Diabetologia (2012) 55:707–718
DOI 10.1007/s00125-011-2369-0

ARTICLE

Islet-enriched gene expression and glucose-induced insulin secretion in human and mouse islets

C. Dai · M. Brissova · Y. Hang · C. Thompson · G. Poffenberger · A. Shostak · Z. Chen · R. Stein · A. C. Powers

Received: 8 July 2011 / Accepted: 11 October 2011 / Published online: 14 December 2011

Abstract
Aims/hypothesis Our understanding of the transcription factors that control the development and function of rodent islet beta cells is advancing rapidly, yet less is known of the role they play in similar processes in human islets.
Methods To characterise the abundance and regulation of key proteins involved in glucose-regulated insulin secretion in human islets, we examined the expression of *MAFA, MAFB, GLUT2* (also known as *SLC2A2*), *βGK* (also known as *GCK*) and *PDX1* in isolated, highly purified human islets with an intact insulin secretory pattern. We also assessed these features in islets from two different mouse strains (C57BL/6J and FVB).
Results Compared with mouse islets, human islets secreted more insulin at baseline glucose (5.6 mmol/l), but less upon stimulation with high glucose (16.7 mmol/l) or high glucose plus 3-isobutyl-1-methyl-xanthine. Human islets had relatively more *MAFB* than *PDX1* mRNA, while mouse islets had relatively more *Pdx1* than *Mafb* mRNA. However, v-maf musculoaponeurotic fibrosarcoma oncogene homologue (MAF) B protein was found in human islet alpha and beta cells. This is unusual as this regulator is only produced in islet alpha cells in adult mice. The expression of insulin, *MAFA, βGK* and *PDX1* was not glucose-regulated in human islets with an intact insulin secretory pattern.
Conclusions/interpretation Our results suggest that human islets have a distinctive distribution and function of key regulators of the glucose-stimulated insulin secretion pathway, emphasising the urgent need to understand the processes that regulate human islet beta cell function.

Keywords Diabetes · Insulin secretion · Islet · Transcription factor

Abbreviations

GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GSIS	Glucose-stimulated insulin secretion
IBMX	3-Isobutyl-1-methyl-xanthine
IEQ	Islet equivalent
MAF	v-Maf musculoaponeurotic fibrosarcoma oncogene homologue
NIH	National Institutes of Health
PDX1	Pancreatic-duodenal homeobox factor-1

Introduction

Glucose-stimulated insulin secretion (GSIS) by islet beta cells is a complex process involving glucose transport and metabolically induced changes in electrical activity that

C. Dai and M. Brissova contributed equally to this study.

Electronic supplementary material The online version of this article (doi:10.1007/s00125-011-2369-0) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

C. Dai · M. Brissova · C. Thompson · G. Poffenberger · A. Shostak · Z. Chen · A. C. Powers (✉)
Division of Diabetes, Endocrinology, and Metabolism, Department of Medicine, Vanderbilt University, 7435 MRBIV, Nashville, TN 37232, USA
e-mail: Al.Powers@Vanderbilt.edu

Y. Hang · R. Stein · A. C. Powers
Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN, USA

A. C. Powers
VA Tennessee Valley Healthcare System, Nashville, TN, USA

result in hormone exocytosis. Recent genome-wide association studies have identified genetic loci associated with type 2 diabetes, most of which are predicted to alter pancreatic islet beta cell function [1, 2]. However, the molecular events that are responsible for normal glucose regulation of insulin secretion in human beta cells, as well as the abnormalities caused by the affected type 2 diabetes genetic loci are poorly defined. This is due partly to deficits in our knowledge of human beta cell function, with interspecies differences between the better studied murine and human islets further confounding this challenge. For example, human and rodent islets differ in architecture, cell composition, insulin secretion properties, proliferative capacity and susceptibility to injury [3–13].

