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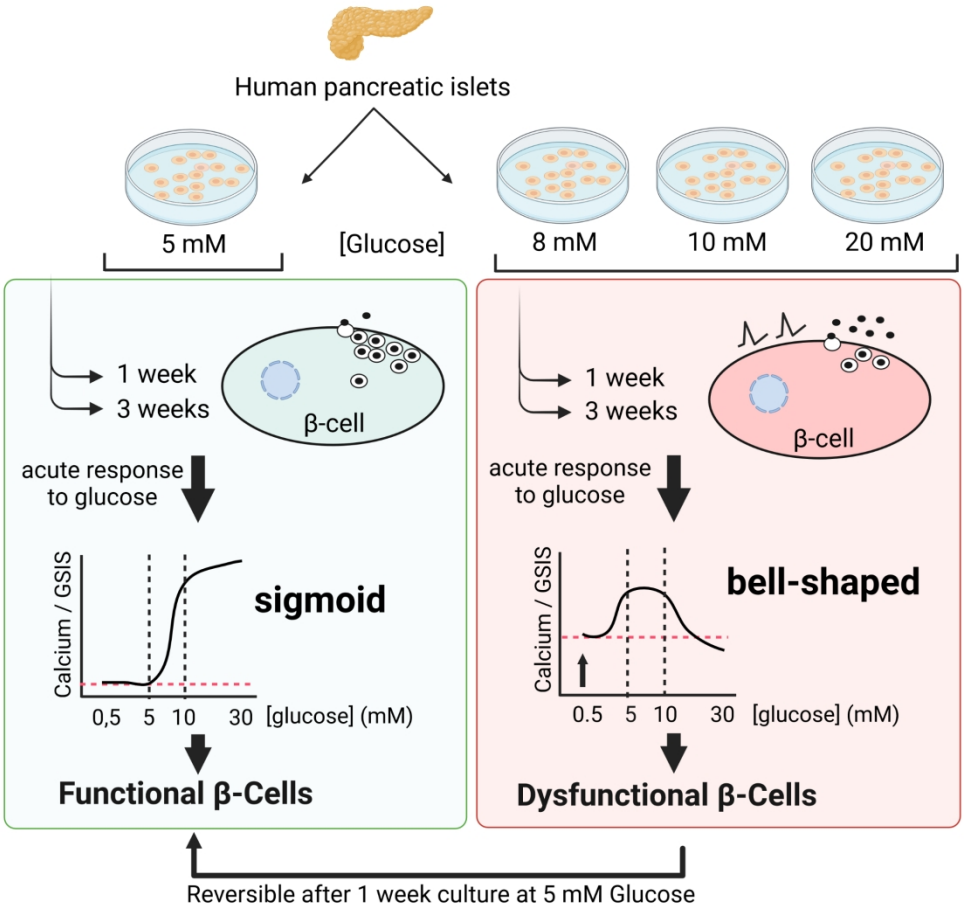

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Prolonged culture of human pancreatic islets under glucotoxic conditions changes their acute beta-cell calcium and insulin secretion glucose-response curves from sigmoid to bell-shaped.

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Prolonged culture of human pancreatic islets under glucotoxic conditions changes their acute beta-cell calcium and insulin secretion glucose-response curves from sigmoid to bell-shaped. A study by the group of @JonasJeanChris1 at @IREC_UCLouvain

Abstract

Background/Aim: The rapid remission of type 2 diabetes (T2D) by very low-calorie diet correlates with a marked improvement of glucose-stimulated insulin secretion (GSIS), emphasising the role of beta-cell dysfunction in the early stages of the disease. In search of novel mechanisms of beta-cell dysfunction after long-term exposure to mild to severe glucotoxic conditions, we extensively characterised the alterations of insulin secretion and upstream coupling events in human islets cultured for 1 to 3 weeks at ~5, 8, 10 or 20 mmol/l glucose and subsequently stimulated by an acute stepwise increase in glucose concentration.

Methods: Human islets from 49 non-diabetic (ND) and 6 T2D donors were obtained from 5 isolation centres. After shipment, the islets were precultured 3-7 days in RPMI medium containing ~5 mmol/l glucose and 10% heat-inactivated FBS with selective islet picking at each medium renewal. They were then cultured for 1 to 3 weeks in RPMI containing ~5, 8, 10 or 20 mmol/l glucose before measurements of insulin secretion during culture, islet insulin and DNA contents, beta-cell apoptosis, cytosolic and mitochondrial glutathione redox state, and assessment of dynamic insulin secretion and upstream coupling events during acute stepwise stimulation with glucose (NAD(P)H autofluorescence, ATP/(ATP+ADP) ratio, electrical activity, cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$)).

Results: Culture of ND-islets for 1-3 weeks at 8, 10 or 20 vs 5 mmol/l glucose did not significantly increase beta-cell apoptosis or oxidative stress but concentration-dependently decreased insulin content and increased the beta-cell sensitivity to subsequent acute stimulation with glucose. The islet glucose responsiveness was larger after culture at 8 or 10 vs 5 mmol/l glucose and markedly reduced after culture at 20 vs 5 mmol/l glucose. In addition, the $[Ca^{2+}]_c$ and insulin secretion responses to acute stepwise stimulation with glucose were no longer sigmoid but bell-shaped, with maximal stimulation at 5 or 10 mmol/l glucose and rapid sustained inhibition above that concentration. This paradoxical inhibition was, however, no longer observed when islets were acutely depolarised by 30 mmol/l extracellular K^+. The glucotoxic alterations of beta-cell function were fully reversible after culture at 5 mmol/l glucose and were mimicked by pharmacological activation of glucokinase during culture at 5 mmol/l glucose. Similar results were obtained in islets from T2D donors compared with ND-islets, except that their rate of insulin secretion during culture at 8 and 20 mmol/l glucose was lower, their cytosolic glutathione oxidation increased rather than decreased after culture at 8 and 20 mmol/l glucose, and the alteration of GSIS and upstream coupling events was stronger after culture at 8 mmol/l glucose.

Conclusions/interpretation: Prolonged culture of human islets under moderate to severe glucotoxic conditions markedly increased their glucose sensitivity and revealed a bell-shaped acute glucose-response curve for changes in $[Ca^{2+}]_c$ and insulin secretion, with maximal stimulation at 5 or 10 mmol/l glucose and rapid inhibition above that concentration. This novel glucotoxic alteration may contribute to beta-cell dysfunction in T2D independently from a detectable increase in beta-cell apoptosis.

Keywords: cytosolic calcium; glucose-response curve; glucotoxicity; human islets; long-term culture

Research in context

What is already known about this subject?

- Culture of rodent islets at high glucose alters beta-cell function and survival.
- Culture of human islets at high glucose alters beta-cell function with little increase in apoptosis.
- Culture at high glucose increases beta-cell glucose sensitivity and lowers beta-cell glucose responsiveness.

What is the key question?

- What are the alterations of beta-cell responses to an acute stepwise stimulation with glucose (insulin secretion and upstream coupling events) in islets from non-diabetic and type 2 diabetic islets cultured for 1-3 weeks at intermediate and high vs normal glucose concentrations?

What are the new findings?

- After 1-3 weeks of culture at 8-20mmol/l glucose, human islets display a paradoxical inhibition of Ca^{2+} and insulin secretion upon acute stimulation above 5 or 10mmol/l glucose.
- This acute inhibition is not associated with a decrease in glucose metabolism but likely results from acute plasma membrane repolarisation.
- In human islets cultured under glucotoxic conditions, the acute Ca^{2+} and insulin secretion glucose-response curves are bell-shaped.

How might this impact on clinical practice in the foreseeable future?

- If this paradoxical glucose response is operational in vivo, characterising its mechanism may help identifying new ways to improve beta-cell function and revert T2D.

1. Introduction

Pancreatic beta-cells are critical to glucose homeostasis by constantly adapting insulin secretion to the concentration of glucose and other nutrients. In humans and rodents, the stimulation of insulin secretion by glucose (GSIS) results from an acceleration of glucose metabolism with rise in NAD(P)H/NAD(P)$^+$ and ATP/ADP ratios, closure of ATP-sensitive K^+ channels, plasma membrane depolarisation, opening of voltage-dependent Ca^{2+} channels, and Ca^{2+} influx with rise in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) that triggers insulin granules exocytosis [1-3]. In addition, glucose metabolism amplifies Ca^{2+}-induced exocytosis [4]. In the context of glucose intolerance and type 2 diabetes (T2D), chronic beta-cells exposure to the diabetic milieu slowly alters their mass and function, thereby aggravating the disease [5, 6]. Although the relative contribution of glucose and lipids to nutrient toxicity is debated, several studies in rodents demonstrated a major contribution of glucotoxicity in the reversible alterations of beta-cell gene expression, loss of insulin content and GSIS, and apoptosis in diabetes, and a beneficial impact of beta-cell rest on some of these alterations [7-13]. In comparison, human beta-cells in culture or in humanised murine models of diabetes are less prone to glucotoxic apoptosis unless concomitantly exposed to high levels of saturated free fatty acids [12, 14, 15]. Moreover, a decrease in beta-cell function rather than number likely contributes to the alterations of GSIS in early T2D. Indeed, in obese patients with recent T2D submitted to severe caloric restriction, the remission of diabetes critically depended on rapid restoration of beta-cell glucose responsiveness [16, 17]. Here, we tested the impact of 1-3 weeks of culture at various glucose concentrations on human islets from non-diabetic (ND) and type 2 diabetic (T2D) donors. As beta-cell apoptosis did not increase, we thoroughly analysed insulin secretion and upstream coupling events during stepwise stimulation with glucose, looking for yet unrecognised glucotoxic alterations of human beta-cell stimulus-secretion events.

2. Materials and Methods

For detailed Methods, please refer to the ESM.

2.1. Islet preparations - Islets were isolated with informed consent from 49 non-diabetic (ND) donors and 6 donors with a history of T2D in 5 isolation centres. Islets were cultured and shipped in CMRL medium containing 5.5mmol/l glucose and 10% FBS or Human Serum Albumin (HAS). Donors and islet preparations characteristics are shown in Table 1 and ESM-Table 1. Experiments were approved by UCL-Saint-Luc ethic committee (project 2017/05JUL/355).

2.2. Islet culture - Islets were precultured 4-7 days in RPMI containing 5.5mmol/l glucose and 10% FBS or 5g/l BSA, with hand-picking to another dish every 2-3 days and removal of islets with detectable central necrosis at 10x magnification. Islets were then cultured 1-3 weeks in RPMI containing ~5.5, 8.5, 10.5 and 20.5mmol/l. They are named G5-, G8-, G10- and G20-islets throughout the paper. Insulin was measured in the culture medium (renewed every 2-3 days) with in-house RIA using human insulin as standard.

2.3. Apoptosis and proportion of alpha- and beta-cells - Islet histone-associated DNA fragments were quantified using a commercial ELISA. The results were normalised to DNA content measured by fluorimetry. The percentage of beta-cell apoptosis was determined by TUNEL assay and insulin immunodetection on islet sections (ESM-Fig.1). Central necrotic areas were excluded from counting. Other islet sections were used to compute the proportion of alpha- and beta-cells after immunodetection of glucagon- and insulin-positive cells.

2.4. Glutathione redox state (E_{GSH}) - Cytosolic and mitochondrial E_{GSH} were measured as indicators of cytosolic and mitochondrial oxidative stress in islets expressing roGFP1 or mt-roGFP1. The probe fluorescence ratio was normalised to the minimal ratio in DTT (0%) and its maximum in aldrithiol (100%) [18, 19].

2.5. Solutions – Acute glucose responses were measured using a Krebs solution (KRB) containing (in mmol/l) NaCl (124), KCl (4.8), $CaCl_2$ (2.5), $MgCl_2$ (1.2), $NaHCO_3$ (20), 1 g/l BSA (fraction V; Roche), and n mmol/l glucose (Gn). When KCl was raised to 30mmol/l, NaCl was reduced to 98.8mmol/l. The solutions were continuously gassed with 5% CO_2 in pure O_2 to maintain pH~7.4 at 37°C.

2.6. ATP measurements – After 30min preincubation in KRB containing 0.5mmol/l glucose, islets were incubated 30min in 0.5 or 10mmol/l glucose before 30min incubation at various glucose concentrations. ATP and ATP+ADP were measured by a luminometric method [20], and ATP/(ATP+ADP) ratio was computed and normalised to the ratio in G5-islets treated with azide.

2.7. NAD(P)H, $[Ca^{2+}]_c$ and insulin secretion – After 30min perifusion with KRB containing 0.5mmol/l glucose, islets were stimulated with increasing glucose concentrations. NAD(P)H autofluorescence and $[Ca^{2+}]_c$ were measured in average sized islets (~75-150µm) [21]. $[Ca^{2+}]_c$ was measured by recording fura2-LR fluorescence ratio in islets loaded with 2µmol/l fura2-LR-acetoxymethylester at the same glucose concentration as during culture [21]. Alternatively, islets were infected with Ad-RIP-D3cpv and the probe fluorescence ratio was recorded. Insulin secretion was measured in 2-min effluent collections from 100-150 perifused islets (diameter ~75-330µm). The islet DNA and insulin contents were measured after perifusion and used for data normalisation.

2.8. Electrical activity - Islets were seeded on Multi-Electrode Arrays (MEAs) coated with Matrigel. After 1week of culture at G5, G8 or G20, they were perifused with Hepes-buffered KRB at various glucose concentrations with final addition of 1mM $CoCl_2$. Extracellular field potentials were acquired and treated as described [22]. Slow potentials (SP), which are multicellular beta-cell-specific signals due to beta-cell coupling [23], were isolated as published. Mean SP frequencies were determined over the last 5min of each condition for each recorded islet.

2.9. Statistics - Data are means ± SD for the number of preparations/donors indicated, with individual data added to column bars. The statistical significance of differences between groups was evaluated by one-way ANOVA followed by a test of Dunnett or a test for linear trend, or by 2-way ANOVA (for repeated measurements when comparing selected time points in the same trace) followed by a test of Tukey, or by mixed-effects model analysis followed by a test of Sidak when few data were missing. We used GraphPad Prism version 9.

3. Results

3.1. Impact of a 1-week culture at increasing glucose concentrations on insulin secretion, islet insulin and DNA content, beta-cell apoptosis, and cytosolic and mitochondrial glutathione redox state – During culture, insulin secretion was significantly higher in G8-islets and G20-islets vs G5-islets, with a time-dependent increase in G8-islets (Fig.1a). After culture, the insulin content was ~33% lower in G8-islets and ~85% lower in G20-islets *vs* G5-islets (Fig.1b), while the DNA content was unaffected (Fig.1c). The proportion of beta- and alpha-cells was also similar in G5-islets and G20-islets (Fig.1d). DNA fragmentation, percentage of TUNEL-positive beta-cells and cytosolic E_{GSH} (a marker of cytosolic oxidative stress) did not significantly differ between groups (Fig.1e-g), and mitochondrial E_{GSH} tended to be lower after culture at higher glucose (Fig.1h). We obtained similar results after culture in FBS-free medium containing BSA, except that DNA fragmentation tended to be higher in G5-islets vs G8-islets and G20-islets (ESM-Fig.2).

3.2. Impact of a 1-week culture at increasing glucose concentrations on subsequent GSIS and upstream coupling events - After culture, the responses to stepwise increases in glucose concentration were measured after a ~30-min exposure to 0.5mmol/l glucose (Fig.1 and ESM-Fig.3). To compare glucose metabolism between groups, NAD(P)H autofluorescence and ATP/(ATP+ADP) ratio were normalised to their minimum in the presence of the mitochondrial poisons FCCP (uncoupler) or azide (complex IV inhibitor).

In G5-islets (black traces), glucose increased NAD(P)H autofluorescence and ATP/(ATP+ADP) ratio across the whole range of glucose concentrations (Fig.1ij) and increased $[Ca^{2+}]_c$ and insulin secretion at 10 and 30mmol/l glucose (Fig.1km and ESM-Fig.3), with a further increase upon cell depolarisation with high K^+. Glucose also increased the slow potential (SP) frequency recorded with multielectrode arrays (Fig.1l and ESM-Fig.4), an effect that was inhibited by blocking all voltage-gated Ca^{2+} currents with Co^{2+} [24].

In G8-islets (green traces) vs G5-islets, NAD(P)H tended to be higher at 0.5 and 2mmol/l glucose, the SP frequency was higher at all glucose concentrations, a rise in $[Ca^{2+}]_c$ and stimulation of insulin secretion was already detected at 5mmol/l glucose, and the maximal amplitude of insulin secretion was 2-5 times larger depending on the mode of expression (per islet, DNA content or insulin content)(ESM-Fig.3). Culture at G8 therefore slightly increased the sensitivity and markedly enhanced the maximal response to subsequent glucose stimulation.

In G20-islets (red traces), the initial NAD(P)H autofluorescence, ATP/(ATP+ADP) ratio, SP frequency, $[Ca^{2+}]_c$ and insulin secretion after 30-60min at 0.5mmol/l glucose were higher or tended to be so vs G5-islets. The higher $[Ca^{2+}]_c$ and insulin secretion resulted from sustained closure of K_{ATP} channels with membrane depolarisation, as attested by their rapid reduction by the K_{ATP} channel opener diazoxide (Dz) (Fig.1n). Despite this persistent stimulation, NAD(P)H, ATP/(ATP+ADP) ratio, $[Ca^{2+}]_c$ and insulin secretion further increased upon stimulation with 5-10mmol/l glucose. Above 10mmol/l glucose, NAD(P)H and ATP/(ATP+ADP) ratio plateaued, whereas SP frequency, $[Ca^{2+}]_c$ and insulin secretion abruptly decreased to a minimum (Fig.1km, Δ(G30-G10)). Thus, the acute $[Ca^{2+}]_c$ and insulin secretion glucose-response curves in G20-islets looked “bell-shaped” rather than “sigmoid” as in G5-islets (Fig.1km). In addition, the maximal average rate of insulin secretion was larger in G20-islets vs G5-islets irrespective of the mode of expression, but it was lower than in G8-islets when normalised to DNA content (ESM-Fig.3b), similar when normalised to islet number (ESM-Fig.3a), and much higher when normalised to insulin content (ESM-Fig.3c). Similar alterations of NAD(P)H, $[Ca^{2+}]_c$ and insulin secretion were observed after culture in FBS-free medium containing BSA (ESM-Fig.5).

We also measured the acute glucose-induced changes in $[Ca^{2+}]_c$ and insulin secretion during sustained depolarisation with 30mmol/l extracellular K^+ in the presence of Dz (K30Dz). As shown in figure 1op, addition of K30Dz to 0.5mmol/l glucose stimulated insulin secretion to a larger extent in G8-islets and G20-islets than in G5-islets despite similar increases in $[Ca^{2+}]_c$ levels. Subsequent stimulation with 10mmol/l glucose reduced $[Ca^{2+}]_c$ to a larger extent in G5-islets whereas it decreased insulin secretion to a larger extent in G8-islets and G20-islets. There were, however, only transient changes in $[Ca^{2+}]_c$ and insulin

secretion in all islet types upon glucose changes from 10 to 30 then back to 10mmol/l. These results suggest that the acute high glucose-induced reduction of $[Ca^{2+}]_c$ in G20-islets (Fig.1k) resulted from acute plasma membrane repolarisation.

