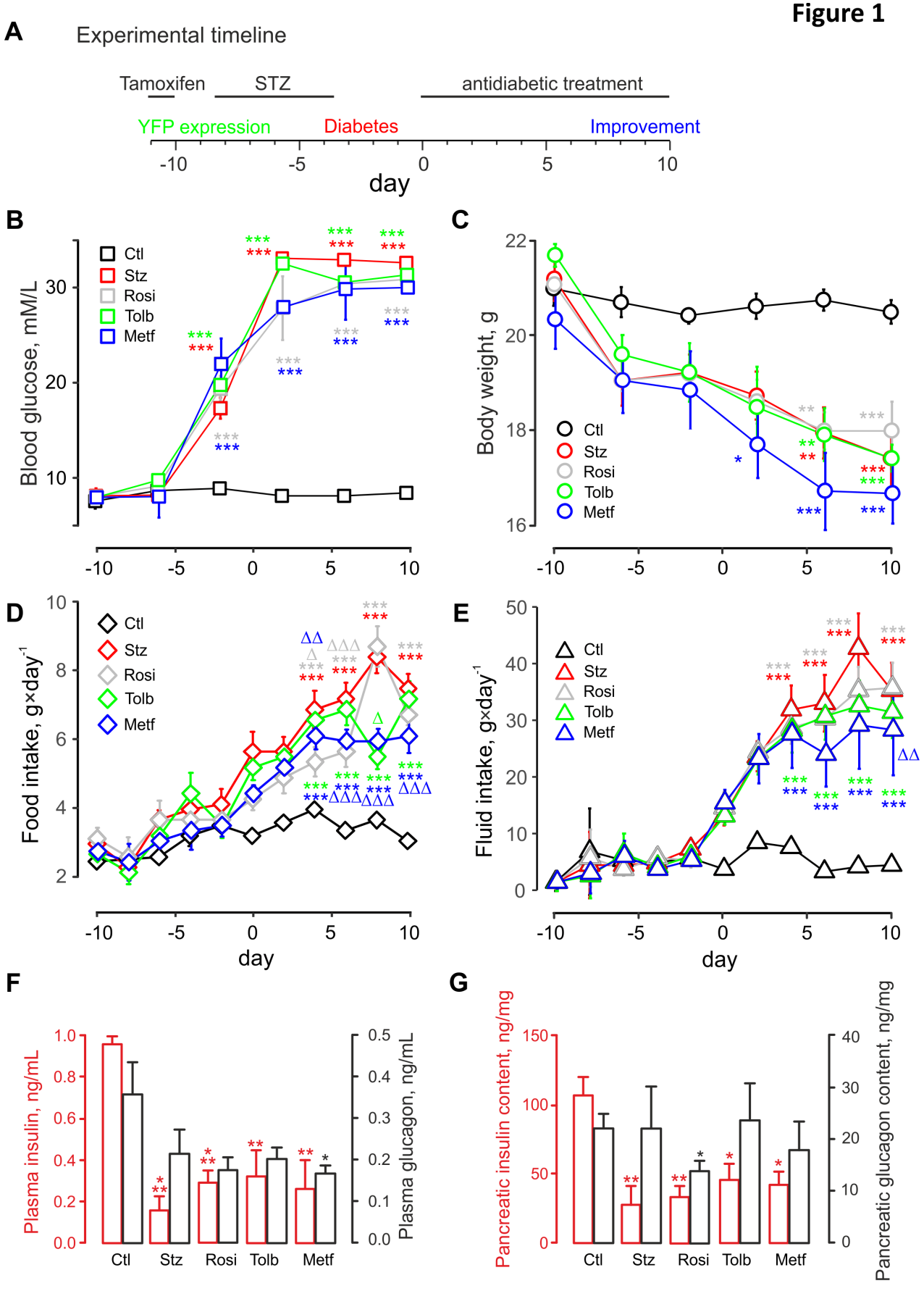

A
Experimental timeline
Tamoxifen
STZ
antidiabetic treatment
YFP expression
Diabetes
Improvement
-10
-5
0
5
10
day
B
Blood glucose, mM/L
Ctl
Stz
Rosi
Tolb
Metf
C
Body weight, g
D
Food intake, g×day-1
E
Fluid intake, g×day-1
F
Plasma insulin, ng/mL
Plasma glucagon, ng/mL
G
Pancreatic insulin content, ng/mg
Pancreatic glucagon content, ng/mg

Figure 2 Diabetic phenotype is associated with changes in the islet composition.

