Human beta cell proliferation by glucose—a complex scenario

M. Tiedge

Received: 13 October 2010 / Accepted: 29 November 2010 / Published online: 21 December 2010
© Springer-Verlag 2010

Keywords Beta cell proliferation · Endoplasmic reticulum stress · Glucose · Glucotoxicity · Growth factors · Human islet transplantation · Incretins · Lipotoxicity

Abbreviations

GLP Glucagon-like peptide
SCID Severe combined immunodeficiency

Beta cell mass regulation is a critical factor in the pathogenesis of type 2 diabetes and offers the possibility of developing innovative strategies for beta cell replacement or preservation of beta cell mass. Several studies have demonstrated that beta cell mass in type 2 diabetes is determined by a critical balance between beta cell loss and regeneration [1, 2]. Beta cell regeneration has been shown to be initiated by differentiation of pancreatic duct cells as well as beta cell proliferation [1, 2]. Glucagon-like peptide (GLP)-1 agonists, insulin and glucose have also been shown to stimulate beta cell proliferation with a realistic possibility of clinical translation [1, 2]. However, most of these studies were performed on rodent models, raising doubts as to whether their results can be extrapolated to humans. Morphometric analyses of human pancreas from autopsies, organ donors and surgical resection have revealed that beta cell replication occurs at a very low level of about

0.05 cells per islet [3] or in the range of 0–1.2% of the beta cells in adults [4]. The mechanisms for proliferation of human beta cells are unknown, as sensitive and robust clinical methods for the quantification of beta cell mass are not available for humans. Transplantation of human islets under the kidney capsule of immunodeficient mice may serve as valuable tool to investigate the effects of defined mitogenic stimuli.

In this issue of *Diabetologia*, Levitt and coworkers provide evidence that human beta cells can proliferate in vivo with dependence on the blood glucose concentration [5]. An islet equivalent of 2,500–4,000 from 12 different human donors were transplanted under the kidney capsule of immunodeficient streptozotocin (STZ)-diabetic NOD-severe combined immunodeficiency (SCID) mice—a model that is widely used to monitor rodent beta cell replication [6]. Notably, the transplanted human islet grafts were functionally active and were able to correct the blood glucose levels of diabetic mice to a normal range. Blood glucose levels were then increased over 4 days by infusion of 50% glucose in the presence of BrdU to quantify beta cell proliferation. The experimental strategy was adapted from previous rodent studies where glucose infusion significantly increased beta cell mass [6, 7]. Morphometric analysis of the human grafts revealed a higher rate of beta cell proliferation in the cohort with average blood glucose concentrations of 5–29 mmol/l. Beta cell replication nearly doubled when blood glucose levels were moderately increased (5–8 mmol/l). The mitogenic effect of glucose was also observed when paired islet grafts from individual donors received infusions of saline or 50% glucose. The increase in beta cell proliferation by glucose infusion did not correlate with human plasma insulin levels. Notably, glucose infusion did not induce beta cell apoptosis in the grafts even at high blood glucose concen-

M. Tiedge (✉)
Institute of Medical Biochemistry and Molecular Biology,
Faculty of Medicine, University of Rostock,
Schillingallee 70,
D-18057 Rostock, Germany
e-mail: markus.tiedge@med.uni-rostock.de

trations of >10 mmol/l over a period of 4 days. Thus, glucose appeared to be a mitogenic stimulus for human beta cells rather than a metabolite that induced cell death. Beta cell proliferation correlated with BMI (but not with the age of the donor), despite large variations in beta cell replication rates that were observed when islets from an individual donor were transplanted into multiple recipients. Overall, the authors were able to demonstrate a mitogenic effect of glucose on human beta cells under in vivo conditions for grafted islets.