3.3. Characterisation of the acute $[Ca^{2+}]_c$ glucose-response curve in human islets cultured under glucotoxic conditions – As beta-cells only represent ~50% of human islet cells, we used a RIP-driven D3cpv Ca^{2+} sensor and confirmed that the glucotoxic alterations of islet $[Ca^{2+}]_c$ measured with fura2-LR occurred in beta-cells (Fig.2a, ESM-Fig.6). Interestingly, in these experiments, both G8-islets and G20-islets presented an acute inhibition of beta-cell $[Ca^{2+}]_c$ above 10mmol/l glucose. Thus, the acute $[Ca^{2+}]_c$ glucose-response curves evolved from sigmoid in G5-islets to bell-shaped with a predominant increase in G8-islets, to bell-shaped with a predominant inhibition in G20-islets (Fig.2b).

We next measured islet $[Ca^{2+}]_c$ in 19 islet preparations different from the 6 preparations used in figure 1k to evaluate the variability of its glucotoxic alterations (Fig.2c-f). The mean $[Ca^{2+}]_c$ traces were as in Fig.1k, except that $[Ca^{2+}]_c$ tended to be acutely inhibited above 10mmol/l glucose not only in G20-islets but also in G8-islets (Fig.2c). The alterations of $[Ca^{2+}]_c$ in G8-islets and G20-islets were not influenced by the isolation centre (ESM-Fig.7) or the donor sex, age and BMI (ESM-Fig.8). However, the glucose concentration at which $[Ca^{2+}]_c$ was maximal in G20-islets ranged from 10 to 0.5mmol/l, generating slightly different types of acute $[Ca^{2+}]_c$ glucose-response curves, with a drop in $[Ca^{2+}]_c$ above 10mmol/l glucose as in Fig.1k (Fig.2d), from 5 to 10mmol/l glucose (*P*=0.083) (Fig.2e), or from 5 to 30mmol/l glucose (Fig.2f).

We also checked what concentration of glucose was required to acutely inhibit $[Ca^{2+}]_c$. In G20-islets, the inhibition was maximal upon stimulation from 10 to 15mmol/l glucose (Fig.2g). In G10-islets (blue trace), the glucose-$[Ca^{2+}]_c$ response curve was also bell-shaped, with a maximum at 10mmol/l glucose and an inhibition at 15mmol/l glucose. Interestingly, the changes in $[Ca^{2+}]_c$ from 5 to 10mmol/l glucose and from 10 to 15mmol/l glucose were significantly lower in G10-islets and G20-islets vs G5-islets.

3.4. Impact of a 3-week culture at increasing glucose concentrations – The islet characteristics and functional responses to acute stimulation with glucose were similar in islets cultured for 1 or 3 weeks at G5 (ESM-Fig.9). After 3 weeks at G8, G10 or G20 instead of G5, beta-cell apoptosis did not increase significantly but insulin secretion during culture and other islet parameters were affected as after one week of culture (Fig.3 and ESM-Fig.10). However, the alterations of GSIS and upstream coupling events were worse. After 3 weeks at G20, there was an almost complete lack of NAD(P)H response to glucose, an acute inhibition of $[Ca^{2+}]_c$ and insulin secretion upon stimulation from 5 to 10-30mmol/l glucose, and

a lower maximal rate of insulin secretion than in G5-islets (except if expressed relative to insulin content). After 3 weeks at G10, the acute glucose-response curve for $[Ca^{2+}]_c$ and insulin secretion also looked bell-shaped rather than sigmoid.

3.5. Acute and chronic reversibility, and role of glucose metabolism in the glucotoxic alterations of beta-cell function – The long-term culture of human islets at G10 or G20 revealed a bell-shaped acute glucose-response curve for $[Ca^{2+}]_c$ and insulin secretion. In G20-islets, the drop in $[Ca^{2+}]_c$ and insulin secretion upon stimulation from 10 to 30mmol/l glucose lasted for at least 1h and was acutely reversible upon return to 10mmol/l glucose, with a large rebound increase in insulin secretion (Fig.4ab). In addition, the long-term alterations of $[Ca^{2+}]_c$ responses in G20-islets were fully reversible after one week of culture at G5 (Fig.4cd), and the alteration by one week of culture at G20 was largely reproduced by pharmacological activation of glucokinase with Ro-280450 during culture at G5 (Fig.4ef). Finally, we tested the impact of acute glucokinase activation on the $[Ca^{2+}]_c$ responses of G5-islets and G20-islets (Fig.4g). As expected, the glucose-induced changes in $[Ca^{2+}]_c$ were left-shifted by Ro-280450 in both G5-islets and G20-islets, with a significant acute inhibition of $[Ca^{2+}]_c$ in G20-islets above 5mmol/l glucose + Ro-28045 rather than 10mmol/l glucose alone.

3.6. Impact of a 1-week culture at increasing glucose concentrations on islets from donors with T2D – The glucotoxic alterations of beta-cell function differ from the lower glucose sensitivity of insulin secretion usually described in T2D patients [25]. They also differ from the decrease of glucose sensitivity and maximal GSIS described in islets from T2D donors and sometimes attributed to defective mitochondrial metabolism [15, 26-29]. However, these results were obtained in freshly isolated islets or after a short culture and did not show whether these functional alterations persisted after prolonged culture. We therefore tested the impact of one week of culture at G5, G8 or G20 on islets from 6 male T2D donors (table 1, ESM-Table1). As with islets from ND donors (Fig.1a), insulin secretion by T2D-islets was higher during culture at G8 and G20 vs G5 (Fig.5a). However, it was significantly lower than in 6 islet preparations from age- and BMI-matched ND male donors with similar cold ischemia duration, islet purity and total culture duration (ESM-Fig.11). As in ND-islets, insulin content in T2D-islets was significantly lower after culture at G20 vs G5 (Fig.5b, ESM-Fig.12a), whereas DNA content, percentage of TUNEL-positive beta-cells and beta-cell proportion were unaffected (Fig.5cd, ESM-Fig.12bc). However, cytosolic E_{GSH} significantly increased after culture at G8 and G20 vs G5 (Fig.5e, ESM-FIG.12d) while mitochondrial E_{GSH} failed to decrease (Fig.5f, ESM-Fig.12e), suggesting that, under glucotoxic conditions, T2D-islets experienced higher cytosolic and mitochondrial oxidative stress than ND-islets.

Regarding the metabolic responses to acute glucose, NAD(P)H recordings in T2D-islets cultured for one week at G5, G8 or G20 were similar to those of ND-islets cultured for 3 weeks at G20, with already high NAD(P)H levels in 0.5mmol/l glucose and little glucose-induced rises in NAD(P)H despite the rapid decrease in FCCP (Fig.5g, ESM-Fig.12f). Accordingly, data from two T2D-preparations suggested that the ATP/(ATP+ADP) ratio at low glucose was higher than in ND-islets and only slightly increased at high glucose (ESM-Fig.13). Nevertheless, the $[Ca^{2+}]_c$ and insulin secretion responses to glucose after culture at G5 were qualitatively and quantitatively the same in T2D-islets and ND-islets (Fig.5hi, ESM-Fig.12gh). Moreover, their alterations after culture at G8 and G20 were qualitatively similar to those in ND-islets, except for 2 major differences in G8-islets: first, their $[Ca^{2+}]_c$ and insulin secretion during perifusion at low glucose was closer to that in G20-islets than G5-islets; second, their insulin secretion significantly decreased upon stimulation from 10 to 30mmol/l glucose (Fig.5hi, ESM-Fig.12h, ESM-Fig.14). These results suggested that T2D-islets present functional signs of glucotoxicity at lower glucose concentration than ND-islets.

4. Discussion

Human islets cultured for up to 3 weeks under glucotoxic conditions (G8, G10- and G20- vs G5-islets) displayed strong alterations of GSIS and upstream coupling events but no significant increase in beta-cell apoptosis (further discussed in ESM) or oxidative stress. Among the glucotoxic alterations of human beta-cell function, the lower insulin content, the left-shifted glucose-response curve with higher mitochondrial metabolism and higher rate of secretion at low glucose, and the lower amplitude of maximal glucose response were reported earlier [15, 30-35]. However, the alteration of the acute $[Ca^{2+}]_c$ and insulin secretion glucose-response curves from sigmoid to bell-shaped, with maximal stimulation at 5 or 10mmol/l glucose and paradoxical inhibition above that concentration, was not reported earlier. It occurred in beta-cells and was almost systematically observed irrespective of the available donor characteristics, including a history of T2D.

4.1. Possible mechanism - The acute glucose-induced inhibition of $[Ca^{2+}]_c$ and insulin secretion in glucotoxic islets unlikely resulted from an increase in beta-cell apoptosis, as shown by its normalisation after another week of culture at G5 and by the lack of large significant increase in beta-cell apoptosis. It likely resulted from an acute repolarisation of the plasma membrane by changes in ion channel activity, as it was not observed in K30-depolarised islets. It did not, however, result from an acute inhibition of glucose metabolism. Indeed, although the glucotoxic alteration of GSIS in human islets is usually

considered to result from defective mitochondrial metabolism [28, 36], measuring NAD(P)H and ATP/(ATP+ADP) ratio normalised to FCCP or azide unequivocally confirmed that, in human as in rat islets cultured under glucotoxic conditions, mitochondrial metabolism was markedly increased at low glucose rather than decreased at high glucose [21, 34]. Such an increase at low glucose was also observed in T2D-islets cultured at G5 for ~10 days and was further aggravated by culture at G20, in contrast with studies showing that mitochondria are markedly altered in T2D-islets [12, 15, 36-38]. The glucotoxic alterations of human beta-cell function we observed may therefore result from overstimulation of glucose metabolism (perhaps resulting from increased glucokinase activity [39]) with eventual activation of a repolarising current upon further rise in glucose concentration. It remains, however, possible that other changes in metabolic coupling factors, e.g. divergent changes in NADH, NADPH and adenine nucleotides between the cytosol and mitochondrial matrix, underlie the acute glucose-induced inhibition of $[Ca^{2+}]_c$ and insulin secretion. Alterations of cyclic AMP signalling and distal Ca^{2+}-exocytosis coupling events may also reduce GSIS independently from alterations in $[Ca^{2+}]_c$ responses [40, 41].

4.2. Pathophysiological relevance - The acute glucose-induced inhibition of $[Ca^{2+}]_c$ and insulin secretion was mainly studied in G20-islets stimulated above 10mmol/l glucose, questioning its pathophysiological relevance. However, we have evidence that it already occurred at lower glucose concentrations that can be encountered in T2D patients submitted to an OGTT [25]. First, in ~50% of the preparations, the drop in $[Ca^{2+}]_c$ in G20-islets was already detected upon stimulation from 5 to 10mmol/l glucose. Second, the drop in $[Ca^{2+}]_c$ was observed in G10-islets, in most beta-cell-specific $[Ca^{2+}]_c$ recordings in G8-islets, and in T2D-islets cultured at G8. The alterations of GSIS and upstream coupling events in islets from ND-donors cultured at G8-G10 merit further discussion. The left-shift of their glucose-response curve and marked increase in maximal GSIS could be a beneficial adaptation to a moderate increase in glucose concentration. However, the bell-shaped curve with acute inhibition of $[Ca^{2+}]_c$ above 10mmol/l glucose and the larger variability of their insulin secretion response to acute stimulation from 10 to 30mmol/l glucose suggests that these islets are close to the tipping point between beta-cell adaptation and decompensation. The corresponding blood glucose level at which such a process would occur in vivo is, however, difficult to estimate.

4.3. How do our results help understanding beta-cell pathophysiology in T2D? – If the role of glucotoxicity in the development of glucose intolerance and T2D remains questionable, its role in established T2D is clearer [13, 42]. Thus, as (gluco)lipotoxic culture conditions trigger apoptosis and irreversible defects in GSIS [15], and because fast recovery of the beta-cell mass is unlikely, the rapid improvement of GSIS and remission of T2D in recently diagnosed patients submitted to very-low-calorie diet [16, 17] likely resulted

from correction of glucotoxic beta-cell dysfunction. If the acute glucose-induced inhibition of insulin secretion we observed above 5-10mmol/l glucose in glucotoxic human islets is operational in T2D patients, it may contribute to the lack of appropriate increase or even to a decrease in insulin secretion after meals. Interestingly, it was previously reported that, in murine models of diabetes with mouse and human islets transplanted under the kidney capsule, glucose injection acutely decreased human plasma insulin while increasing mouse insulin, suggesting that this glucotoxic phenotype is operational in vivo [14]. The bell-shaped insulin secretion glucose-response curve observed in Zucker Diabetic Fatty rats pancreas perfusion [43] reinforces the hypothesis that this glucotoxic alteration is operational in diabetic islets in vivo. In that scenario, a reduction in beta-cell glucose metabolism could restore a sigmoid acute glucose-response curve (for $[Ca^{2+}]_c$ and insulin secretion) and restore a rise in insulin secretion after meals. In support of this hypothesis, it has been shown that inhibition of glucokinase or mitochondrial pyruvate transporter in models of beta-cell glucotoxicity or diabetes reduced beta-cell stimulation at low glucose, right-shifted the $[Ca^{2+}]_c$ and insulin secretion glucose-response curves, and thereby improved $[Ca^{2+}]_c$ oscillations and GSIS [34, 44, 45]. Thus, identifying the molecular mechanism of the acute glucose-induced inhibition of $[Ca^{2+}]_c$ and insulin secretion beyond acute membrane repolarisation may help identifying new putative therapeutic targets to improve beta-cell function in recent onset T2D.

4.4. Considerations about donors, islet shipment, culture conditions, and islet characteristics

This study was carried out with islets from 5 different sources covering a large range of ages and BMI with or without a history of T2D. The islet preparations were selected based on their high islet purity and the possibility to ship islets in maximum 3 days. The size of islets without central necrosis detected at 10x magnification was rather large, limiting the validity of the study to islets with a diameter between 75 and 330µm. However, except for the high male/female sex ratio and high prevalence of obesity (Table 1), the clinical characteristics of the donors were unremarkable. Although we used different sets of preparations for each type of measurements, the alterations of the $[Ca^{2+}]_c$ responses to glucose were largely similar across the 27 islet preparations tested.

The time between isolation and reception in the lab varied considerably between islet sources. Because we cultured islets for at least 10 days, we consider that this variable should not affect our results. Moreover, the long preculture and culture time can be considered as a strength of our study, as it allowed to assess beta-cell function at distance from the events preceding donor death, and to study the intrinsic alteration of beta-cell function that persist in T2D-islets after 10-15 days of culture at G5.

Finally, isolated islets are no longer vascularised and innervated, which may alter their response to glucose, thereby limiting the pathophysiological relevance of in vitro studies. In this context, the islet size is important, as oxygen/nutrients diffusion to the centre decreases with the increase in diameter. From their DNA content, we estimated that the islets contained on average ~12000 cells (among which ~6000 beta-cells) for a diameter of ~250µm. Although we cannot exclude that limited access to nutrients/oxygen contributed to the glucotoxic phenotype, the alterations of the $[Ca^{2+}]_c$ responses occurred at the islet periphery where fura2-LR-AM loading and adenoviral infection are optimal.

5. Conclusion

Prolonged culture of human islets from ND- or T2D-donors under moderate to severe glucotoxic conditions markedly increased their glucose sensitivity and changed their acute glucose-response curve for changes in $[Ca^{2+}]_c$ and insulin secretion from sigmoid to bell-shaped, with maximal stimulation at 5 or 10mmol/l glucose and paradoxical inhibition above that concentration. If the latter glucose-induced inhibition is operational in vivo, characterising its underlying mechanism may help identifying new ways to improve beta-cell function and reverse recently diagnosed T2D.

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Data availability

All data generated or analysed during this study are included in this published article and its ESM material. For further information, please write to the corresponding author.

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Authors' relationships

J-CJ is a member of the Diabetologia Editorial Board. The other authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

Author contributions

JCJ, AW, CB, MR and PG conceived and designed the experiments.

MT, AS, HC, MJ, MB, AFC, JPD and LRBS performed the experiments.

MT, MR and JCJ analysed the data.

MT and JCJ wrote the paper, and all authors edited and approved the paper.

AW and CB set up human islet isolation procedures in Montpellier.

AB and NIM isolated human islets in Brussels.

JCJ is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1 : Summary of the characteristics of donors and islet preparations. Details for each donor and islet preparation can be found in ESM-Table 1. The number of donors/preparations for which the data were available are shown on the first line of each column.

	Age	**Sex ratio M/F**	**BMI (kg/m²)**	**HbA1c (%)**	**HbA1c (mmol/mol)**	**Cold ischemia duration (h)**	**Islet purity (%)**	**Culture time until reception (h)**
Donors with no history of diabetes								
n=	49	30/16	49	12	12	41	46	49
min	17		20.5	4.5	25.5	2	75	6
max	74		40.2	6.1	43.1	23	95	170
median	55		25.7	5.5	36.5	8	90	96
mean	54		26.6	5.4	35.4	9	87	95
Donors with a history of T2D								
n=	6	6/0	6	2	2	6	6	6
min	43		25.4	6.0	42	4	80	60
max	68		30.5	6.5	47.5	11	90	168
median	61		27.9	6.3	45.3	8	83	125
mean	59		27.9	6.3	45.3	8	84	116

Figure legends

Figure 1. **Long-term (one week) effects of glucose on cultured human islets -** After preculture at G5, human islets were cultured for one week in RPMI containing G5, G8 or G20 (G5-islets, black symbols and traces; G8-islets, green symbols and traces; G20-islets, red symbols and traces). a, insulin secreted in the culture medium collected every 2-3 days and expressed in ng/h per islet. Means ± SD and individual data for the 22 preparations used for experiments shown in figure 1. b-c, islet insulin to DNA content ratio and DNA content at the end of experiments shown in panel m. Means ± SD and individual data for 6 preparations. d-e, proportion of beta- and alpha-cells and percentage of TUNEL-positive beta-cells in islet sections. Means ± SD and individual data for 3-4 preparations. f, islet histone-associated cytosolic DNA fragments after culture, absorbance arbitrary units normalised for differences in islet DNA content. Means ± SD and individual data for 3 preparations. g-h, cytosolic and mitochondrial E_{GSH} as reflected by the roGFP1 and mt-roGFP1 normalised fluorescence ratio (see Methods). Means ± SD and individual data for 4 (g) or 6 (h) preparations. i, NAD(P)H autofluorescence during stimulation with a stepwise increase in glucose concentration (Gn, n mmol/l glucose), as indicated on top of the figure. Data from each islet were normalised to the fluorescence level measured 10 min after addition of 10 µmol/l FCCP. Means ± SD for 5 preparations, each with one or two islets. Δ(G30-G10) graph, the change in NAD(P)H autofluorescence between 10 and 30 mmol/ glucose steps was calculated by subtracting the averaged data over the last 3 min of each step. j, ATP/(ATP+ADP) ratio in islets incubated for 2 consecutive periods of 30 min at the indicated glucose concentrations. In each experiment, the ratios were normalised to that measured in G5-islets incubated at 30 mmol/l glucose + 5 mmol/l Na^+-azide. Means ± SD and individual data for 5 preparations, each with 2-3 batches of 10 islets per condition. km, fura2-LR fluorescence ratio (as an indicator of $[Ca^{2+}]_c$) and insulin secretion rate during stepwise glucose stimulation followed by membrane depolarisation with 30 mmol/l extracellular K^+ in the presence of 250 µmol/l diazoxide (K30Dz). Means ± SD for 5-6 preparations. Δ(G30-G10) graph, the change in fura2-LR fluorescence ratio and insulin secretion rate between 10 and 30 mmol/ glucose steps was calculated by subtracting the averaged data over the last 3 min (k) or 6 min (m) of each step; units are as in the graph showing the corresponding dynamic traces. l, electrical activity recorded with MEAs in islets stimulated by a stepwise increase in glucose concentration, as indicated at the top of the figure. The mean SP frequencies was computed over the last 5 min of each condition. Co^{2+}, 1 mmol/l $CoCl_2$. Data are means ± SD for 14-27 measurements from 3 islet preparations. For more details, see ESM-Fig.4. n, effect of 100 µmol/l diazoxide (Dz) on $[Ca^{2+}]_c$ and insulin secretion during perifusion at 0.5 mmol/l glucose. Means ± SD for 4-5 preparations. o-p, fura2-LR fluorescence ratio and insulin secretion rate during acute

depolarisation with 30 mmol/l extracellular K^+ (K30) in the presence of 0.5 mmol/l glucose and 100 µmol/l diazoxide (Dz) followed by stepwise glucose stimulation. Means ± SD for 4-5 preparations.