Impact of the administration of STZ to $Glu^{CreERT2}$;ROSA26-eYFP.mice and subsequent treatment with antidiabetic drugs, as indicated, on: islet number (black, n=150 islets from 6 mice) and islet area (red, n=150 islets from 6 mice) (*A*); β- (red, n=150 islets from 6 mice) and α-cell (black, n=150 islets from 6 mice) percentage among the islet cells (*B)*. *C:* Representative immunostaining of mouse pancreatic sections for DAPI (blue), glucagon (green) and insulin (red). *p<0.05 and ***p<0.001 compared to the saline control group. Δp<0.05compared to streptozotocin treated group. Scale bars: 50μm.

Figure 2

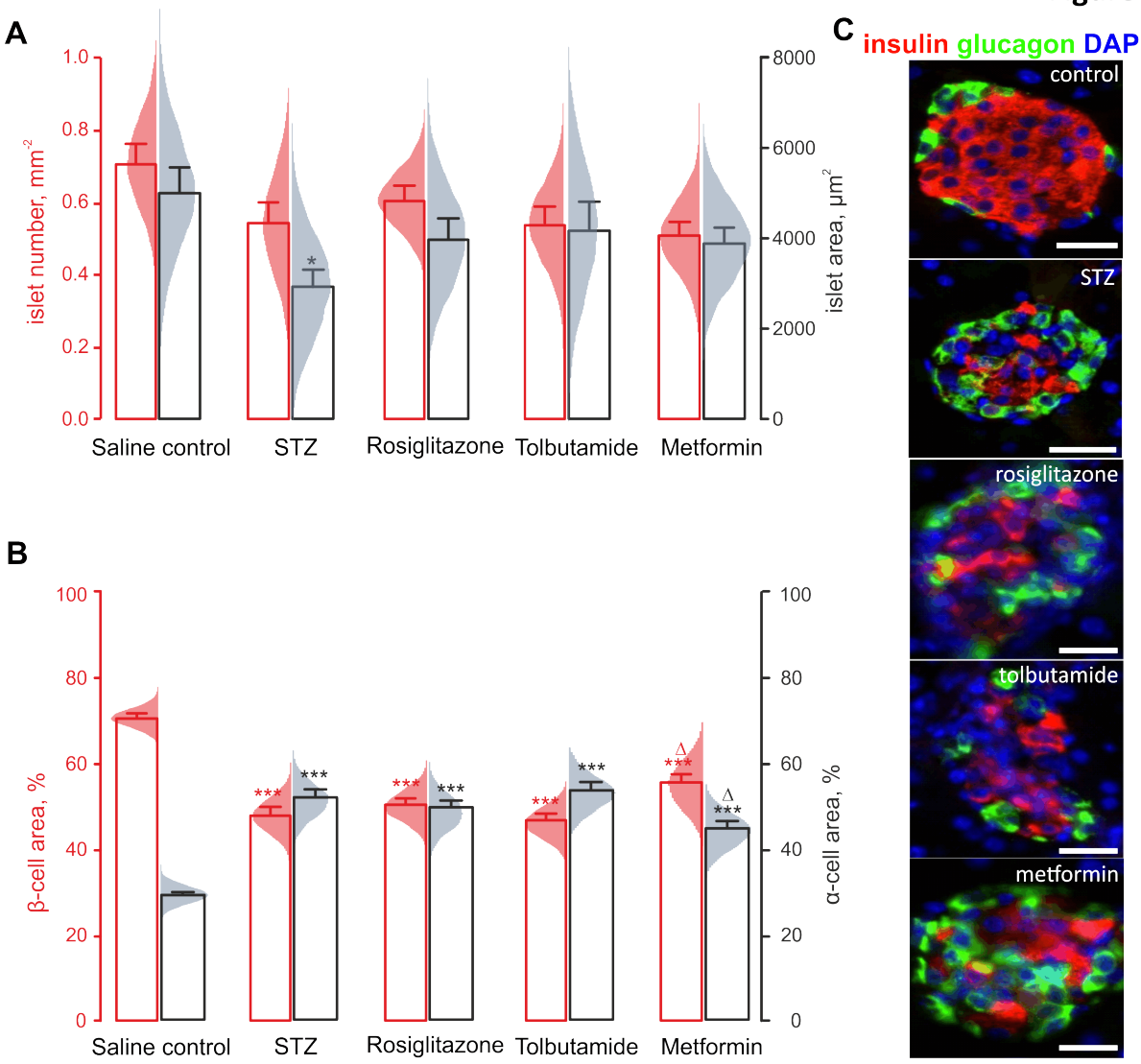
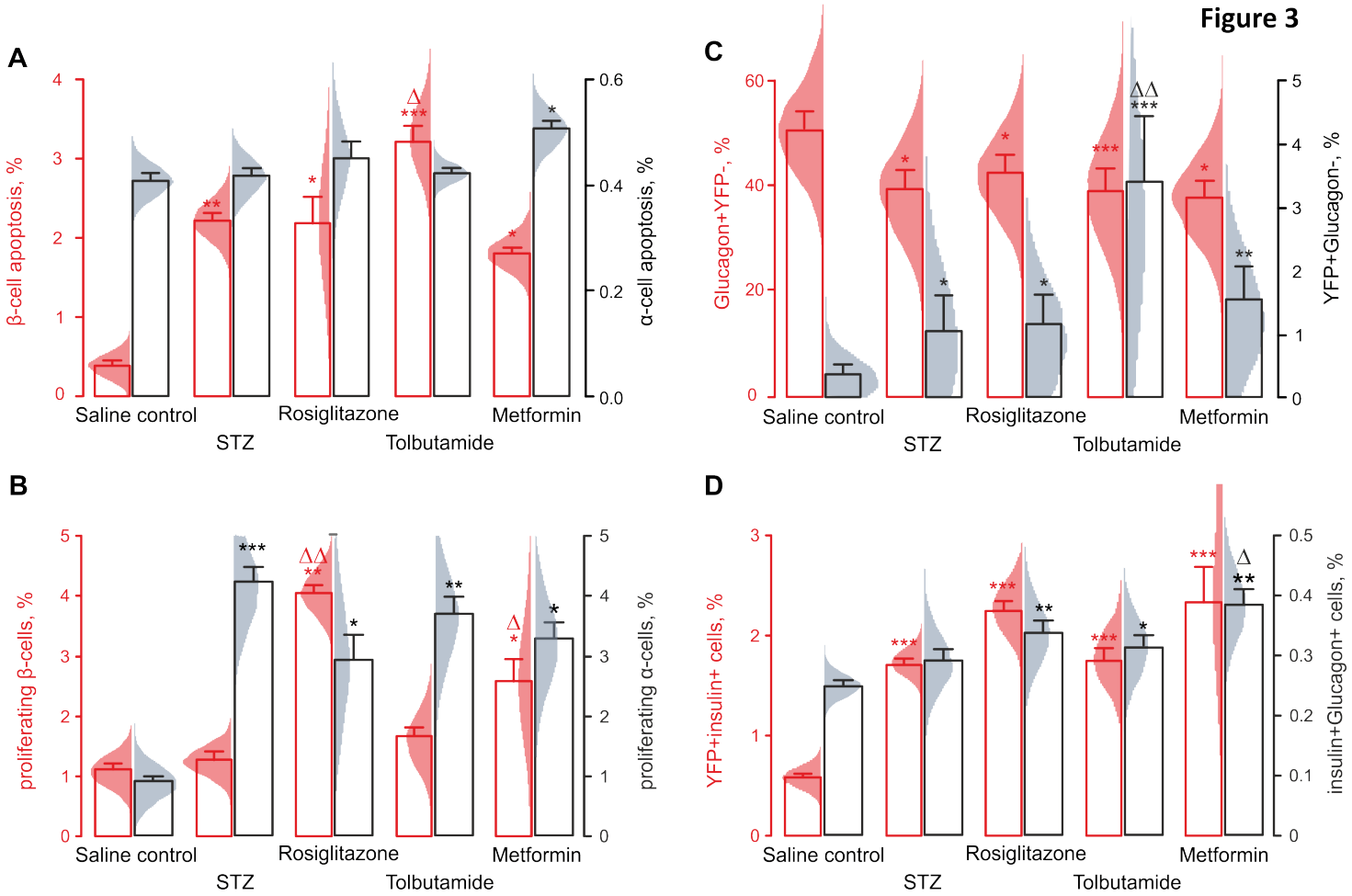