As a simplified model it could be argued that mild hyperglycaemia in diabetic patients may provide a strong stimulus for regeneration or at least preservation of beta cell mass. However, the effects of glucose on beta cell replication appear to be complex, and several factors, such as duration of hyperglycaemia, determine the fate of the beta cell. Notably, autopsy studies of type 2 diabetic patients convincingly documented a progressive loss of beta cell mass in comparison with non-diabetic controls [8–10]. In cellular model systems this beta cell loss is plausibly explained by a complex interaction between high glucose concentrations, lipotoxicity and inflammatory mechanisms [11]. Because a 4 day glucose infusion in NOD-SCID mice does not mirror the metabolic situation in human type 2 diabetes, the mitogenic effects of glucose must be interpreted in this context. Thus, it cannot be excluded that prolonged hyperglycaemia compromises cell proliferation with an ultimate loss of beta cells, as shown in models of glucotoxicity [12]. The signal pathways that are operative during glucose-stimulated beta cell replication are less clear. In addition to upregulation and nuclear shift of cyclin D, autocrine effects of insulin secretion have been proposed as possible mechanisms [6, 13, 14]. It is also likely that in NOD-SCID mice human islet grafts are exposed to a rodent-specific internal milieu of growth factors [15, 16] that may contribute to the mitogenic effect of glucose, e.g. the release of incretin hormones from the gut [10, 17, 18]. As pointed out by Levitt et al., the highest level of beta cell proliferation was observed at moderately increased glucose concentrations below 10 mmol/l [5]. Glucose concentrations >19 mmol/l resulted in variant levels of beta cell proliferation and may indicate that glucose could have negative effects upon beta cell mass [5].

How does the in vivo data on replication of human beta cells correspond to in vitro studies on isolated islets? In 2008 a comprehensive study published in this journal clearly demonstrated that FACS-purified human beta cells do not share the replicative potential of rodent beta cells when exposed to identical proliferative stimuli [19]. The stimuli comprised extracellular matrix components, a GLP-1 agonist, epidermal growth factor (EGF), human growth hormone (hGH), gastrin and betacellulin [19]. All compounds increased the number of BrdU⁺ rat beta cells under in vitro conditions but showed no mitogenic effect on human beta

cells [19]. In vitro exposure of human beta cells to different glucose concentrations did not give discrepant results: in 1996 it was demonstrated that human beta cells had a higher replication rate when the glucose concentration in the culture medium was increased from 2.8 to 5.6 mmol/l [20]. However, in a more recent study high glucose concentration (33.3 mmol/l) increased Ki67⁺ human beta cells at day 2 but resulted in a significant decrease at day 4 [21]. Assuming that the absolute numbers of Ki67⁺ beta cells did not differ at 5.5 mmol/l glucose between day 1 and 4, these data argue in favour of negative effects on beta cell replication after prolonged exposure to high glucose concentrations [21].

Although human islets are widely accepted as the gold standard to investigate mechanisms of beta cell proliferation, the quality of the islet tissue appears to be a critical factor for the outcome and interpretation of the data. This is exemplified by the variation in beta cell proliferation rate of 0.3–1.5% that was observed in different islet preparations without any infusion of glucose [5]. Today we assume that human islet isolation is a standard procedure, but cellular stressors by collagenase digestion, purification and shipment are clearly greater than in rodent model systems, explaining differences in basal beta cell proliferation rates.

Glucose initiates complex signalling pathways that regulate the balance between beta cell proliferation and beta cell apoptosis, as summarised in Fig. 1. While the proliferative response of rodent beta cells to glucose seems to be significantly greater than that of human beta cells, positive modulators (incretins, growth factors, insulin) as well as negative regulators (lipotoxicity, inflammation) are effective in both species. Glucose appears to be a double-edged sword that can stimulate beta cell proliferation at moderate concentrations and after short-term exposure in synergism with incretins and growth factors. On the other hand, prolonged exposure of beta cells to high glucose concentrations initiates inflammatory pathways in beta cells

Fig. 1 Effects of glucose on beta cell mass in rodents and humans. ER, endoplasmic reticulum

in synergism with lipotoxicity and endoplasmic reticulum stress.

What are the implications of the work by Levitt et al.? First, it presents a valuable model system to study strategies for modulation of human beta cell proliferation under in vivo conditions. Second, it provides evidence that human beta cell proliferation is positively regulated by moderate increases in blood glucose concentrations. Thus, extreme hypo—and hyperglycaemia should be avoided in the therapy of type 2 diabetes in order to preserve beta cell mass in endogenous endocrine pancreas or islet grafts. Third, the study shows that in spite of quantitative differences in proliferation rates, human beta cells share regulatory principles with rodent beta cells. There are several challenges for future studies (see Text box).