Statistical analysis: a and i-p, dynamic recordings and ATP measurements, 2-way ANOVA + test of Tukey: *, ** and ***, *P*<0.05, *P*<0.01 and *P*<0.001 vs G0.5 step under the same condition; †, 2† and 3†, *P*<0.05, *P*<0.01 and P<0.001 vs G5-islets; ‡ and 3‡, *P*<0.05 and *P*<0.001 vs previous step; § and 3§, *P*<0.05 and *P*<0.001 vs azide (j) or vs initial step before K30 stimulation (o-p). b-h and ikm, Δ graphs, 1-way ANOVA + test of Dunnett or test for linear trend from left to right: †, 2† and 3†, *P*<0.05, *P*<0.01 and P<0.001 vs G5-islets; *P*=, exact P value for linear trend.

Figure 2. Detailed analysis of the glucose-$[Ca^{2+}]_c$ response curve in islet from ND-donors – Islets were cultured as in figure 1 before measuring their acute functional responses to stepwise glucose stimulation (G5-islets, black symbols and traces; G8-islets, green symbols and traces; G10-islets, blue symbols and traces; G20-islets, red symbols and traces). a, changes in beta-cell D3cpv fluorescence ratio (Ad-RIP-D3cpv infected islets) in response to glucose and K30Dz. Δ graphs, changes in D3cpv fluorescence ratio between the indicated glucose steps computed as for fura2-LR data in figure 1k. Means ± SD for 6 islet preparation, each with 2 islets of each kind. b, acute $[Ca^{2+}]_c$ glucose-response curves drawn from data in figure 2a. c-f, fura2-LR fluorescence ratio during stepwise glucose stimulation in 19 islet preparations different from the 6 preparations used in figure 1k (c) and in subgroups sorted according to the glucose step at which the average $[Ca^{2+}]_c$ was maximal in G20-islets (d, max at 10 mmol/l glucose for 7 preps; e, max at 5 mmol/l glucose for 5 preps; f, max at 0.5 mmol/l glucose for 7 preps). Average $[Ca^{2+}]_c$ was computed over the 2 min preceding the last min of each step. Data are means for the number of islet preparations. g, same legend as for figure 1k (n=4). Δ graphs, the change in fura2-LR fluorescence ratio between the indicated glucose steps was calculated by subtracting the averaged data over the last 2 min of each step.

Statistical analysis, dynamic recordings, 2-way ANOVA + test of Tukey: *, ** and ***, *P*<0.05, *P*<0.01 and *P*<0.001 vs G0.5 step; †, 2† and 3†, *P*<0.05, *P*<0.01 and *P*<0.001 vs G5-islets; ‡, 2‡ and 3‡, *P*<0.05, *P*<0.01 and *P*<0.001 vs previous step; Δ graphs, 1-way ANOVA + test of Dunnett: †, 2† and 3†, *P*<0.05, *P*<0.01 and P<0.001 vs G5-islets

Figure 3. Very long-term effects of glucose on cultured human islets - Islets were cultured for 3 weeks under the same conditions as in figures 1 (G5-islets, black symbols and traces; G8-islets, green symbols and traces; G10-islets, blue symbols and traces; G20-islets, red symbols and traces). a, insulin secreted in the culture medium collected every 2-3 days, 7 days-pooled data are shown for the last 2 weeks of

culture. Means ± SD and individual data for the 10 preparations used for this figure. b-c, islet insulin to DNA content ratio and DNA content after culture and perifusion shown in panel h. Means ± SD and individual data for 5-6 preparations. d-e, proportion of beta- and alpha-cells and percentage of TUNEL-positive beta-cells in islet sections. Means ± SD and individual data for 3-4 preparations. f, NAD(P)H autofluorescence during stepwise glucose stimulation normalised to the fluorescence after 10 min in FCCP. Means ± SD for 7 preparations, each with one or two islets. g-h, fura2-LR fluorescence ratio and insulin secretion rate during stepwise glucose stimulation followed by membrane depolarisation with K30Dz. Means ± SD for 7 (g) or 5 (h) preparations. f-h, Δ graphs, change in NAD(P)H autofluorescence, fura2-LR fluorescence ratio and insulin secretion rate between the indicated glucose steps calculated as in figure 1.

Statistical analysis: a, 2-way ANOVA + test of Tukey: * and **, *P*<0.05 and P<0.01 vs day 1-2; 2† and 3†, *P*<0.01 and *P*<0.001 vs G5-islets. b-e and Δ graphs in f-h, 1-way ANOVA + test of Dunnett or test for linear trend from left to right: †, 2† and 3†, *P*<0.05, *P*<0.01 and *P*<0.001 vs G5-islets; §, *P*<0.02 for linear trend.

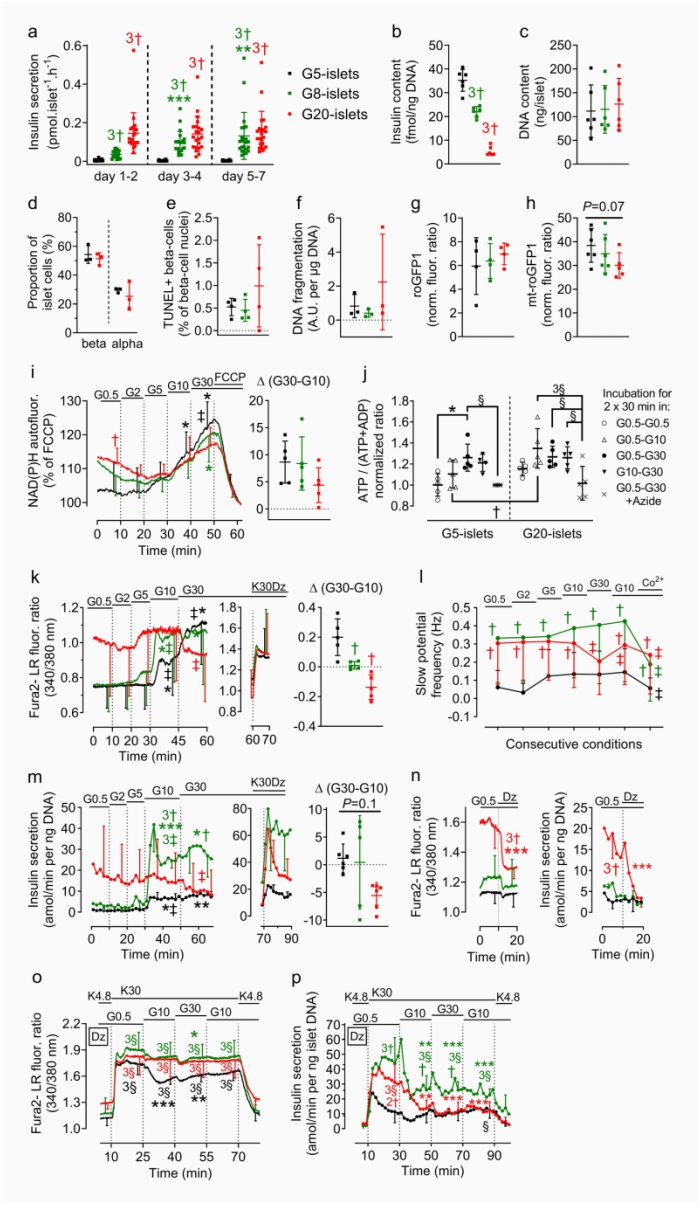
Figure 4. Detailed analysis of the glucotoxic alterations of human beta-cell function – a-b, acute reversibility. Islets were cultured for 1 week at G5 or G20 before measuring $[Ca^{2+}]_c$ and insulin secretion in response to various glucose concentrations as shown on top of the figure. The insulin to DNA content ratio was measured after perifusion. Data are means ± SD for 3-4 preparations. Dashed trace, mean from 2 preparations shown as reference but not used for statistical analysis. **c-d, long-term reversibility.** Islets were cultured for a total of 3 weeks at the glucose concentrations shown for each consecutive week: G5-G5-G5 (3 weeks at G5, black symbols and traces), G5-G5-G20 (last week at G20, red symbols and traces), G5-G20-G5 (second week at G20 then 1 week at G5, orange symbols and traces). c, insulin secretion during culture with data pooled over 3-4 days-periods, means ± SD for 9 preparations. d, $[Ca^{2+}]_c$ responses to stepwise glucose stimulation and K30Dz, means ± SD for 5 preparations; Δ graph, difference in fura2-LR fluorescence ratios between 10 and 30 mmol/l glucose. **e-f, impact of pharmacological glucokinase activation during culture.** Islets were cultured for 1 week at G5 + DMSO 1/1000 (G5-islets, black trace), G20 + DMSO 1/1000 (G20-islets, red trace) or G5 + 3 µmol/l Ro 28-0450, a glucokinase activator (G5+GKA-islets, purple trace). e, insulin secretion in the medium collected at the end of day 2, day 4 and day 7 of culture, means ± SD and individual data for 5 preparations. f, $[Ca^{2+}]_c$ responses to stepwise glucose stimulation and K30Dz, means ± SD for 6 preparations; Δ graph, difference in fura2-LR fluorescence ratios between 10 and 30 mmol/l glucose. **g, impact of acute pharmacological glucokinase**

activation. Islets were cultured for 1 week in RPMI containing G5 (black traces) or G20 (red traces). $[Ca^{2+}]_c$ responses to acute glucose stimulation in the presence of DMSO 1/1000 (dashed traces) or 3 µmol/l GKA (solid traces). Δ graphs, difference in fura2-LR fluorescence ratios between the indicated glucose concentrations in the presence of vehicle or GKA. Means ± SD for 4 preparations.

Statistical analysis: 2-way ANOVA + test of Tukey: *, ** and ***, P<0.05, P<0.01 and *P*<0.001 vs G0.5 step under the same condition; †, 2† and numbers, *P*<0.05, *P*<0.01 and exact *P* values vs G5-islets; ‡, 2‡, 3‡ and §, *P*<0.05, *P*<0.01, *P*<0.001 and *P*=0.062 vs previous step. Δ graphs, 1-way ANOVA + test of Dunnett: †, 2†, 3† and numbers, *P*<0.05, *P*<0.01, *P*<0.001 and exact *P* values vs G5-islets.

Figure 5. Long-term effects of glucose on cultured islets from type 2 diabetic donors - After preculture, islets from T2D donors were cultured for one week in RPMI containing G5 (black trace), G8 (green trace) or G20 (red trace). a, insulin secreted in the culture medium collected every 2-3 days. Means ± SD and individual data for 5-6 preparations. b-c, islet insulin and DNA contents after perifusion shown in panel i. Means ± SD and individual data for 6 preparations. d, percentage of apoptotic beta-cells and proportion of beta-cells measured on the same islet sections. Means ± SD and individual data for 4 preparations. e-f, cytosolic and mitochondrial glutathione oxidation measured as in figure 1 g-h. Means ± SD and individual data for 3-4 (e) and 5 (e) preparations. g, NAD(P)H autofluorescence during stepwise glucose stimulation normalised to the fluorescence after 10 min in FCCP. Means ± SD for 5 preparations. h-i, fura2-LR fluorescence ratio and insulin secretion rate during stepwise glucose stimulation followed by membrane depolarisation with K30Dz. The islet insulin to DNA content ratio was measured after perifusion. Means ± SD for 6 preparations. g,h,i Δ graphs, change in NAD(P)H autofluorescence, fura2-LR fluorescence ratio and insulin secretion rate between the indicated glucose steps.

Statistical analysis: a, g, h, i, 2-way ANOVA + test of Tukey: *, ** and ***, *P*<0.05, *P*<0.01 and *P*<0.001 vs G0.5 step; †, 2† and 3†, *P*<0.05, *P*<0.01 and *P*<0.001 vs G5-islets; ‡, 2‡ and 3‡, *P*<0.05, *P*<0.01 and *P*<0.001 vs previous step; §, *P*<0.05 vs G2 step. b-e and Δ graphs in g, h, i, 1-way ANOVA + test of Dunnett or test for linear trend from left to right: † and 2†, *P*<0.05 and *P*<0.01 vs G5-islets; ¶, *P*=0.036 for linear trend.



Long-term (one week) effects of glucose on cultured human islets

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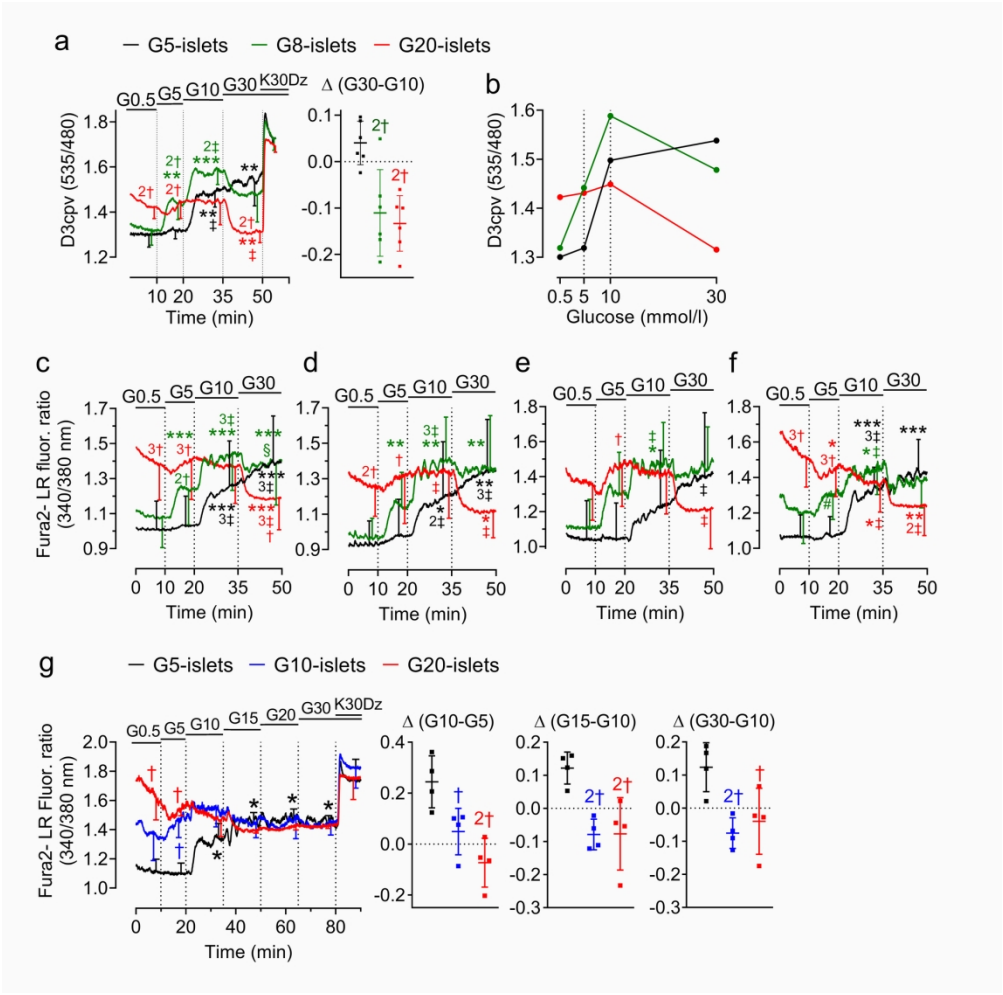

Detailed analysis of the glucose-[Ca2+]c response curve in islet from ND-donors

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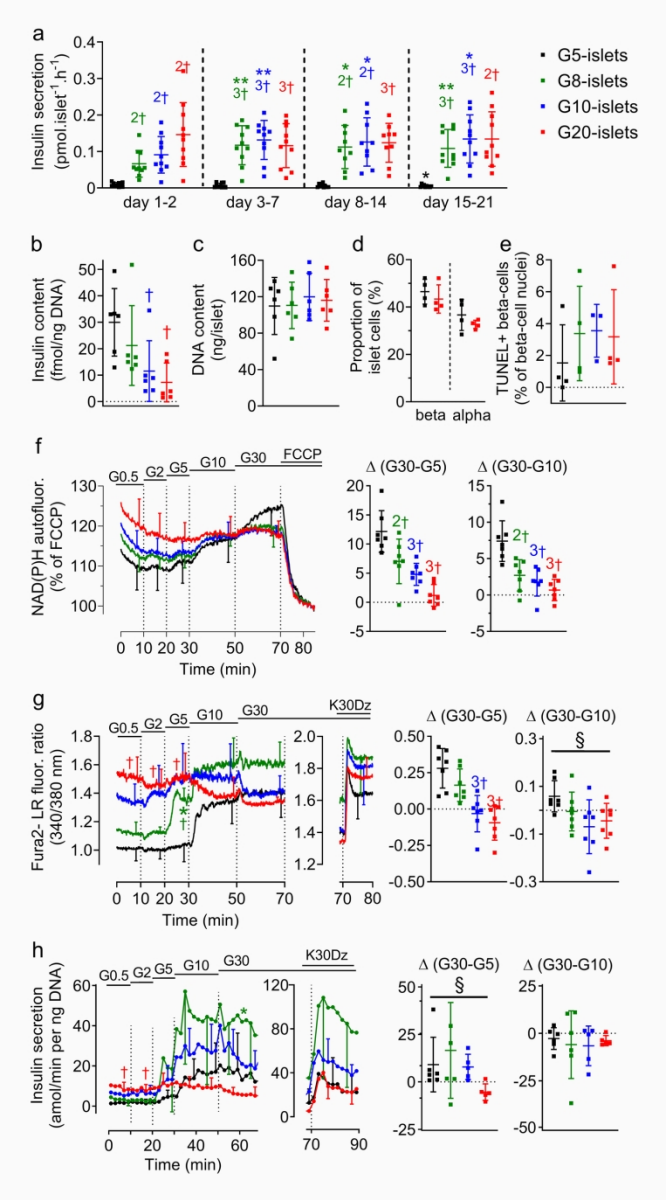