Pancreas and islet development is a complex process involving signalling pathways and transcription factors that determine early pancreatic specification and later differentiation [14, 15]. Most information about islet-enriched transcription factors has emerged from studies in rodent islet cell lines and genetically modified mice. In contrast, little is known about the abundance or function of these transcription factors in human islet cells because of the absence of human islet cell lines and the low availability of human islets. Hence, the role of transcription factors in human beta cell function is largely based on linkage to the monogenic forms of diabetes, collectively termed MODY [16]. A challenge in defining how these factors mediate control of beta cell function is the failure of most mutations to mimic the human phenotype in rodent models. For example, while mice and humans heterozygous for *Pdx1*/*PDX1* mutations are phenotypically similar [17–20], heterozygous mutations in *Hnf1a*, *Hnf4a* and other MODY transcription factors do not appear to result in similar islet dysfunction or diabetes in mice [16]. In fact, v-maf musculoaponeurotic fibrosarcoma oncogene homologue (MAF)A is the only other islet-enriched transcription factor that causes adult beta cell dysfunction and diabetes under heterozygous conditions in mice [21].

A central role in mouse islet development and/or beta cell function has been established for the pancreatic-duodenal homeobox factor-1 (PDX1), MAFA and MAFB transcription factors [21–27]. PDX1 is not merely important for regulating insulin secretion in adult islet beta cells, but is also essential for early exocrine and endocrine progenitor cell formation, since PDX1-deficient mice and humans exhibit pancreatic agenesis [22, 28, 29]. MAFA is exclusively produced in the mouse beta cell during development and in adult animals [25, 30, 31]. In contrast, MAFB, the only other large MAF family member produced in rodent islets, is only found in islet alpha cells [26, 27], although its transient production in developing beta cells is critical to their formation [26]. MAFA is not required for beta cell development, probably reflecting compensation by MAFB [23, 26, 27]. In addition, PDX1 and MAFA are critical activators of glucose-responsive insulin gene transcription [24, 25], with transcription factor abundance and/or activity regulated by glucose in mice [21, 26, 27].

Here, we assessed the functional properties and gene expression characteristics of key beta cell regulators in highly purified, glucose-responsive mouse and human islet preparations. Human islets were found to have distinctive properties with regard to GSIS, glucose-regulated gene expression and islet cell distribution of the MAFB transcription factor.

Methods

Human and mouse islets Mouse islets were isolated from 10 to 12-week-old male FVB and C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) as described [32]. Non-diabetic human islet preparations (n=59) were obtained from islet isolation centres supported by the Integrated Islet Distribution Network (http://iidp.coh.org/) [33]. We chose 24 preparations from 15 male and nine female donors (age 38.6±2.9 years [range, 17–58], weight 75.8±3.2 kg [range, 46–105 kg], BMI 25.5±0.7 kg/m^2 [range, 21–29.3 kg/m^2]) for further study. Cause of death was listed as: head trauma (n=7), neurological event (stroke, subarachnoid haemorrhage etc., n=10), anoxia (n=1) and unknown (n=6). The cold ischaemia time before pancreas isolation was 9.4±1.2 h (range, 1.5–14.5 h). Because the purity of these preparations varied greatly (30–90% as designated by the isolation centre), islets were handpicked on the day of arrival prior to further analysis. Some islet preparations were incubated in dithizone for 30 min and then viewed by light microscopy [34]. All animal studies were approved by the Vanderbilt Institutional Animal Care and Use Committee; de-identified human samples were obtained as approved by the Vanderbilt Institutional Review Board.

Assessment of islet function Following isolation, human islets were usually cultured overnight at the islet isolation centre before overnight shipment to Vanderbilt. Human and mouse islets were then cultured in RPMI-1640 containing 10% FBS and 5 mmol/l glucose at 37°C, after which mRNA or protein was collected. The time for human islet shipment (approximately 24 h) was included in the culture time. GSIS of islets was assessed by perifusion [19]. Human islets were perifused on the day of arrival or after 24 h of culture at Vanderbilt. Mouse islets were perifused after 48 or 72 h of culture; GSIS at both time points was similar. Perifusion responses were measured using size-matched mouse and human islets. Insulin secretion was normalised to islet equivalents (IEQ), representing islet volume, or normalised to islet insulin content in some

samples. Human islet preparations were designated as having intact insulin secretion based on the following: stable baseline response at 5.6 mmol/l glucose, at least a threefold response to 16.7 mmol/l glucose and at least a fivefold response to 16.7 mmol/l glucose+100 μmol/l 3-isobutyl-1-methyl-xanthine (IBMX). To assess the effects of shipping on islet function, C57BL/6J mouse islets were isolated at Vanderbilt then shipped by overnight courier to the University of Massachusetts and then back to Vanderbilt.