Some suggestions for future research

- Investigate the effects of glucose on cell cycle signalling in human and rodent beta cells
- Explore long-term effects of moderate and high glucose concentrations on beta cell mass and replication in humans
- Clarify if moderate increases of blood glucose concentrations really preserves beta cell mass in islet grafts
- Study how BMI of graft donors affects the proliferative potential of human beta cells in vivo
- Standardise isolation procedures and culture conditions for human islets to minimise disturbing effects of media supplements on beta cell viability and proliferation

In particular, the mechanism(s) of glucose-mediated stimulation of beta cell proliferation is still unclear in both rodents and humans. There is also a need for clarification of how beta cell proliferation adapts to a long-term increase in glucose concentrations and how this affects beta cell mass in balance with apoptosis.

Duality of interest The author declares that there is no duality of interest associated with this manuscript.

References

1. Bouwens L, Rooman I (2005) Regulation of pancreatic beta-cell mass. *Physiol Rev* 85:1255–1270
2. Bonner-Weir S, Li W-C, Ouziel-Yahalom L, Guo L, Weir GC, Sharma A (2010) β -cell growth and regeneration: replication is only part of the story. *Diabetes* 59:2340–2348
3. Reers C, Erbel S, Esposito I et al (2009) Impaired islet turnover in human donor pancreata with aging. *Eur J Endocrinol* 160:185–191
4. Meier JJ, Butler AE, Saisho Y et al (2008) Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. *Diabetes* 57:1584–1594
5. Levitt HE, Cyphert TJ, Pascoe JL et al (2010) Glucose stimulates human beta cell replication in vivo in islets transplanted into NOD-severe combined immunodeficiency (SCID) mice. *Diabetologia*. doi:10.1007/s00125-010-1919-1
6. Alonso LC, Yokoe T, Zhang P et al (2007) Glucose infusion in mice: a new model to induce beta-cell replication. *Diabetes* 56:1792–1801
7. Bonner-Weir S, Deery D, Leahy JL, Weir GC (1989) Compensatory growth of pancreatic beta-cells in adult rats after short-term glucose infusion. *Diabetes* 38:49–53
8. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC (2003) Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110
9. Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC (2008) Pancreatic beta-cell mass in European subjects with type 2 diabetes. *Diab Obes Metab* 10(Suppl 4):32–42
10. Meier JJ (2008) Beta cell mass in diabetes: a realistic therapeutic target? *Diabetologia* 51:703–713
11. Prentki M, Nolan CJ (2006) Islet beta cell failure in type 2 diabetes. *J Clin Invest* 116:1802–1812
12. Maedler K, Sergeev P, Ris F et al (2002) Glucose-induced beta cell production of IL-1 beta contributes to glucotoxicity in human pancreatic islets. *J Clin Invest* 110:851–860
13. Georgia S, Bhushan A (2004) Beta cell replication is the primary mechanism for maintaining postnatal beta cell mass. *J Clin Invest* 114:963–968
14. Fatrai S, Elghazi L, Balcazar N et al (2006) Akt induces beta-cell proliferation by regulating cyclin D1, cyclin D2, and p21 levels and cyclin-dependent kinase-4 activity. *Diabetes* 55:318–325
15. Vasavada RC, Garcia-Ocana A, Zawulich WS et al (2000) Targeted expression of placental lactogen in the beta cells of transgenic mice results in beta cell proliferation, islet mass augmentation, and hypoglycemia. *J Biol Chem* 275:15399–15406
16. Garcia-Ocana A, Takane KK, Syed MA, Philbrick WM, Vasavada RC, Stewart AF (2000) Hepatocyte growth factor overexpression in the islet of transgenic mice increases beta cell proliferation, enhances islet mass, and induces mild hypoglycemia. *J Biol Chem* 275:1226–1232
17. Xu G, Stoffers D, Habener J, Bonner-Weir S (1999) Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 48:2270–2276
18. Buteau J, Foisy S, Joly E, Prentki M (2003) Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. *Diabetes* 52:124–132
19. Parnaud G, Bosco D, Berney T et al (2008) Proliferation of sorted human and rat beta cells. *Diabetologia* 51:91–100
20. Tyrberg B, Eizirik DL, Hellerstrom C, Pipeleers DG, Andersson A (1996) Human pancreatic beta-cell deoxyribonucleic acid-synthesis in islet grafts decreases with increasing organ donor age but increases in response to glucose stimulation in vitro. *Endocrinology* 137:5694–5699
21. Maedler K, Oberholzer J, Bucher P, Spinas GA, Donath MY (2003) Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic beta-cell turnover and function. *Diabetes* 52:726–733