Very long-term effects of glucose on cultured human islets

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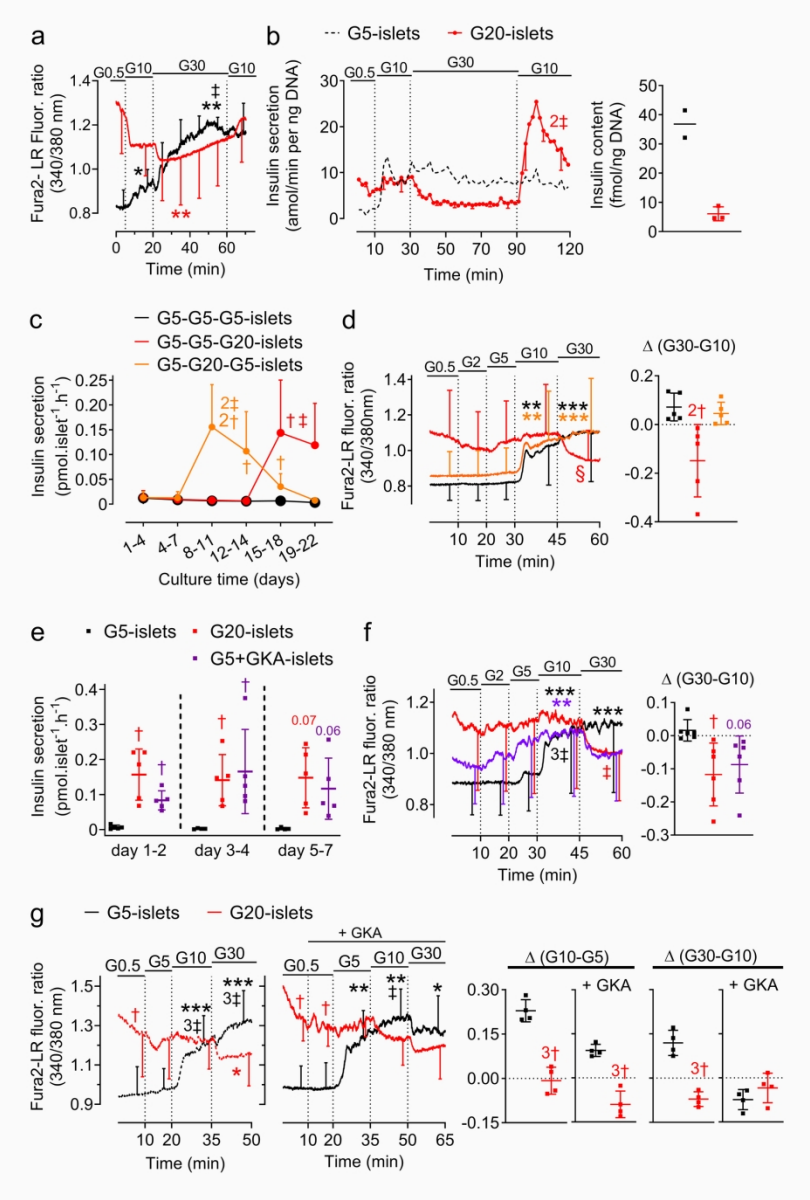

Detailed analysis of the glucotoxic alterations of human beta-cell function

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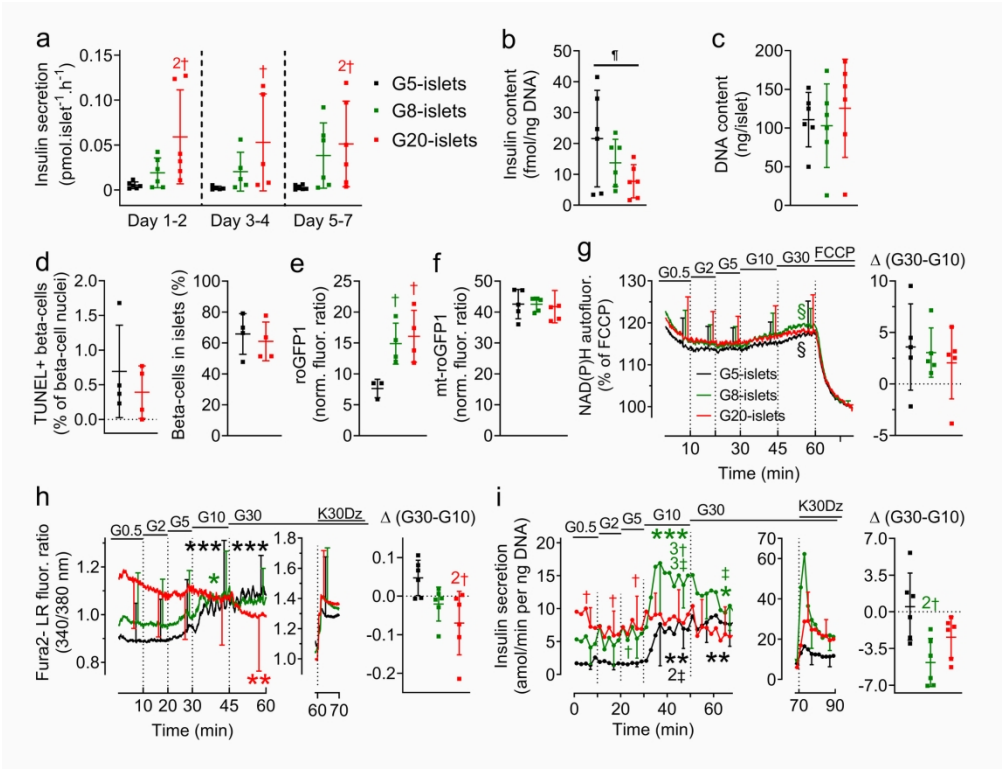

Long-term effects of glucose on cultured islets from type 2 diabetic donors

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ESM Materials and Methods

1. Reagents - Dithiotreitol (DTT), aldrithiol (AT2), diazoxide (Dz), sodium azide, dimethylsulfoxide (DMSO) and carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) were from Sigma, the glucokinase activator (GKA) Ro 28-0450 was from Axon Medchem (Groningen, The Netherlands), D-mannoheptulose was from Carbosynth (UK), and fura2-LR acetoxymethyl ester was from TEF labs / Ion Biosciences (Austin, Tx, USA). The adenoviruses coding roGFP (Ad-roGFP) and mt-roGFP (Ad-mt-roGFP) under the control of the CMV promoter have been described previously [1]. The adenovirus coding the Ca^{2+} sensor D3cpv [2] under the control of a rat insulin promoter (Ad-RIP-D3cpv) was constructed and packaged by VectorBuilder (Cyagen Biosciences, USA, vector ID VB210923-1281bzj).

2. Human islet preparations - Islets from 49 non-diabetic (ND) donors and 6 donors with a history of T2D were obtained from 5 different isolation centers between January 2012 and June 2022 (Informed consent was obtained for all pancreases at each isolation center). The characteristics of donors and islet preparations are detailed in ESM-Table 1 and summarised in Table 1. Of note, the islets from Alberta and Montpellier were rather large, with a diameter before shipment from Alberta ranging from 54 to 540 µm. After isolation, islets were cultured and shipped in CMRL medium containing 5.5 mmol/l glucose and supplemented with penicillin (100 UI/ml), streptomycin (100 µg/ml), glutamine (2 mmol/l), Hepes and 10% FBS or Human Serum Albumin (HAS) (FBS for Montpellier, HSA for ECIT, or Prodo lab medium for SAMN preps). Islets were usually received within 24h of shipment from European centers, or within 48h of shipment from Canada or USA, with occasional 24h additional delay. The experiments were approved by the ethic committee of UCL-Saint-Luc (project number 2017/05JUL/355) and the samples were registered at the Biothèque UCLouvain.

3. Culture of human islets - Upon reception, the islets were rinsed twice then precultured for 4-7 days in RPMI 1640 medium containing 5.5 mmol/l glucose supplemented with penicillin (100 UI/ml), streptomycin (100 µg/ml), glutamine (2 mmol/l) and 10% FBS (ThermoFisher, catalog # 10500-064). In 9 islet preparations, part of the islets was cultured in the same culture medium supplemented with 5 g/l BSA fraction V (Roche) instead of FBS. During preculture, the islets were hand-picked to another dish containing fresh medium every 2-3 days, leaving behind cell aggregates that were firmly attached to the dish and discarding big islets with signs of central necrosis on visual inspection with a stereomicroscope. After preculture, islets were cultured for 1-3 weeks in the same medium containing 5.5, 8.8, 11.1 or 22.2 mmol/l glucose before supplementations. When the culture media were supplemented with FBS, assuming that the glucose concentration in FBS was ~7 mmol/l, the final glucose concentration was estimated at about 5.6, 8.5, 10.5 and 20.5 mmol/l. When the culture media were supplemented with BSA, the final glucose concentration was 5.1, 8.2, 10.3 and 20.6

mmol/l. For ease of description, these islets are consistently referred to as G5-, G8-, G10- and G20-islets. During culture, the islets were transferred to fresh medium every 2-3 days.

4. Apoptosis

4.1. DNA fragmentation: Islet cell death was determined by quantification of cytoplasmic histone-associated DNA fragments using the "Cell Death Detection ELISA" (Roche Diagnostics, Castle Hill, NSW, Australia). For each culture condition, 60 islets were placed in 60 µl of lysis buffer and processed as per manufacturer instructions. At the end of the procedure, the islet cell pellets were rinsed with Tris-EDTA buffer and their total DNA content was measured by fluorimetry using SYBR Green I as a DNA stain (Roche Diagnostics, Castle Hill, NSW, Australia) and used for normalisation of the ELISA absorbance values.

4.2. TUNEL and insulin staining: For TUNEL assay after 1 to 3 weeks of culture, 100-150 islets were fixed in 4% paraformaldehyde for 4 h and embedded in paraffin. The percentage of apoptotic β-cells was determined on 5 µm-thick islet sections using the "In Situ Cell Death Detection Kit" (Roche Diagnostics) followed by insulin immunodetection. Briefly, islet sections were washed and blocked with BSA (50 g/l) followed by incubation with guinea pig anti-insulin antibody (DAKO; A0564) diluted 1:500 or rabbit anti-insulin antibody (Cell Signaling Technology; C27C9) diluted 1:500 at 4˚C overnight. Islet sections were then incubated for 1 hour at room temperature with anti-guinea pig or anti-rabbit Alexa Fluor 594-conjugated secondary antibody diluted 1:2000 [3]. Islet cell nuclei were stained with DAPI. Images were captured with a 20x objective on an Axioimager fluorescence microscope (ESM-Fig.1). Cells were manually counted using ImageJ analysis software, excluding central necrotic area. Except in 3 samples in which we could only count 478 cells from 4 islets, 733 cells from 7 islets and 673 cells from 7 islets, we systematically counted at least 10 islet sections and more than 1000 cells from each sample. The total number of cells counted in all preparations are indicated in the legend to the figures.

5. Measurement of alpha- and beta-cell proportion in islets - Islet sections were prepared as described for TUNEL assay. After 10-20 min microwaving (100 W) in citrate buffer pH 5.5 for antigen retrieval and 1h incubation at RT in PBS containing 0.05% (vol:vol) Triton and 50 g/l BSA to reduce unspecific binding, sections were incubated overnight at 4°C with rabbit anti-insulin (C27C9) antibody diluted 1:500 in PBS containing Triton and 10 g/l BSA, then with mouse monoclonal anti-glucagon (Sigma G2654) diluted 1:1000 for 2h at RT before consecutive incubation with anti-rabbit AlexaFluor 594-conjugated antibody diluted 1:2000 for 1h at RT, then anti-mouse Alexa 488 conjugated antibody diluted 1:2000 for 1h at RT. Sections were washed 3 times in PBS containing Triton 0.05% vol/vol between each incubation. Images were captured and analysed as for TUNEL assay.

6. Solutions - Experiments to evaluate islet cell function were carried out in a Krebs solution (KRB) containing (in mmol/l) NaCl (124), KCl (4.8), $CaCl_2$ (2.5), $MgCl_2$ (1.2), $NaHCO_3$ (20), 1 g/l BSA (fraction V; Roche), and n mmol/l glucose (Gn). When KCl was raised to 30 mmol/l, NaCl was reduced to 98.8 mmol/l. The solutions were continuously gassed with 5% CO_2 in pure O_2 to maintain pH ~7.4.

7. ATP measurements - After culture, islets were preincubated for 30 min in KRB buffer containing 0.5 mmol/l glucose. They were then incubated 30 min in groups of 10 in 500 µl KRB containing 0.5 or 10 mmol/l glucose before a further 30 min incubation in 1 ml of KRB containing various glucose concentrations. After removal of 625 µl of medium, the islets were disrupted with 125 µl trichloroacetic acid (20 %), vortexed and centrifuged at maximum speed for 5 min at 4˚C. The supernatant (450 µl) was submitted to a triple diethyl ether extraction, diluted with 450 µl of a buffer containing 20 mmol/l HEPES, 3 mmol/l $MgCl_2$, and KOH to adjust pH at 7.75, and stored at −80°C. ATP was measured by a luminometric method, as previously described [4]. Blanks and ATP standard curves in KRB were run through the entire procedure. The sum (ATP+ADP) was measured after conversion of ADP into ATP by pyruvate kinase in the presence of phosphoenolpyruvate, and the ATP/(ATP+ADP) ratio was computed and normalised to the ratio in G5-islets exposed to azide in the same experiment/islet preparation.

8. Fluorescence imaging of NAD(P)H, $[Ca^{2+}]_c$ and glutathione redox state (E_{GSH}) - After culture, islets were perifused with KRB at a flow rate of ~1ml/min at 37˚C on the stage of an inverted fluorescence microscope. The duration of perifusion before recording was ~20 min in all experiments, allowing ~30 min recovery at 0.5 mmol/l glucose before stimulation. NAD(P)H autofluorescence ($\lambda_{ex}/\lambda_{em}$ 360/470 nm) was measured every 10 s during perifusion as described previously [7]. For $[Ca^{2+}]_c$ recordings, islets were loaded for 2 h with 2µmol/l Fura2-LR acetoxymethyl ester in a medium containing the same glucose concentration as during culture, then the Fura2-LR fluorescence ratio (λ_{ex} 340/380 nm; λ_{em} 510 nm) was recorded every 5 s during perifusion. Alternatively, islets were infected with Ad-RIP-D3cpv (multiplicity of infection ~100) 2 days before the experiment, and the fluorescence ratio of the probe (λ_{ex} 430 nm; λ_{em} 535/480 nm) was recorded every 5 s during perifusion. To measure cytosolic and mitochondrial E_{GSH}, islets were infected with Ad-roGFP1 or Ad-mt-roGFP1 (MOI ~50) 2 days before the end of culture. The fluorescence of the probe was then recorded at 535 nm during alternate excitation at 405/485 nm every 30 s during perifusion. The fluorescence ratio was normalised to the ratio measured in the same islets consecutively exposed to 10 mmol/l DTT (set to 0%) and to 100 µmol/l AT2 (set to 100%) at the end of the experiment as described [5].

9. Measurement of insulin secretion, insulin content and DNA content - For measurement of insulin secretion during culture, the medium was collected after islet transfer to fresh medium or at the end

of culture and centrifuged at 800 rpm for 3 min to remove cell debris. The supernatant was diluted for measurement of insulin concentration by in-house RIA using human insulin as standard. For dynamic measurement of insulin secretion, batches of 100-150 islets were perifused with KRB at a flow rate of ~1 ml/min, and insulin was measured by RIA in the effluent collected every 2 min. The diameter of perifused islets without signs of central necrosis ranged from 75 to 330 µm (corresponding to approximately 320-24400 cells) in insulin secretion measurements, and from 75 to 200 µm in NAD(P)H and $[Ca^{2+}]_c$ measurements. As for NAD(P)H and $[Ca^{2+}]_c$ measurements, islets were perifused for ~20 min before starting sample collection, allowing ~30 min recovery at 0.5 mmol/l glucose before stimulation. Islets were recovered from the perifusion chamber and freeze-thawed / sonicated 3 x 5 s in 500 µl Tris-EDTA buffer before measurement of insulin by RIA and of DNA using the Pico488 dsDNA quantification kit (Lumiprobe Gmbh, Hannover, Germany).

10. Membrane potential measurement by Multi-Electrode Arrays (MEAs) - Human islets were seeded on MEAs (60MEA200/30iR-Ti-gr, 60 electrodes; Multichannel Systems, MCS, Reuttlingen, Germany) coated with Matrigel (2% v/v; BD Biosciences, San Diego, CA, USA). After 1 week of culture in media containing 5.5, 8.8 and 22.2 mmol/l glucose (see above), islets on MEAs were continuously perifused (0.5 ml/min; Ismatec Reglo ICC, Glattbrugg, Switzerland) at 37°C in a buffer containing (in mmol/l): NaCl 135, KCl 4.8, $MgCl_2$ 1.2, $CaCl_2$ 2.5, HEPES 10, BSA 0.1% and glucose as indicated (pH 7.4 adjusted with NaOH). When specified, 1 mM $CoCl_2$ was applied to block Ca^{2+} channels. Images of islets on MEAs were taken before and after each recording to localise islets on electrodes. Extracellular field potentials were acquired at 10 kHz as published [6], amplified (gain, 1200) and band-pass filtered (0.1-3000 Hz) with a USB-MEA60-InvSystem-E amplifier controlled by MC_Rack software (MCS). Slow potentials (SP), which are multicellular β cell-specific signals due to β cell coupling [7], were isolated as published with MC_Rack using a 0.2-2 Hz 2nd-order Butterworth digital filter and the threshold module of MC_Rack with a dead time (minimal period between 2 SPs) of 300 ms. Mean SP frequencies over the last 5 min of each condition were determined for each islet on electrodes.

11. Statistics - Data are means ± SD for the number of islet preparations/donors indicated, with individual data added to column bars. The statistical significance of differences between groups was evaluated by one-way ANOVA followed by a test a Dunnett or a test for linear trend, or by 2-way ANOVA (for repeated measurements when the comparison was made between selected time points in the same trace) followed by a test of Tukey, or by mixed-effects model analysis followed by a test of Sidak when there were few missing data. We used GraphPad Prism version 9.

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ESM Discussion

About the lack of increase in beta-cell apoptosis in glucotoxic human islets and the possible role of amyloid formation - In contrast with studies in rodent islets [1], we did not detect a significant increase in beta-cell apoptosis in human islets cultured for up to 3 weeks under glucotoxic conditions. This observation is in good agreement with the lack of detectable increase in beta-cell apoptosis in human islets exposed to insulin resistance and hyperglycaemia in several humanised mouse models of diabetes [2]. Nevertheless, considering the variability between donors and the relatively small number of islet preparations in which we measured beta-cell apoptosis, the larger dispersion of the data in G20- vs G5- and G8-islets suggests that beta-cell apoptosis may increase in a subgroup of islet preparations. Therefore, we want to insist that our data do not question previous studies showing that the beta-cell mass is significantly reduced in subjects with a history of T2D in part through an increase in cell apoptosis [3, 4], nor do we exclude a possible role of amyloid formation in human beta-cell loss in T2D [5, 6] . However, we think that beta-cell apoptosis and amyloid formation are unlikely contributing to the rapidly reversible glucotoxic alterations of beta-cell function we describe in our study.