Quantitative RT-PCR Quantitative RT-PCR was performed using the primer–probe approach from Applied Biosystems (Foster city, CA, USA) using the primers and conditions described in the electronic supplementary material (ESM) Methods and ESM Table 1. Quantitative PCR experiments followed the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines [35].

Histological assessment of pancreas and isolated islets Human and mouse islets were embedded in collagen I (Bectin-Dickinson, San Jose, CA, USA) and then fixed on ice in 4% (wt/vol.) paraformaldehyde in 1X PBS for 20 min, followed by three 20-min washes with 1X PBS and 3 h equilibration in 30% sucrose/1X PBS. The islets were cryopreserved in Optimum Cutting Temperature Compound (VWR Scientific Products, Willard, OH, USA). Adult mouse and human pancreatic tissues were preserved as described previously [32], except that human pancreas fixation and washing times were doubled. Human pancreas samples were obtained from a 23-year-old female donor and a number of 1-cm^3 specimens dissected from the head, body and tail of the pancreas. Immunocytochemical studies on 8-μm cryosections were performed as described previously [32]. The primary antibody–antigen complex (ESM Table 2) was visualised using secondary antibodies conjugated with Cy2, Cy3 or Cy5 fluorophors (Jackson ImmunoResearch, West Grove, PA, USA). Islet beta cell death was measured in histological sections using a kit (ApopTag Red S7165; Millipore, Bedford, MA, USA). Digital sample images were acquired with a confocal laser-scanning microscope (LSM510 META; Carl Zeiss MicroImaging, Thornwood, NY, USA) at 1 μm optical depth and analysis performed using a software package (MetaMorph 6.0; Molecular Devices, Downingtown, PA, USA).

Immunoblotting Whole-islet protein extracts were prepared as described previously [19], with proteins resolved (25 μg/lane) using a 10% (wt/vol.) NuPAGE Bis-Tris gel (Invitrogen, Carlsbad, CA, USA) and then electroblotted on to Immobilon-P membrane (Millipore) [36]. The primary antibody–horseradish peroxidase-conjugated secondary antibody complex was detected with a chemiluminescence system (Amersham Biosciences, Piscataway, NJ, USA). Each membrane was probed first for MAFA, and then reprobed for MAFB, PDX1 or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (ESM Fig. 1, ESM Table 2).