A
islet number, mm-2
islet area, µm2
1.0
0.8
0.6
0.4
0.2
0.0
8000
6000
4000
2000
0
*
Saline control
STZ
Rosiglitazone
Tolbutamide
Metformin
B
β-cell area, %
α-cell area, %
100
80
60
40
20
0
***
Δ
C
insulin glucagon DAPI
control
STZ
rosiglitazone
tolbutamide
metformin

Figure 3 [35]

A, B: Percentage of β-cells (red, n=60 islets from 6 mice) and α-cells (black, n=60 islets from 6 mice) undergoing apoptosis (*A*), as determined by TUNEL staining, or proliferation (*B)*, ki67 staining, in response to the administration of STZ to $Glu^{CreERT2}$;ROSA26-eYFP.mice and subsequent treatment with antidiabetic drugs, as indicated. Grey bars in *B* represent the net difference in the proliferating fractions of α- and β-cells. *C, D*: Trans-differentiation of YFP+ cells within $Glu^{CreERT2}$;ROSA26-eYFP.mice. The YFP expression, originally specifically induced in α-cells, was detectable within α-cells (*C*, red, n=60 islets from 6 mice), non-α-cells (*C*, black, n=60 islets from 6 mice) and β-cells (*D*, red, n=60 islets from 6 mice) after to the administration of STZ and subsequent anti-diabetic treatment. In addition, double-positive (insulin+glucagon+) cells were detectable (*D*, black, n=60 islets from 6 mice). *p<0.05, **p<0.01 and ***p<0.001 compared to saline control group. Δp<0.05 and ΔΔp<0.01, ΔΔΔp<0.001 compared to STZ-treated group.

Figure 3


A
β-cell apoptosis, %
α-cell apoptosis, %
Saline control
Rosiglitazone
Metformin
STZ
Tolbutamide
C
Glucagon+YFP-, %
YFP+Glucagon-, %
B
proliferating β-cells, %
proliferating α-cells, %
D
YFP+insulin+ cells, %
insulin+Glucagon+ cells, %

Supplementary data

Table S1 Primer sequence for PCR genotyping of $Glu^{CreERT2}$;ROSA26-eYFP mice

Primers	**Sequence**
β-catenin (Housekeeping control, 220BP)	Forward: AAGGTAGAGTGATGAAAGTTGTT Reverse: CACCATGTCCTCTGTCTATTC
iCre002/003 fragment (Cre lines, 537BP)	Forward: GACAGGCAGGCCTTCTCTGAA Reverse: CTTCTCCACACCAGCTGTGGA
GLUCre-ERT2 (759BP)	Forward: CCACCTTCTAGAATGTGCCTG Reverse: CATCTGCATGCAAAGCAATATAGC
EYFP (442BP)	Forward: GACGTAAACGGCCACAAGTT Reverse: GGATCTTGAAGTTCGCCTTG

Table S2 Primary and secondary antibodies used for immunohistochemistry

Primary antibody	**Dilution**	**Source**
Mouse anti-insulin	1:1000	Abcam, ab6995
Guinea pig anti-glucagon	1:200	Raised in-house: PCA2/4
Rabbit anti-GFP	1:1000	Abcam, ab6556
Rabbit anti-Ki-67	1:200	Abcam, ab15580
TUNEL enzyme	1:10	Sigma Aldrich 11684795910
Secondary antibody	**Dilution**	**Source**
Goat anti-mouse	1:400	Alexa Fluor 488, Invitrogen, UK
Goat anti-mouse	1:400	Alexa Fluor 594, Invitrogen, UK
Goat anti-guinea pig	1:400	Alexa Fluor 488, Invitrogen, UK
Goat anti-guinea pig	1:400	Alexa Fluor 594, Invitrogen, UK
Goat anti-rabbit	1:400	Alexa Fluor 488, Invitrogen, UK
Donkey anti-rabbit	1:500	Alexa Fluor 594, Invitrogen, UK

Figure S1 Representative images showing immunostaining for:

A: DAPI (blue), TUNEL (green) and insulin or glucagon (red) (see Figure 3A); *B:* DAPI (blue), Ki67 (green) and insulin or glucagon (red) (see Figure 3B); *C:* YFP (red), DAPI (blue) and insulin or glucagon (green) (see Figure 3C,D). D: insulin (red), DAPI (blue) and glucagon (green) (see Figure 3D). Scale bars: 50 μm.