References to ESM Discussion

[1] Bensellam M, Laybutt DR, Jonas JC (2012) The molecular mechanisms of pancreatic beta-cell glucotoxicity: Recent findings and future research directions. Mol Cell Endocrinol 364(1-2): 1-27

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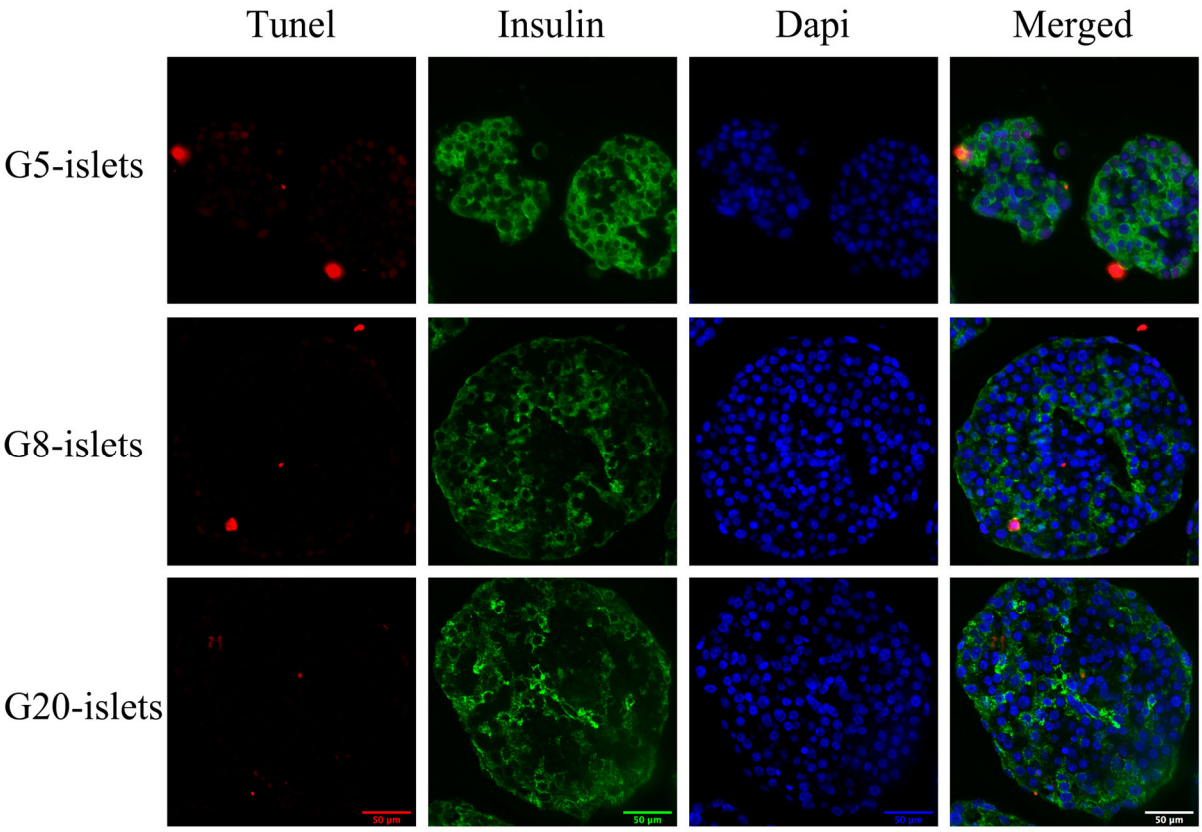
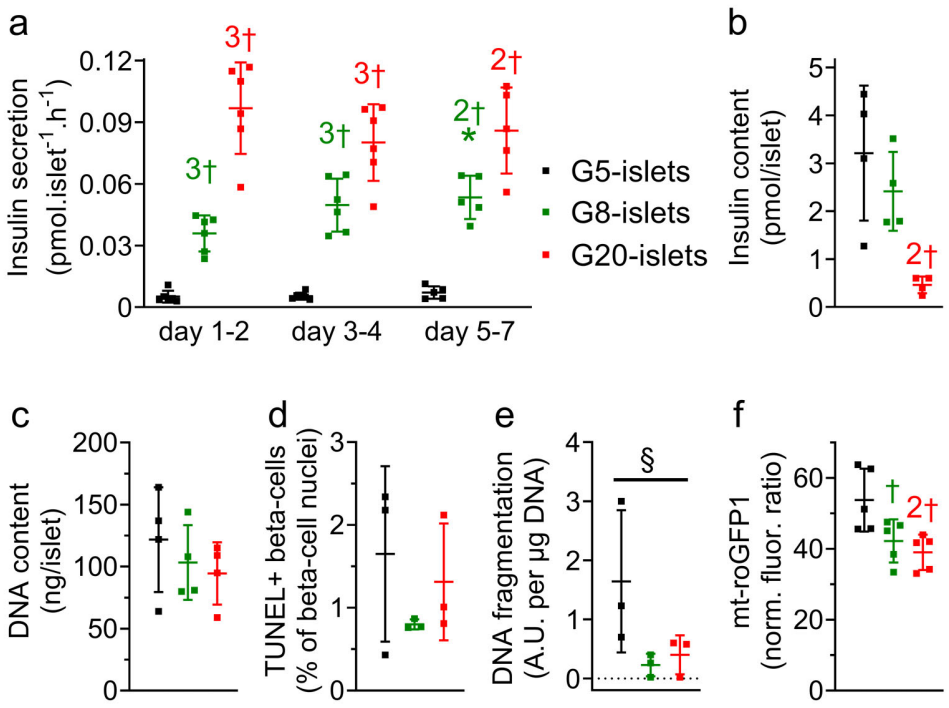
ESM figures

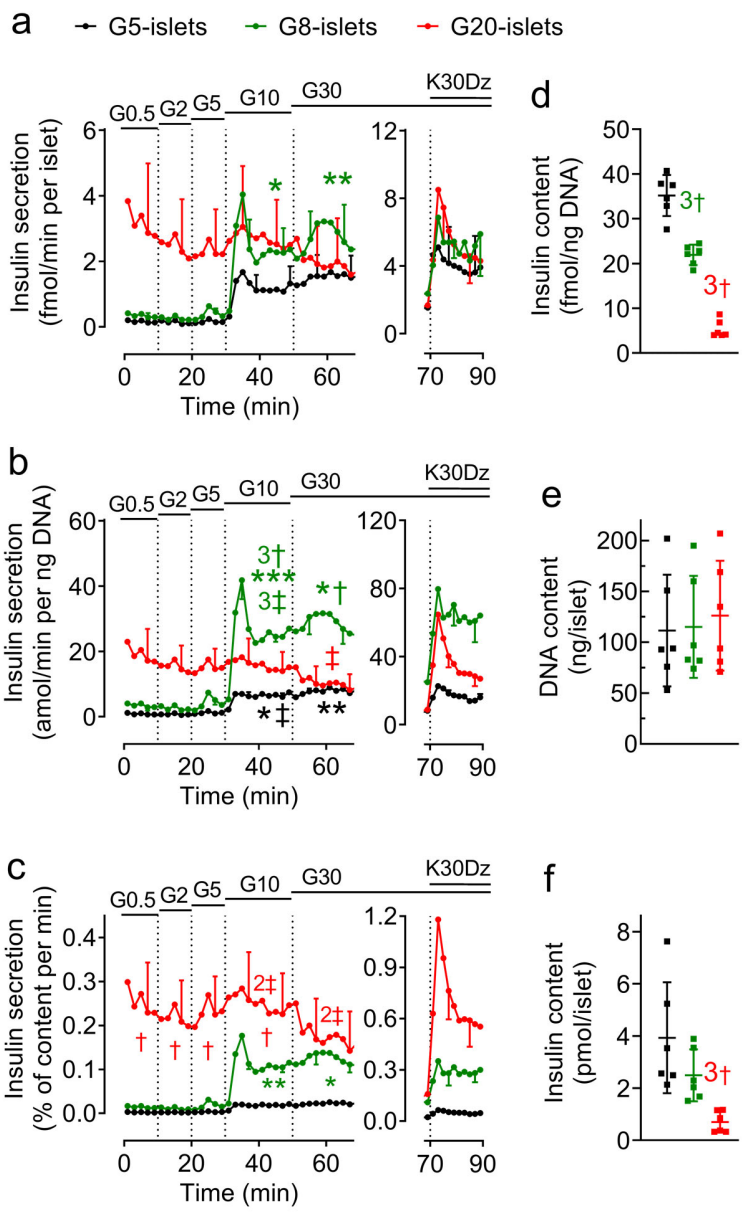


ESM-Fig.1. Measurement of beta-cell apoptosis on islet sections – After culture, islets were fixed and processed for TUNEL assay, insulin immunodetection and DNA stain as described in ESM methods. Insulin-positive (beta) cells (green signal), TUNEL-positive cells (red signal) and cell nuclei (blue signal) were counted using ImageJ software. Shown are representative images of islets from one preparation of islets cultured for 3 weeks at G5, G8 and G20. Bar scale = 50 µm.

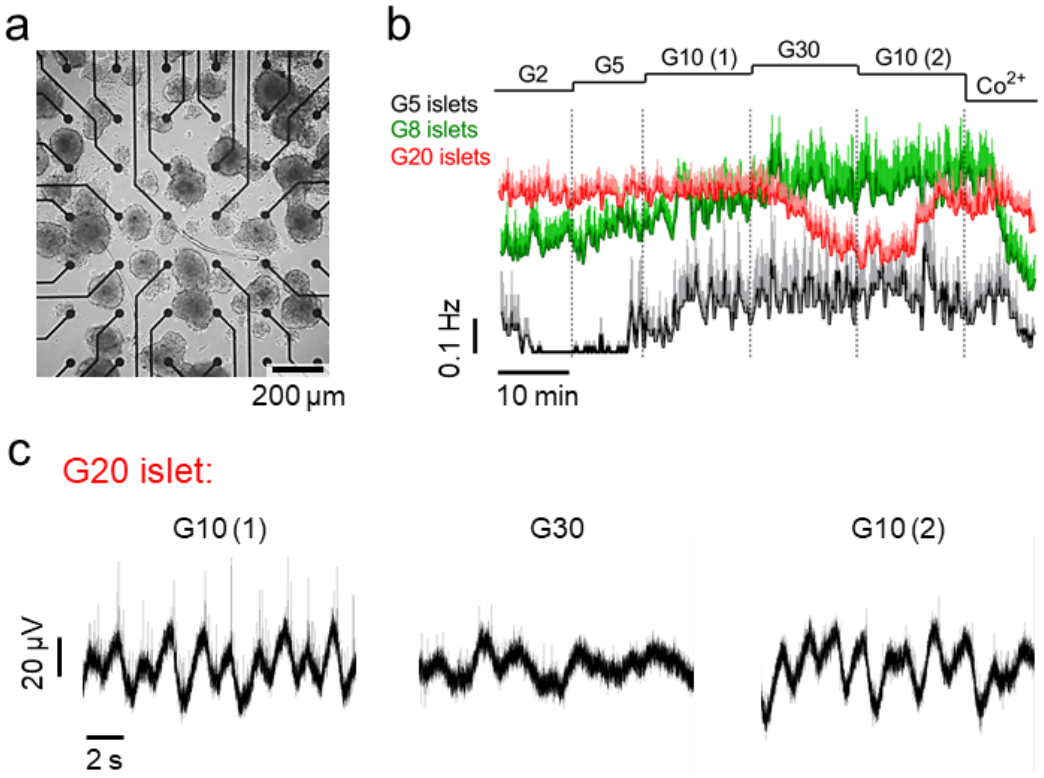


ESM-Fig. 2 (related to Fig.1a-h). Long-term (one-week) effects of glucose on insulin secretion, insulin content, apoptosis and mitochondrial glutathione oxidation in human islets cultured in RPMI medium supplemented with 0.5g/l BSA instead of 10% FCS - Islets were cultured as for figure 1 but the RPMI medium was supplemented with 0.5 g/l BSA instead of 10% FBS. a, insulin secreted in the culture medium collected every 2-3 days. Means ± SD and individual data for the 6 preparations. b-c, islet insulin and DNA content after culture. Means ± SD and individual data for 4 preparations. d, percentage of TUNEL-positive beta-cells on islet sections. Means ± SD and individual data for 3 preparations. e, islet histone-associated cytosolic DNA fragments after culture, absorbance arbitrary units normalised for differences in islet DNA content. Means ± SD and individual data for 3 preparations. f, mitochondrial glutathione oxidation as reflected by mt-roGFP1 normalised fluorescence ratio (see Methods). Means ± SD and individual data for 5 preparations.

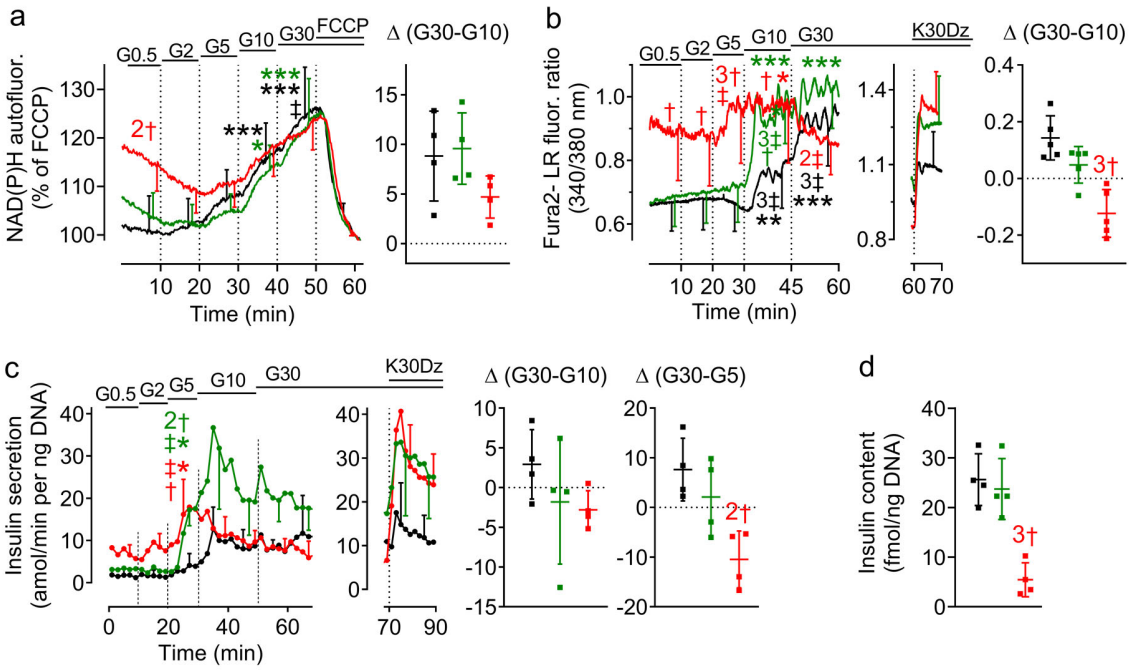
Statistical analysis: a, 2-way ANOVA + test of Tukey: *, $P<0.05$ vs day 1-2; 2† and 3†, $P<0.01$ and $P<0.001$ vs G5-islets. b-f, 1-way ANOVA + test of Dunnett: † and 2†, $P<0.05$ and $P<0.01$ vs G5-islets; §, $P=0.082$ by 1-way ANOVA + test for linear trend.



ESM-Fig.3 (related to Fig.1m). Long-term effects of glucose on dynamic insulin secretion in cultured human islets – Comparison of dynamic insulin secretion in G5-, G8- and G20-islets (Fig.1m) expressed in fmol/min per islet (a), in amol/min per ng of islet DNA content (b, same data as in Fig.1m), and in percent of the islet insulin content (c) (G5-islets, black symbols and traces; G8-islets, green symbols and traces; G20-islets, red symbols and traces). Δ graphs, change in insulin secretion rate between the indicated glucose steps calculated as in figure 1m. The islet insulin content, DNA content and insulin to DNA content ratio were measured at the end of perifusion. Statistical analysis: Dynamic recordings, 2-way ANOVA + test of Tukey: *, ** and ***, $P<0.05$, $P<0.01$ and $P<0.001$ vs G0.5 step under the same condition; † and 3†, $P<0.05$ and P<0.001 vs G5-islets; ‡, 2‡ and 3‡, $P<0.05$, $P<0.01$ and $P<0.001$ vs previous step. Δ graphs, 1-way ANOVA + test of Dunnett or test for linear trend from left to right: 3†, $P<0.001$ vs G5-islets; §, $P<0.05$ for linear trend

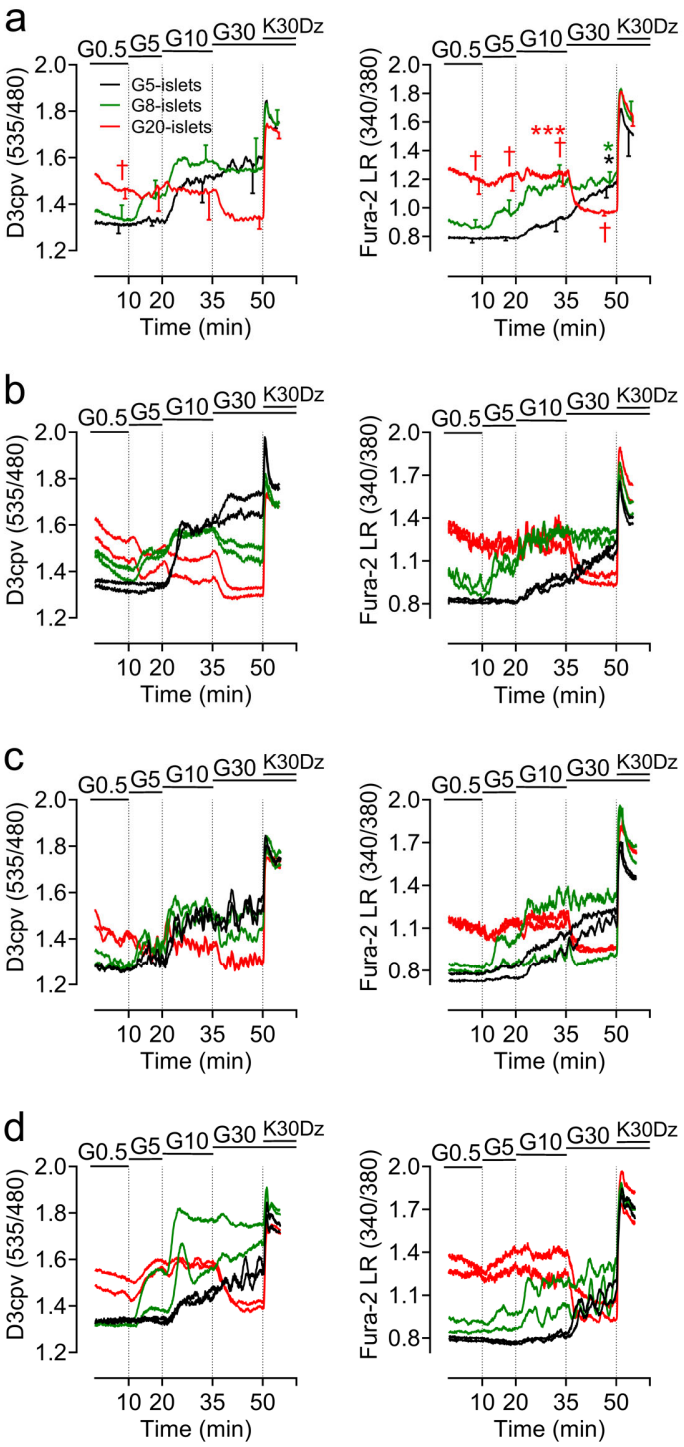


ESM-Fig.4 (related to Fig.1l) Dynamic recording of islet electrical activity on multi-electrode arrays - Representative MEA experiment on human islets. a, image of islets on a MEA. b, SP frequencies of islets from the same donor after 1 week of culture at G5, G8, and G20 (n=3-16). The electrical activity of islets was recorded in the presence of increasing glucose concentrations as indicated at the top of the figure. Co^{2+}: 1 mmol/l $CoCl_2$ applied at G0.5. c, representative recordings of a G20-islet in 3 successive conditions indicated in b: G10 (1), G30 and G10 (2), showing the reversible decrease in SP frequency upon acute stimulation with G30.