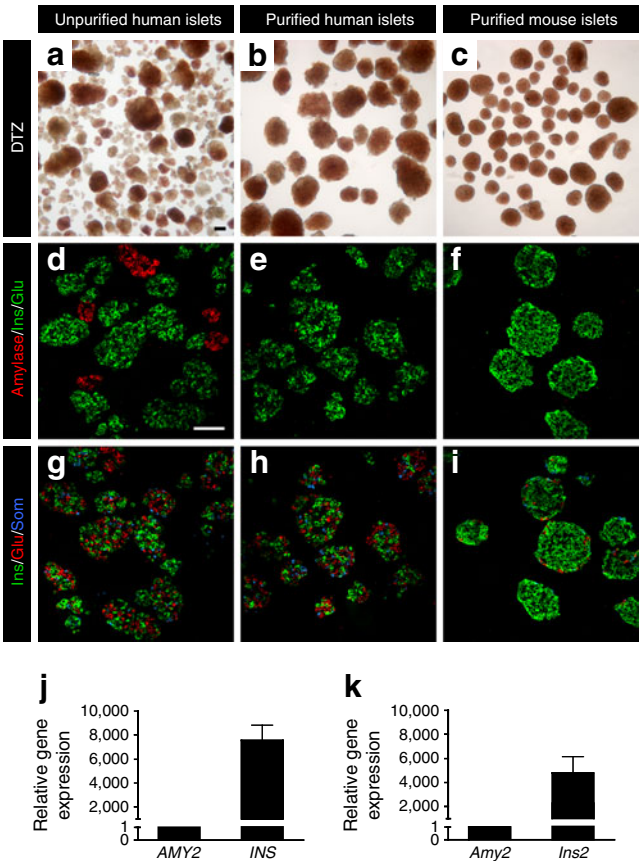


Fig. 1 Human islets were isolated by handpicking under microscopic guidance. **a** An example of a human islet preparation received from an islet isolation facility and stained by dithizone (DTZ) before and (**b**) after the handpicking procedure. While this example was one of the more impure preparations studied (stated purity 50%), all islet preparations contained ductal and acinar fragments. During culture, acinar fragments rounded up and became similar in size and shape to human islets. These acinar structures could only be distinguished from islets by the lighter brown colour of human islets noted by experienced observers. In contrast, isolated mouse islets were much easier to distinguish from acinar tissue fragments and could readily be identified for handpicking. **c** An example of mouse islets stained by DTZ after handpicking. Scale bar (**a**–**c**) 100 μm. To further evaluate the purity of handpicked islets (**d**–**f**), selected islet preparations were processed for cryosections and labelled for insulin (Ins, green), glucagon (Glu, green) and α-amylase (red). The islet cell composition of human and mouse islets was similar to that observed in a previous report [3]. Note (**d**) that the size of some acinar fragments (amylase-positive) is similar to that of islets. Adjacent sections (**g**–**i**) were labelled for insulin (green), glucagon (red) and somatostatin (Som, blue). Note the difference in islet cell distribution between human and mouse islets. Scale bar (**d**–**i**) 100 μm. **j** High enrichment of human (n=6) and (**k**) FVB mouse islet preparations (n=4) for beta cells vs acinar cells was demonstrated by quantitative RT-PCR

Statistical analysis The Student's *t* test was used for comparisons of two groups and one-way ANOVA with Newman–Keuls post-test was applied to multiple group comparisons. All values represent mean±SEM.

Results

Characteristics of human islet preparations Human islet preparations were shipped to Vanderbilt University from various islet isolation facilities as part of the islet distribution programme supported by the National Institute of Diabetes and Digestive and Kidney Diseases/National Institutes of Health (NIH) and Juvenile Diabetes Research Foundation (http://iidp.coh.org/) [33]. Within 24 h of arrival, human islets were evaluated for insulin secretion in a cell perifusion system. Appreciating that it is impossible to isolate human and rodent islets of like age or under identical conditions, only human islet preparations with intact insulin secretory response were selected for this study. Based upon insulin secretory responsiveness, the human islet preparations could be divided into three distinct categories: intact or high responders (n=33, 56%), lower responders (n=15, 25%) and non-responders (n=11, 19%). The 24 islet preparations that had an intact secretory profile and were from non-obese individuals (BMI 25.5±0.7 kg/m^2; range, 21–29.3 kg/m^2) were included in this study.

The islets were handpicked to minimise the ductal and acinar cell contamination commonly found in human islet preparations (Fig. 1). Significantly, their purity was now similar to that of isolated mouse islets as judged by dithizone staining (Fig. 1a–c), immunocytochemistry (Fig. 1d–i) and quantitative RT-PCR for insulin and α-amylase gene expression (Fig. 1j, k). Enrichment of insulin mRNA in comparison to α-amylase mRNA appeared to be slightly lower in purified mouse islets (4,783-fold) than in human islets (7,554-fold), probably because the mRNA contribution was assessed from only one of the two mouse insulin genes (i.e. insulin II; Fig. 1j, k). The viability of mouse and human islets was very similar in TUNEL assays (both cultured in 5 mmol/l glucose), with a very low level of beta cell death detected in both species (Fig. 2a). A caveat is that apoptotic rates are very low in both types of islets making differences difficult to quantify. The expression pattern of various apoptosis-related genes was unchanged in these samples upon incubation in 5 or 11 mmol/l glucose (Fig. 2b, c). In human islets, this change in glucose concentration did not affect the expression of apoptosis-related genes, with only slight differences in *Bid* and *Chop* (also known as *Ddit3*) mRNA levels being noted in mouse islets. Collectively, these results indicate that the purity and viability of the glucose-responsive human and mouse islet preparations were similar.