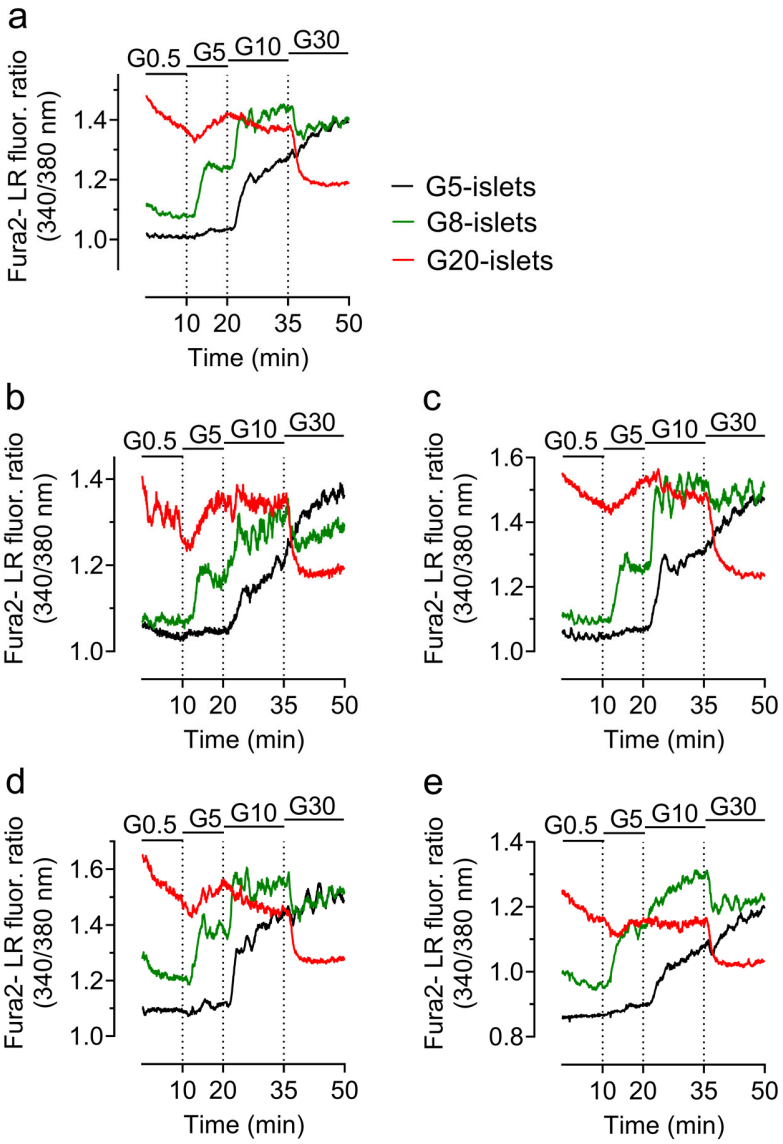
ESM-Fig.5 (related to Fig.1). Long-term effects of glucose on stimulus-secretion coupling events in human islets cultured in RPMI medium supplemented with 0.5g/l BSA instead of 10% FCS - Islets were cultured as for Suppl. figure 1 before measuring their acute functional responses to stepwise glucose stimulation (G5-islets, black symbols and traces; G8-islets, green symbols and traces; G20-islets, red symbols and traces). a, NAD(P)H autofluorescence during stepwise glucose stimulation. Data were normalised to the fluorescence after 10 min in FCCP. Means ± SD for 4 preparations, each with one or two islets. b-c, fura2-LR fluorescence ratio and insulin secretion rate during stepwise glucose stimulation followed by membrane depolarisation with K30Dz. The islet insulin to DNA content ratio was measured after perifusion. Means ± SD for 4-5 preparations. Δ graphs, the change in NAD(P)H autofluorescence, fura2-LR fluorescence ratio and insulin secretion rate between the indicated glucose steps was calculated by subtracting the averaged data over the last 3 min of each step.

Statistical analysis for dynamic recordings: 2-way ANOVA + test of Tukey: *, ** and ***, $P<0.05$, $P<0.01$ and $P<0.001$ vs G0.5 step; †, 2† and 3†, $P<0.05$, $P<0.01$ and $P<0.001$ vs G5-islets; ‡, 2‡ and 3‡, $P<0.05$, $P<0.01$ and $P<0.001$ vs previous step. Δ graphs, 1-way ANOVA + test of Dunnett: † and 3†, $P<0.05$ and $P<0.001$ vs G5-islets

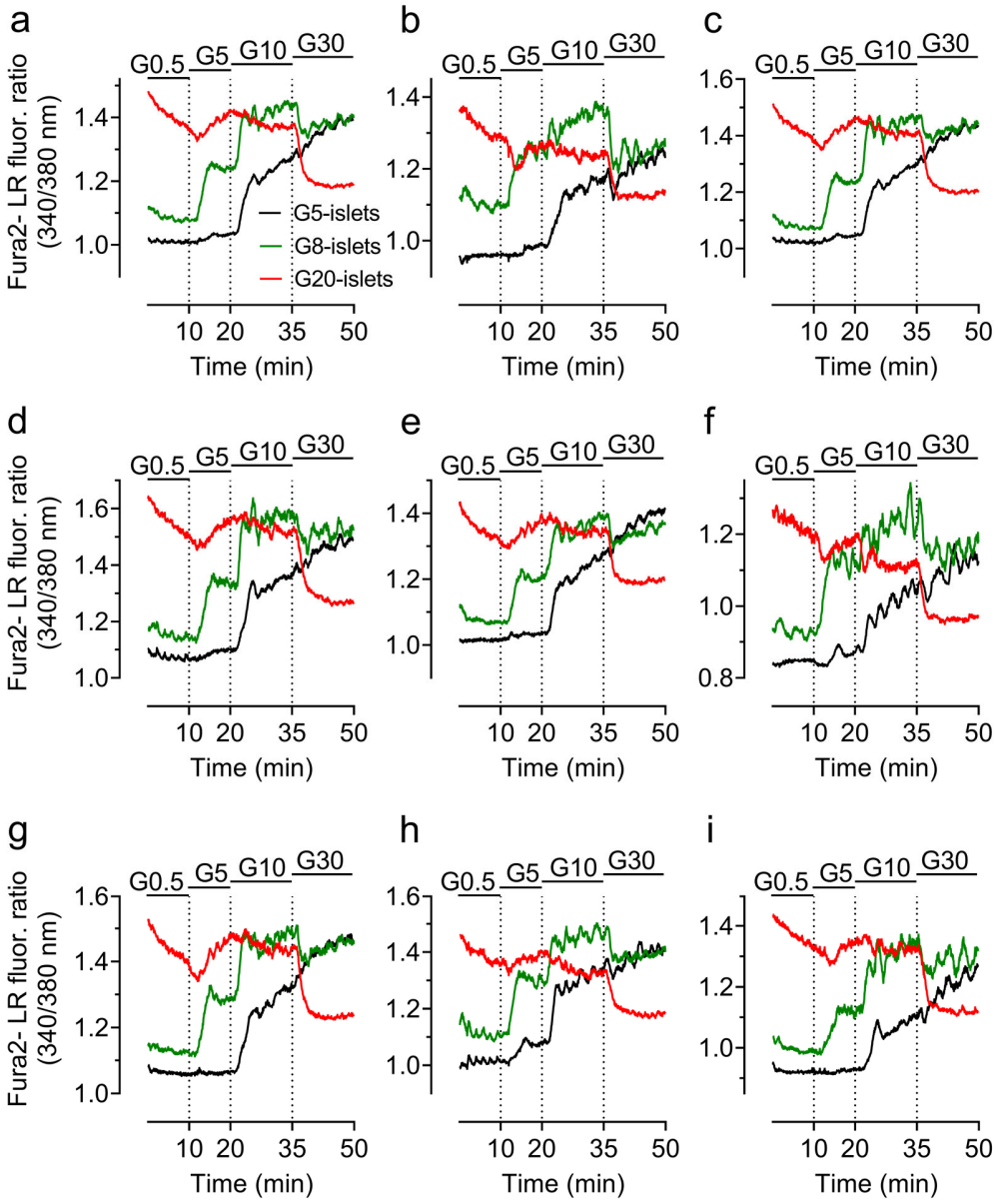


ESM-Fig.6 (related to Fig.2a) Comparison of glucose-induced changes in $[Ca^{2+}]_c$ recorded in Ad-RIP-D3cpv infected islets (beta-cell specific) and fura2-LR loaded islets (all islet cell types) - Islets were cultured as in figure 1 before measuring their acute functional responses to stepwise glucose stimulation (G5-islets, black symbols and traces; G8-islets, green symbols and traces; G20-islets, red symbols and traces). a, mean ± SD for the same 3 preparations. b-d, individual traces for 2 islets of each kind in each islet preparation.

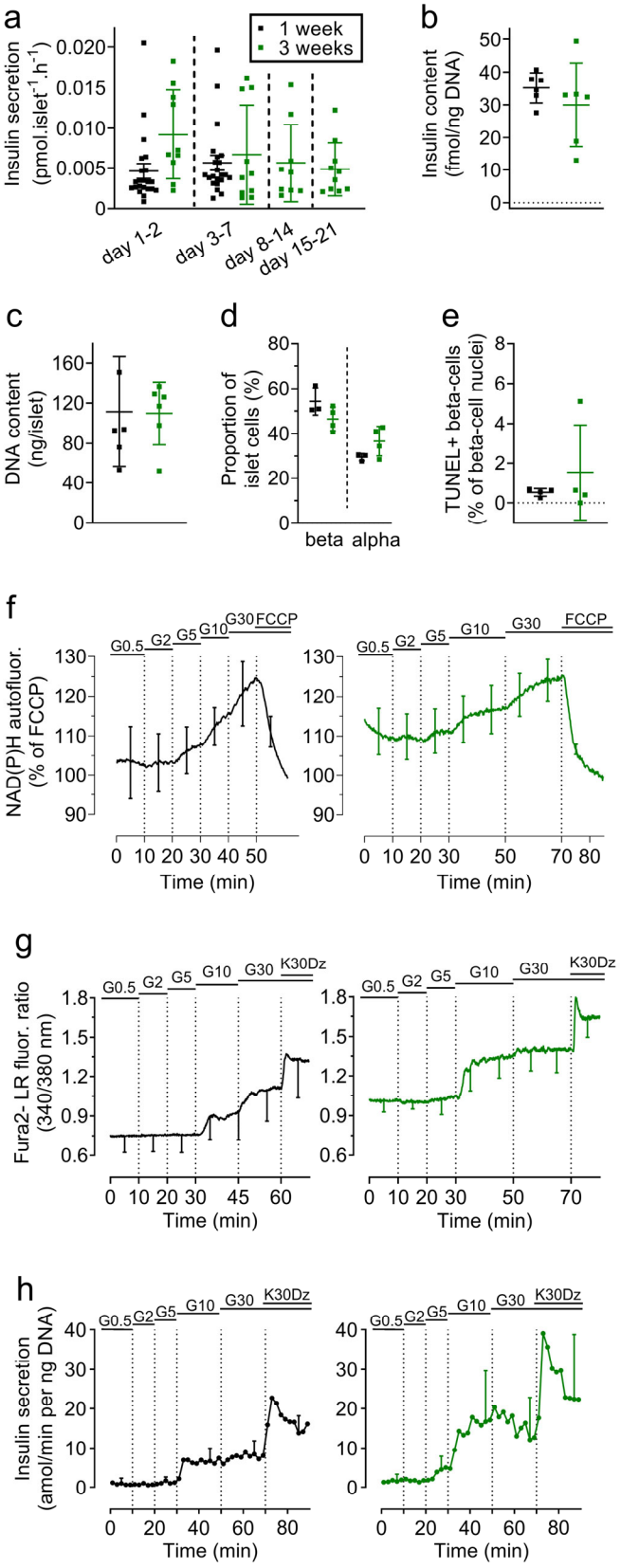
Statistical analysis for dynamic recordings: 2-way ANOVA + test of Tukey: * and ***, $P<0.05$ and $P<0.001$ vs G0.5 step; †, $P<0.05$ vs G5-islets



ESM-Fig.7 (related to Fig.2c) The glucotoxic alteration of $[Ca^{2+}]_c$ responses to an acute glucose stimulation are independent from the isolation center - Fura2-LR fluorescence ratio during stepwise glucose stimulation in 19 islet preparations from 4 different isolation centers (a)(2 from Montpellier (b), 7 from the Alberta Islet Core (c), 5 from the ECIT (d), and 5 from the IIDP (e)). These preparations were different from the 5 preparations from Montpellier (3 male and 2 female donors) used in Fig.1k. Data are means for the number of preparations.

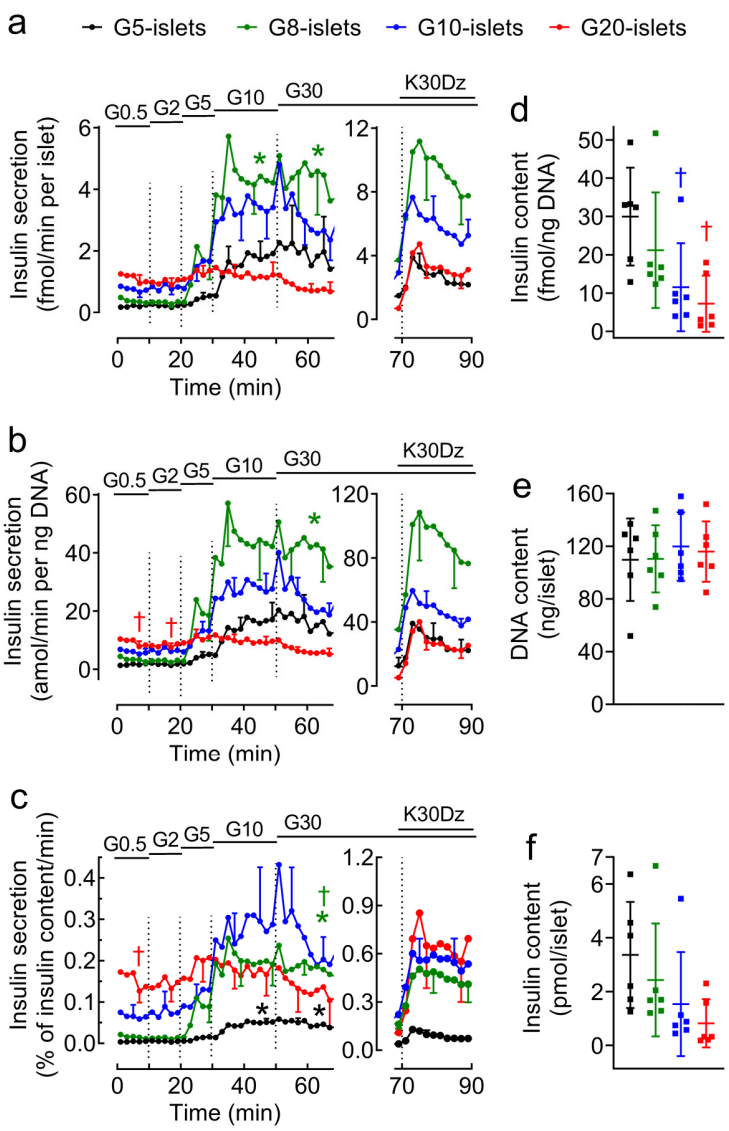


ESM-Fig.8 (related to Fig.2c) The glucotoxic alteration of $[Ca^{2+}]_c$ responses to an acute glucose stimulation are independent from the sex, age and BMI of the donor - Fura2-LR fluorescence ratio during stepwise glucose stimulation in the 19 islet preparations used in suppl. figure 3 (a) sorted according to the donor sex (b, 4 females; c, 15 males), the donor age (d, 7 donors below 50 year-old; e, 9 donors between 50 and 60 year-old; f, 3 donors above 60 year-old) or the donor BMI (g, 8 donors with BMI<25; h, 6 donors with BMI between 25 and 30; i, 5 donors with BMI>30). Data are means for the number of donors.



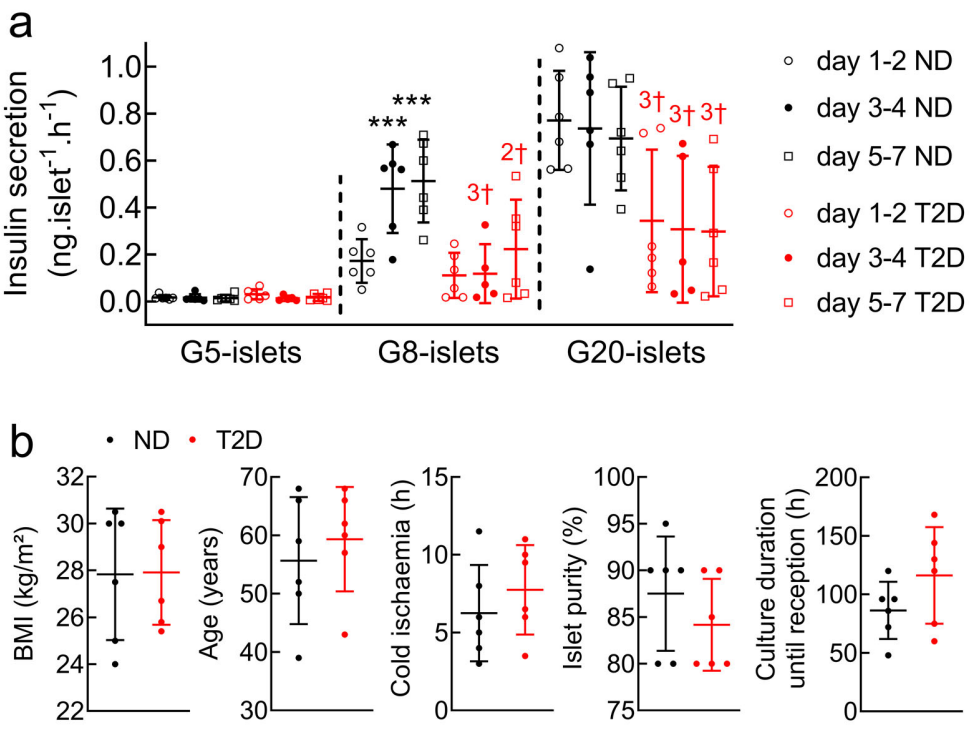
ESM-Fig.9 (related to Figs.1 and 3)

Comparison of islets cultured for 1 and 3 weeks at G5 – Data for G5-islets shown in figures 1 (black symbols and traces) and 3 (green symbols and traces) are shown side by side or in the same graph for easy comparison. Please note that the islets cultured for 1 and 3 weeks were from different preparations. a, insulin secreted in the culture medium collected every 2-3 days, with data pooled for days 3-7, 8-14 and 15-21. Means ± SD and individual data for 9-22 preparations. b-c, islet insulin and DNA content after culture. Means ± SD and individual data for 6 preparations. d-e, proportion of β- and α-cells and percentage of TUNEL-positive beta-cells in islet sections. Means ± SD and individual data for 3-4 preparations. f, NAD(P)H autofluorescence during stepwise glucose stimulation. Data were normalised to the fluorescence after 10 min in FCCP. Means ± SD for 4-5 preparations, each with one or two islets. g-h, fura2-LR fluorescence ratio and insulin secretion rate during stepwise glucose stimulation followed by membrane depolarisation with K30Dz. Means ± SD for from 3-5 preparations (g) or for 6 preparations (h).



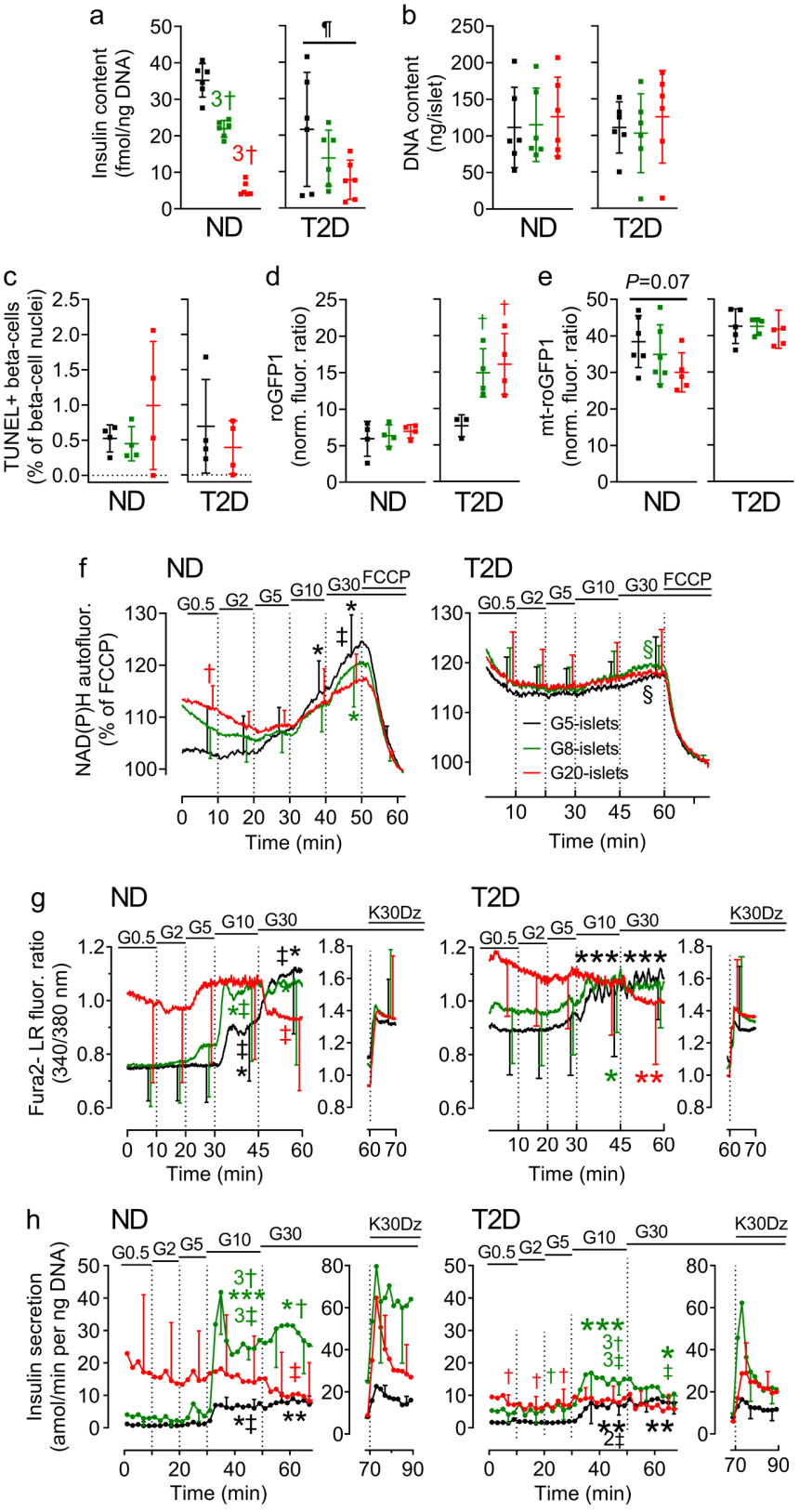
ESM-Fig.10 (related to Fig.3h). Very long-term effects of glucose on dynamic insulin secretion in cultured human islets – Comparison of dynamic insulin secretion in G5-, G8- and G20-islets (Fig.3h) expressed in fmol/min per islet (a), in amol/min per ng of islet DNA content (b, same data as in Fig.3h), and in percent of the islet insulin content (c) (G5-islets, black symbols and traces; G8-islets, green symbols and traces; G10-islets, blue symbols and traces; G20-islets, red symbols and traces). Right panels, change in insulin secretion rate between the indicated glucose steps calculated as in figure 3h. The insulin content, DNA content and insulin to DNA content ratio in islets at the end of perifusion are shown on the far-right panels.

Statistical analysis: Dynamic recordings, 2-way ANOVA + test of Tukey: *, $P<0.05$ vs G0.5 step under the same condition; †, $P<0.05$ vs G5-islets. Δ graphs, 1-way ANOVA + test of Dunnett: †, $P<0.05$ vs G5-islets.



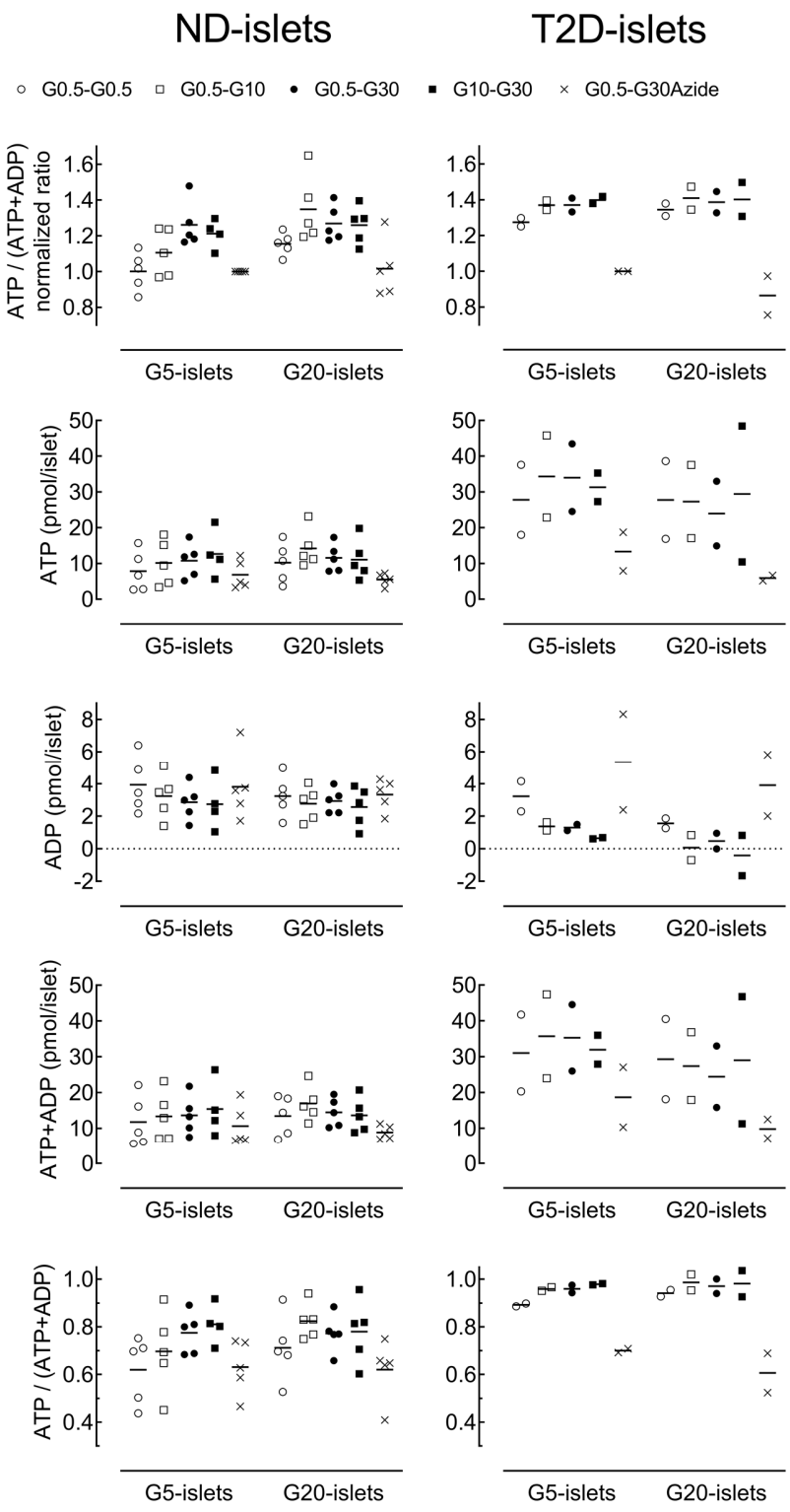
ESM-Fig.11 (related to Fig.5a) Insulin secretion during culture of islets from T2D donors and islets from ND donors matched for sex, age and BMI - a, see legend to figure 5a. Means ± SD and individual data for 6 male T2D-preparations (red symbols) compared to 6 male ND-preparations (black symbols). b, BMI and age of the donors, and duration of cold ischaemia, islet purity and total culture duration between isolation and reception of the islets in the laboratory.

Statistical analysis: a, Mixed-effects model (for paired data with missing values) followed by a test of Sidak. ***, P<0.001 vs day 1-2 at the same glucose concentration; 2† and 3†, *P*<0.01 and *P*<0.001 vs ND-islets at the same time and glucose concentration. b, there were no differences between groups by t-test.

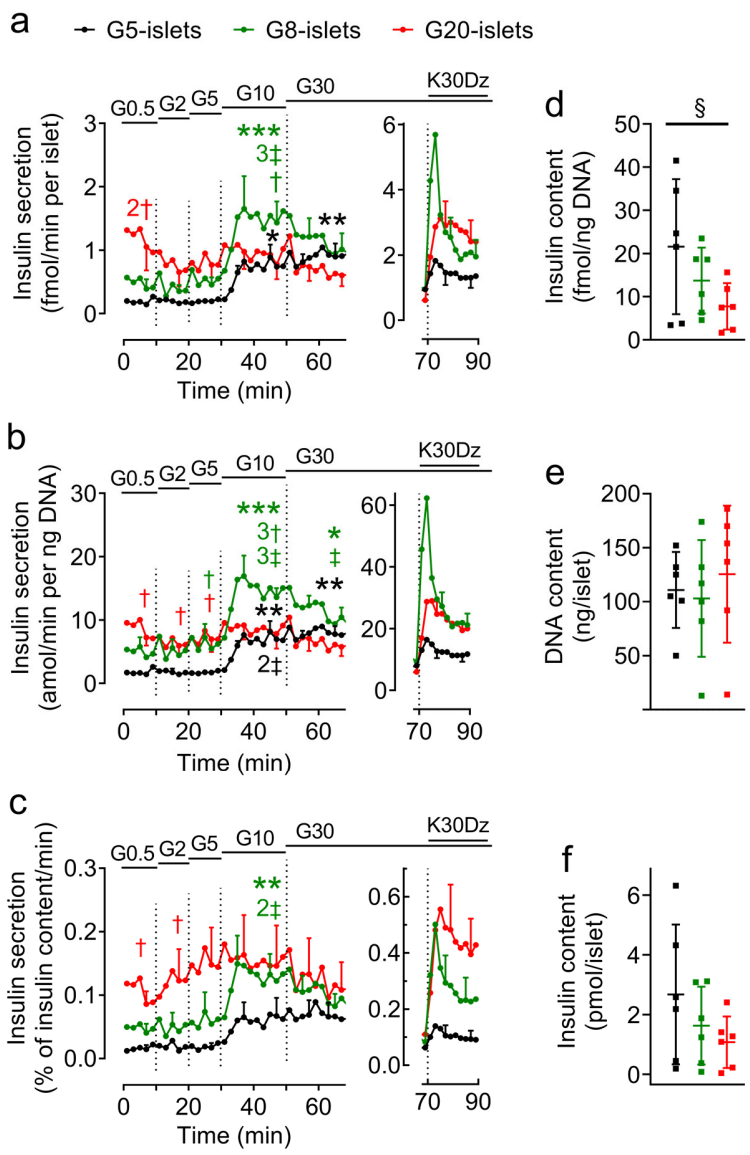


ESM-Fig.12 (related to Figs.1 and 5) Comparison of islets from ND- and T2D donors – Side-by-side comparison of data from ND-islets and T2D-islets cultured for 1 week at G5, G8 or G20. Data are the same as shown in figures 1 and 5, but the Y scales have been modified to ease the comparison between ND- and T2D-islets. Please note that, in contrast with ESM.Fig.11 comparing insulin secretion during culture, the ND- and T2D-donors used here were not matched for age, sex and BMI. a-b, islet insulin and DNA content after culture. Means ± SD and individual data for 6 preparations. c, percentage of TUNEL-positive beta-cells in islet sections. Means ± SD and individual data for 4 preparations. d-e, cytosolic and mitochondrial E_{GSH} as reflected by the roGFP1 and mt-roGFP1 normalised fluorescence ratio. Means ±

SD and individual data for 3-6 preparations. f, NAD(P)H autofluorescence during stepwise glucose stimulation. Data were normalised to the fluorescence after 10 min in FCCP. Means ± SD for 5 preparations. g-h, fura2-LR fluorescence ratio and insulin secretion rate during stepwise glucose stimulation followed by membrane depolarisation with K30Dz. Means ± SD for from 5-6 preparations (h). Statistical analysis: a-e, 1-way ANOVA + test of Dunnett or test for linear trend from left to right: †, $P<0.05$ vs G5-islets; ¶, $P=0.036$ for linear trend. f-h, 2-way ANOVA + test of Tukey: *, ** and ***, $P<0.05$, $P<0.01$ and $P<0.001$ vs G0.5 step; † and 3†, $P<0.05$ and $P<0.001$ vs G5-islets; ‡, 2‡ and 3‡, $P<0.05$, $P<0.01$ and $P<0.001$ vs previous step; §, $P<0.05$ vs G2 step.



ESM-Fig.13 (related to Figs.1 and 5) Adenine nucleotide levels in ND- and T2D-islet preparations - After preculture, islets from ND- and T2D-donors were cultured for one week in RPMI containing G5 or G20. Their ATP and ATP+ADP contents were measured after 2 consecutive 30-min incubation periods at the indicated glucose concentrations, with or without 5 mmol/l of the mitochondrial complex IV inhibitor azide. The ADP contents and ATP/(ATP+ADP) ratios were computed, and the ratios were normalised to that measured in G5-islets incubated with 5 mmol/l azide in each experiment. Means and individual data for 5 ND- and 2 T2D-islet preparations, each with 2-3 batches of 10 islets per condition. The normalised ratios with statistical differences between groups of ND-islets are shown in Fig.1j.



ESM-Fig.14 (related to Fig.5j) Long-term effects of glucose on dynamic insulin secretion in cultured islets from T2D donors – Comparison of dynamic insulin secretion in G5-, G8- and G20-islets (Fig.5j) expressed in fmol/min per islet (a), in amol/min per ng of islet DNA content (b, same data as in Fig.5j), and in percent of the islet insulin content (c) (G5-islets, black symbols and traces; G8-islets, green symbols and traces; G20-islets, red symbols and traces). Right panels, change in insulin secretion rate between the indicated glucose steps calculated as in figure 5j. The islet insulin content, DNA content and insulin to DNA content ratio were measured after perifusion.

Statistical analysis: Dynamic secretion, 2-way ANOVA + test of Tukey: *, ** and ***, $P<0.05$, $P<0.01$ and $P<0.001$ vs G0.5 step under the same condition; †, 2† and 3†, $P<0.05$, $P<0.01$ and $P<0.001$ vs G5-islets; ‡, 2‡ and 3‡, $P<0.05$, $P<0.01$ and $P<0.001$ vs previous step. Δ graphs, 1-way ANOVA + test of Dunnett: †, $P<0.05$ vs G5-islets.

ESM-Table 1: Human islet checklist

Islet preparation	**1**	**2**	**3**	**4**	**5**	**6**
MANDATORY INFORMATION						
Unique identifier	HM12	HM14	HM19	HM21	HM23	HM33
Donor age (years)	70	50	17	63	57	64
Donor sex (M/F)	M	M	F	M	F	M
Donor BMI (kg/m^2)	21.6	30	21	32	22.5	33
Donor HbA_{1c} in mmol/mol (%) or blood glucose (BG) in mmol/l [a]	N/A	N/A	N/A	N/A	N/A	N/A
Origin/source of islets [b]	LCTD	LCTD	LCTD	LCTD	LCTD	LCTD
Islet isolation centre	Montpellier	Montpellier	Montpellier	Montpellier	Montpellier	Montpellier
Donor history of diabetes? Yes/No	No	No	No	No	No	No
If Yes, complete the next two lines if this information is available						
Diabetes duration (years)						
Glucose-lowering therapy at time of death						

RECOMMENDED INFORMATION						
Donor cause of death	Stroke	Anoxia	Trauma	Vascular	Vascular	Trauma
Warm ischaemia time (h)	< 2	< 2	< 2	< 2	< 2	< 2
Cold ischaemia time (h)	6	8	9	12	9	7
Estimated purity (%)	80	80	80	ND	80	85
Estimated viability (%)	N/A	N/A	N/A	N/A	N/A	75
Total culture time (h) [c]	144	120	72	168	48	48
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes	Treated with acetylsalicylate	High BG in ICU before death				

[a] Information about the donor glucose tolerance is provided if available at the time of paper revision. N/A: no data. ICU, intensive care unit. The lack of information about HbA1c or BG levels for most islet preparations from ECIT and Montpellier is due to the local organ procurement policy. Donors were considered non-diabetic based on investigation with relatives, family physician, and medical record. The ICU BG level should not be considered as informative of the glucose tolerance of the donor before ICU admission.
[b] Sources of islets: LCTD: Laboratory of Cell Therapy for Diabetes (Hôpital St Eloi, Montpellier, France) ; ADI Islet core (Alberta, Canada); ECIT (San Raffaele Diabetes Research Institute, Milan, Italy); Integrated Islet Distribution Program (IIDP, United States of America); Lab of experimental surgery (UCLouvain, Brussels, Belgium)
[c] Duration of islet culture at the isolation centre and during shipment

Islet preparation	**7**	**8**	**9**	**10**	**11**	**12**
MANDATORY INFORMATION						
Unique identifier	HM34	HM35	HM36	HM37	HM42	HM50
Donor age (years)	59	60	66	59	39	66
Donor sex (M/F)	F	M	F	M	M	M
Donor BMI (kg/m^2)	22.3	29	30.5	26	25	30.5
Donor HbA_{1c} in mmol/mol (%) or blood glucose (BG) in mmol/l [a]	N/A	ICU BG 12	N/A	N/A	N/A	N/A
Origin/source of islets [b]	LCTD	LCTD	LCTD	LCTD	LCTD	LCTD
Islet isolation centre	Montpellier	Montpellier	Montpellier	Montpellier	Montpellier	Montpellier
Donor history of diabetes? Yes/No	No	Yes	No	No	No	No
If Yes, complete the next two lines if this information is available						
Diabetes duration (years)		Recent				
Glucose-lowering therapy at time of death		Metformin				
RECOMMENDED INFORMATION						
Donor cause of death	Trauma	Trauma	Vascular	Vascular	Asphyxia	Trauma
Warm ischaemia time (h)	< 2	< 2	< 2	< 2	< 2	< 2
Cold ischaemia time (h)	10	6	3	5	5	6
Estimated purity (%)	80	80	75	90	90	90
Estimated viability (%)	N/A	N/A	>75	>70	>80	N/A
Total culture time (h) [c]	144	168	168	168	96	48
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes	Alcoholism, 8 days in ICU before death			Alcoholism, Smoker		

[a] Information about the donor glucose tolerance is provided if available at the time of paper revision. N/A: no data. ICU, intensive care unit. The lack of information about HbA1c or BG levels for most islet preparations from ECIT and Montpellier is due to the local organ procurement policy. Donors were considered non-diabetic based on investigation with relatives, family physician, and medical record. The ICU BG level should not be considered as informative of the glucose tolerance of the donor before ICU admission.