Glucose-stimulated insulin secretory profile of human islets Steady insulin secretion was observed in human islets at 5.6 mmol/l glucose, increasing rapidly upon stimulation with 16.7 mmol/l glucose alone or with IBMX in the dynamic islet perifusion system (Fig. 3a). An intact second phase of insulin secretion in human islets was also observed (ESM Fig. 2). However, size-matched human islets secreted more insulin at the 5.6 mmol/l glucose baseline and had a lower stimulation index in 16.7 and 16.7 mmol/l glucose plus IBMX than islets from the two different control mouse strains when normalised to IEQ or islet insulin content (note that insulin secretion was greater in FVB than C57BL/6J islets; Fig. 3 a–e, ESM Fig. 3). The greater basal insulin secretion by human islets does not represent insulin 'leaking' from beta cells, as the perifusion protocol established a stable baseline and excluded islet preparations without this property. We also considered the possibility that these differences between human and mouse islets might have resulted from the shipping of human islets from the isolation centre. As a result, C57BL/6J islets

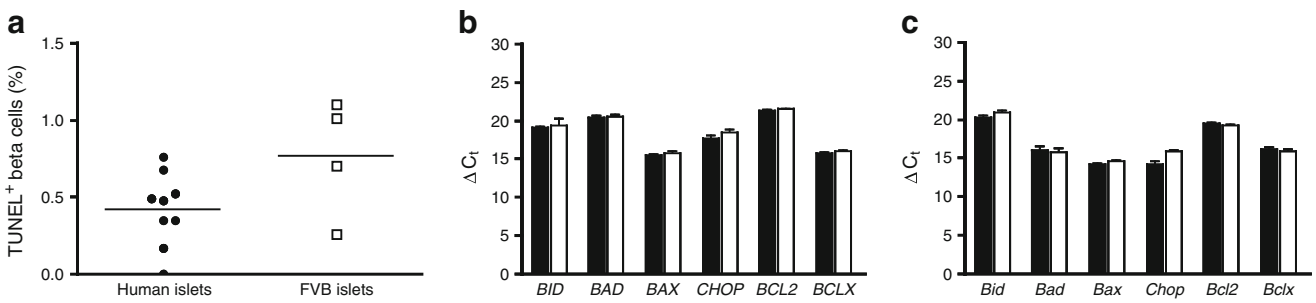


Fig. 2 Assessment of cell viability in human and mouse islet preparations. **a** The numbers of TUNEL-positive beta cells in representative human (black circles, n=9) and mouse FVB (white squares, n=4) islet preparations were determined. Analysis was based on counting approximately 3,000 and 1,200 beta cells per human and mouse islet sample, respectively; p=0.0673. **b** The expression profile of apoptosis-related genes as labelled in human (n=6) and (**c**) FVB mouse islets (n=5) cultured in 5 (black bars) or 11 mmol/l (white bars) glucose. *BCLX*/*Bclx*, also known as *BCL2L1*/*Bcl2l1*

isolated at Vanderbilt were first cultured for 24 h and then shipped, by the procedures used for shipping human islets, to the University of Massachusetts, which then returned them by overnight courier to Vanderbilt. Notably, similar GSIS properties were found in the control (unshipped) and shipped mouse islet preparations (Fig. 3d).