[b] Sources of islets: LCTD: Laboratory of Cell Therapy for Diabetes (Hôpital St Eloi, Montpellier, France) ; ADI Islet core (Alberta, Canada); ECIT (San Raffaele Diabetes Research Institute, Milan, Italy); Integrated Islet Distribution Program (IIDP, United States of America); Lab of experimental surgery (UCLouvain, Brussels, Belgium)

[c] Duration of islet culture at the isolation centre and during shipment

Islet preparation	**13**	**14**	**15**	**16**	**17**	**18**
MANDATORY INFORMATION						
Unique identifier	HM52	HM54	HM56	HM57	HM61	HI12
Donor age (years)	74	36	68	52	66	60
Donor sex (M/F)	F	M	M	M	M	F
Donor BMI (kg/m^2)	29.4	22.9	30	24	25.8	22.2
Donor HbA_{1c} in mmol/mol (%) or blood glucose (BG) in mmol/l [a]	N/A	N/A	N/A	N/A	ICU BG 11	N/A
Origin/source of islets [b]	LCTD	LCTD	LCTD	LCTD	LCTD	ECIT
Islet isolation centre	Montpellier	Montpellier	Montpellier	Montpellier	Montpellier	Italy
Donor history of diabetes? Yes/No	No	No	No	No	Yes	No
If Yes, complete the next two lines if this information is available						
Diabetes duration (years)					26	
Glucose-lowering therapy at time of death					Metformin	

RECOMMENDED INFORMATION						
Donor cause of death	Trauma	Vascular injury	Trauma	Stroke	Stroke	Cerebral bleeding
Warm ischaemia time (h)	< 2	< 2	< 2	< 2	< 2	
Cold ischaemia time (h)	6	2	3	4	3.5	
Estimated purity (%)	90	95	90	90	80	94
Estimated viability (%)	80	N/A	N/A	N/A	N/A	N/A
Total culture time (h) [c]	72	96	72	96	144	106
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes	Simvastatin treatment	No medical history	No medical history	High blood pressure		

[a] Information about the donor glucose tolerance is provided if available at the time of paper revision. N/A: no data. ICU, intensive care unit. The lack of information about HbA1c or BG levels for most islet preparations from ECIT and Montpellier is due to the local organ procurement policy. Donors were considered non-diabetic based on investigation with relatives, family physician, and medical record. The ICU BG level should not be considered as informative of the glucose tolerance of the donor before ICU admission.

[b] Sources of islets: LCTD: Laboratory of Cell Therapy for Diabetes (Hôpital St Eloi, Montpellier, France) ; ADI Islet core (Alberta, Canada); ECIT (San Raffaele Diabetes Research Institute, Milan, Italy); Integrated Islet Distribution Program (IIDP, United States of America); Lab of experimental surgery (UCLouvain, Brussels, Belgium)

[c] Duration of islet culture at the isolation centre and during shipment

Islet preparation	19	20	21	22	23	24
MANDATORY INFORMATION						
Unique identifier	HP1302	R251	HP1305	R253	HP1318	HP1332
Donor age (years)	50	49	31	57	62	62
Donor sex (M/F)	F	M	F	M	M	F
Donor BMI (kg/m^2)	23.9	21.3	25.7	25.5	24.5	29.3
Donor HbA_{1c} in mmol/mol (%) or blood glucose (BG) in mmol/l [a]	N/A	31 (5)	N/A	31 (5)	N/A	N/A
Origin/source of islets [b]	ECIT	Alberta IsletCore	ECIT	Alberta IsletCore	ECIT	ECIT
Islet isolation centre	Italy	Canada	Italy	Canada	Italy	Italy
Donor history of diabetes? Yes/No	No	No	No	No	No	No
If Yes, complete the next two lines if this information is available						
Diabetes duration (years)						
Glucose-lowering therapy at time of death						

RECOMMENDED INFORMATION						
Donor cause of death	Anoxia	NDD-Neurological	Massive pulmonary embolism	NDD-Neurological	Cerebral bleeding	Cerebral bleeding
Warm ischaemia time (h)						
Cold ischaemia time (h)	8.5			14.3		5
Estimated purity (%)	90	80	80	90	80	90
Estimated viability (%)	95	N/A	90	N/A	N/A	95
Total culture time (h) [c]	42	62	96	60	39	44
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes						

[a] Information about the donor glucose tolerance is provided if available at the time of paper revision. N/A: no data. ICU, intensive care unit. The lack of information about HbA1c or BG levels for most islet preparations from ECIT and Montpellier is due to the local organ procurement policy. Donors were considered non-diabetic based on investigation with relatives, family physician, and medical record. The ICU BG level should not be considered as informative of the glucose tolerance of the donor before ICU admission.
[b] Sources of islets: LCTD: Laboratory of Cell Therapy for Diabetes (Hôpital St Eloi, Montpellier, France) ; ADI Islet core (Alberta, Canada); ECIT (San Raffaele Diabetes Research Institute, Milan, Italy); Integrated Islet Distribution Program (IIDP, United States of America); Lab of experimental surgery (UCLouvain, Brussels, Belgium)
[c] Duration of islet culture at the isolation centre and during shipment

Islet preparation	25	26	27	28	29	30
MANDATORY INFORMATION						
Unique identifier	HP1333	R292	HM78	CHEX-H6	R307	R310
Donor age (years)	54	47	50	67	43	25
Donor sex (M/F)	M	M	F	F	M	M
Donor BMI (kg/m^2)	24.2	27.6	28.8	26	25.4	26.4
Donor HbA_{1c} in mmol/mol (%) or blood glucose (BG) in mmol/l [a]	N/A	34.7 (5.6)	35.3 (5.7)	27-37 (4.4-6)	40.3 (6.5)	33.5 (5.4)
Origin/source of islets [b]	ECIT	Alberta IsletCore	LCTD	Lab of experimental surgery	Alberta IsletCore	Alberta IsletCore
Islet isolation centre	Italy	Canada	Montpellier	UCLouvain	Canada	Canada
Donor history of diabetes? Yes/No	No	No	No	No	Yes	No
If Yes, complete the next two lines if this information is available						
Diabetes duration (years)					2 Years	
Glucose-lowering therapy at time of death					Metformin	

RECOMMENDED INFORMATION						
Donor cause of death	Cerebral trauma	NDD-Neurological	Aneurysm	Terminal pulmonary fibrosis	NDD-Neurological	NDD-Neurological
Warm ischaemia time (h)			<2			
Cold ischaemia time (h)		12.5	7		6.5	11
Estimated purity (%)	90	90	95		80	90
Estimated viability (%)	95	N/A	>80	N/A	N/A	N/A
Total culture time (h) [c]	48	61	120		75	89
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes						

[a] Information about the donor glucose tolerance is provided if available at the time of paper revision. N/A: no data. ICU, intensive care unit. The lack of information about HbA1c or BG levels for most islet preparations from ECIT and Montpellier is due to the local organ procurement policy. Donors were considered non-diabetic based on investigation with relatives, family physician, and medical record. The ICU BG level should not be considered as informative of the glucose tolerance of the donor before ICU admission.
[b] Sources of islets: LCTD: Laboratory of Cell Therapy for Diabetes (Hôpital St Eloi, Montpellier, France) ; ADI Islet core (Alberta, Canada); ECIT (San Raffaele Diabetes Research Institute, Milan, Italy); Integrated Islet Distribution Program (IIDP, United States of America); Lab of experimental surgery (UCLouvain, Brussels, Belgium)
[c] Duration of islet culture at the isolation centre and during shipment

Islet preparation	31	32	33	34	35	36
MANDATORY INFORMATION						
Unique identifier	HP1354	R318	HM80	HP1357	HP1371	R341
Donor age (years)	58	54	55	20	46	42
Donor sex (M/F)	M	M	M	M	F	M
Donor BMI (kg/m^2)	27.2	20.5	22.5	21.8	23.5	30
Donor HbA_{1c} in mmol/mol (%) or blood glucose (BG) in mmol/l [a]	N/A	31 (5)	37.8 (6.1)	N/A	ICU BG 5.6	N/A
Origin/source of islets [b]	ECIT	Alberta IsletCore	LCTD	ECIT	ECIT	Alberta IsletCore
Islet isolation centre	Italy	Canada	Montpellier	Italy	Italy	Canada
Donor history of diabetes? Yes/No	No	No	No	No	No	No
If Yes, complete the next two lines if this information is available						
Diabetes duration (years)						
Glucose-lowering therapy at time of death						

RECOMMENDED INFORMATION						
Donor cause of death	Anoxia	NDD-Neurological	Anoxia	Anoxia	Cerebral bleeding	NDD-Neurological
Warm ischaemia time (h)			< 2			
Cold ischaemia time (h)	4	16	22	8	6	16
Estimated purity (%)	80	90	75	90	90	95
Estimated viability (%)	90	N/A	>80	N/A	95	N/A
Total culture time (h) [c]	120	60	38	95	48	130
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes						

[a] Information about the donor glucose tolerance is provided if available at the time of paper revision. N/A: no data. ICU, intensive care unit. The lack of information about HbA1c or BG levels for most islet preparations from ECIT and Montpellier is due to the local organ procurement policy. Donors were considered non-diabetic based on investigation with relatives, family physician, and medical record. The ICU BG level should not be considered as informative of the glucose tolerance of the donor before ICU admission.
[b] Sources of islets: LCTD: Laboratory of Cell Therapy for Diabetes (Hôpital St Eloi, Montpellier, France) ; ADI Islet core (Alberta, Canada); ECIT (San Raffaele Diabetes Research Institute, Milan, Italy); Integrated Islet Distribution Program (IIDP, United States of America); Lab of experimental surgery (UCLouvain, Brussels, Belgium)
[c] Duration of islet culture at the isolation centre and during shipment

Islet preparation	37	38	39	40	41	42
MANDATORY INFORMATION						
Unique identifier	R344	HM84	HP1375	HM92	SAMN 15724795	SAMN 15770453
Donor age (years)	59	62	48	68	56	48
Donor sex (M/F)	M	M	F	M	M	F
Donor BMI (kg/m^2)	27.5	30.5	40.2	30.1	32.8	30.9
Donor HbA_{1c} in mmol/mol (%) or blood glucose (BG) in mmol/l [a]	34.1 (5.5)	37.2 (6)	BG 5.1	ICU BG 8.1	32.8 (5.3)	36.0 (5.8)
Origin/source of islets [b]	Alberta IsletCore	LCTD	ECIT	LCTD	IIDP	IIDP
Islet isolation centre	Canada	Montpellier	Italy	Montpellier	USA	USA
Donor history of diabetes? Yes/No	No	Yes	No	Yes	No	No
If Yes, complete the next two lines if this information is available						
Diabetes duration (years)		ND		ND		
Glucose-lowering therapy at time of death		Metformin		Metformin		

RECOMMENDED INFORMATION						
Donor cause of death	NDD-Neurological	Anoxia	Trauma	Trauma	Anoxia	Stroke
Warm ischaemia time (h)			< 2	< 2	0.25	
Cold ischaemia time (h)	11.5	5	11	9.5	7	5.5
Estimated purity (%)	95	85	90	90	88	90
Estimated viability (%)	N/A	90	>80	80	95	98
Total culture time (h) [c]	86	120	144	60	120	86
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes				High blood pressure treatment		

[a] Information about the donor glucose tolerance is provided if available at the time of paper revision. N/A: no data. ICU, intensive care unit. The lack of information about HbA1c or BG levels for most islet preparations from ECIT and Montpellier is due to the local organ procurement policy. Donors were considered non-diabetic based on investigation with relatives, family physician, and medical record. The ICU BG level should not be considered as informative of the glucose tolerance of the donor before ICU admission.
[b] Sources of islets: LCTD: Laboratory of Cell Therapy for Diabetes (Hôpital St Eloi, Montpellier, France) ; ADI Islet core (Alberta, Canada); ECIT (San Raffaele Diabetes Research Institute, Milan, Italy); Integrated Islet Distribution Program (IIDP, United States of America); Lab of experimental surgery (UCLouvain, Brussels, Belgium)
[c] Duration of islet culture at the isolation centre and during shipment

Islet preparation	43	44	45	46	47	48
MANDATORY INFORMATION						
Unique identifier	SAMN 16515959	R396	CHEX-SL2	HP1406	R402	SAMN 20926064
Donor age (years)	51	32	70	65	57	62
Donor sex (M/F)	F	M	M	M	M	F
Donor BMI (kg/m^2)	25.2	24.8	23.5	26.1	26.7	26.8
Donor HbA_{1c} in mmol/mol (%) or blood glucose (BG) in mmol/l [a]	34.7 (5.6)	27.9 (4.5)	N/A	N/A	34.7 (5.6)	38.4 (6.2)
Origin/source of islets [b]	IIDP	Alberta IsletCore	Lab of experimental surgery	ECIT	Alberta IsletCore	IIDP
Islet isolation centre	USA	Canada	UCLouvain	Italy	Canada	USA
Donor history of diabetes? Yes/No	No	No	No	No	Yes	No
If Yes, complete the next two lines if this information is available						
Diabetes duration (years)					3 months	
Glucose-lowering therapy at time of death					Diet	

RECOMMENDED INFORMATION						
Donor cause of death	Stroke	NDD - Neurological	Stroke	Trauma	NDD - Neurological	Stroke
Warm ischaemia time (h)						
Cold ischaemia time (h)	7.5	22.5	14.5	5	10	11
Estimated purity (%)	90	90			85	90
Estimated viability (%)	96	N/A	N/A	N/A	N/A	96
Total culture time (h) [c]	170	127	6	120	130	140
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes					Unusual, spontaneously corrected T2D	

[a] Information about the donor glucose tolerance is provided if available at the time of paper revision. N/A: no data. ICU, intensive care unit. The lack of information about HbA1c or BG levels for most islet preparations from ECIT and Montpellier is due to the local organ procurement policy. Donors were considered non-diabetic based on investigation with relatives, family physician, and medical record. The ICU BG level should not be considered as informative of the glucose tolerance of the donor before ICU admission.

[b] Sources of islets: LCTD: Laboratory of Cell Therapy for Diabetes (Hôpital St Eloi, Montpellier, France) ; ADI Islet core (Alberta, Canada); ECIT (San Raffaele Diabetes Research Institute, Milan, Italy); Integrated Islet Distribution Program (IIDP, United States of America); Lab of experimental surgery (UCLouvain, Brussels, Belgium)

[c] Duration of islet culture at the isolation centre and during shipment

Islet preparation	**49**	**50**	**51**	**52**	**53**	**54**
MANDATORY INFORMATION						
Unique identifier	R413	SAMN 24579752	HM102	R427	HPC03	HPC04
Donor age (years)	41	55	70	52	73	64
Donor sex (M/F)	M	M	M	M	F	F
Donor BMI (kg/m^2)	33.1	24.9	24.7	35.8	22	24
Donor HbA_{1c} in mmol/mol (%) or blood glucose (BG) in mmol/l [a]	34.7 (5.6)	33.5 (5.4)	N/A	36 (5.8)	ICU BG 24.4	N/A
Origin/source of islets [b]	Alberta IsletCore	IIDP	LCTD	Alberta IsletCore	Lab of experimental surgery	Lab of experimental surgery
Islet isolation centre	Canada	USA	Montpellier	Canada	UCLouvain	UCLouvain
Donor history of diabetes? Yes/No	No	No	No	No	No	No
If Yes, complete the next two lines if this information is available						
Diabetes duration (years)						
Glucose-lowering therapy at time of death						

RECOMMENDED INFORMATION						
Donor cause of death	NDD - Neurological	Stroke	Vascular	Medical Assistance in Dying	Trauma	Stroke
Warm ischaemia time (h)			<2			
Cold ischaemia time (h)	14	10.5	12	10	10.5	9.5
Estimated purity (%)	90	90	85	90	90	90
Estimated viability (%)	N/A	95	90	N/A	95	85
Total culture time (h) [c]	72	160	120	124	120	168
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes			High blood pressure treatment		High blood pressure treatment	

[a] Information about the donor glucose tolerance is provided if available at the time of paper revision. N/A: no data. ICU, intensive care unit. The lack of information about HbA1c or BG levels for most islet preparations from ECIT and Montpellier is due to the local organ procurement policy. Donors were considered non-diabetic based on investigation with relatives, family physician, and medical record. The ICU BG level should not be considered as informative of the glucose tolerance of the donor before ICU admission.

[b] Sources of islets: LCTD: Laboratory of Cell Therapy for Diabetes (Hôpital St Eloi, Montpellier, France) ; ADI Islet core (Alberta, Canada); ECIT (San Raffaele Diabetes Research Institute, Milan, Italy); Integrated Islet Distribution Program (IIDP, United States of America); Lab of experimental surgery (UCLouvain, Brussels, Belgium)

[c] Duration of islet culture at the isolation centre and during shipment

Islet preparation	**55**					
MANDATORY INFORMATION						
Unique identifier	HM105					
Donor age (years)	54					
Donor sex (M/F)	M					
Donor BMI (kg/m^2)	32.9					
Donor HbA_{1c} in mmol/mol (%) or blood glucose (BG) in mmol/l [a]	37.2 (6%)					
Origin/source of islets [b]	LCTD					
Islet isolation centre	Montpellier					
Donor history of diabetes? Yes/No	No					
If Yes, complete the next two lines if this information is available						
Diabetes duration (years)						
Glucose-lowering therapy at time of death						

RECOMMENDED INFORMATION						
Donor cause of death	Vascular					
Warm ischaemia time (h)						
Cold ischaemia time (h)	10.5					
Estimated purity (%)	80					
Estimated viability (%)	80					
Total culture time (h) [c]	84					
Handpicked to purity? Yes/No	Yes					
Additional notes	High blood pressure treatment					

[a] Information about the donor glucose tolerance is provided if available at the time of paper revision. N/A: no data. ICU, intensive care unit. The lack of information about HbA1c or BG levels for most islet preparations from ECIT and Montpellier is due to the local organ procurement policy. Donors were considered non-diabetic based on investigation with relatives, family physician, and medical record. The ICU BG level should not be considered as informative of the glucose tolerance of the donor before ICU admission.
[b] Sources of islets: LCTD: Laboratory of Cell Therapy for Diabetes (Hôpital St Eloi, Montpellier, France) ; ADI Islet core (Alberta, Canada); ECIT (San Raffaele Diabetes Research Institute, Milan, Italy); Integrated Islet Distribution Program (IIDP, United States of America); Lab of experimental surgery (UCLouvain, Brussels, Belgium)
[c] Duration of islet culture at the isolation centre and during shipment