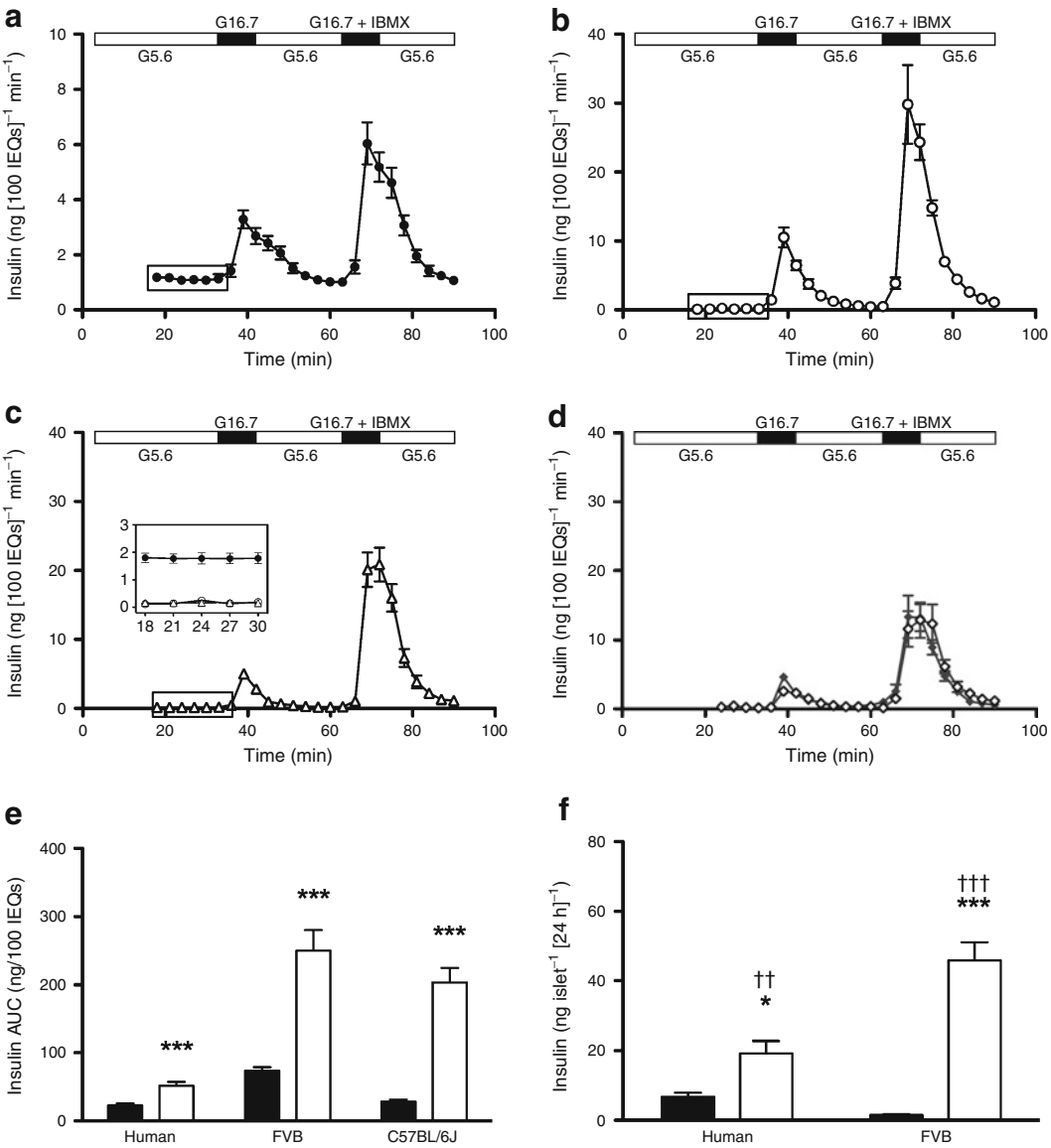
A lower level of insulin secretion in response to glucose stimulus was also observed in human islets during static stimulation with glucose (Fig. 3f). The relatively lower beta/alpha cell ratio of human islets may have contributed to the reduced human islet insulin secretion levels [3], although the higher basal insulin secretion by human islets indicates that other factors are also involved.

Expression and regulation of genes encoding proteins that are important for GSIS in human islets Quantitative PCR gene profiling of gene expression in human and mouse islets showed a similar pattern of islet hormone mRNA levels relative to glucokinase (Fig. 4a–c), with glucagon and somatostatin mRNA being relatively greater in human islets, and insulin (I+II) and *Iapp* mRNA being greater in mouse islets. As expected, human islets had a higher level of *GLUT1* (also known as *SLC2A1*) than of *GLUT2* (also known as *SLC2A2*) mRNA (Fig. 4a) [37, 38].

In contrast to the FVB and C57BL/6J islets, glucokinase, insulin and somatostatin mRNA expression was not stimulated by glucose (at 11 or 16.7 mmol/l) in human islets (Fig. 4d–f; ESM Fig. 4a). Notably, glucose stimulated the expression of *IAPP/Iapp* in human and mouse islets (Fig. 4d–f), as described previously [39]. Mouse islet mRNA expression was induced as early as 6 h and was very similar in the two mouse strains, but was stimulated to a lesser degree by 16.7 mmol/l glucose (ESM Fig. 4b,c). The lack of glucose-stimulated gene expression in human islets paralleled a difference in beta cell function and insulin secretion following culture at elevated extracellular glucose [11]. Thus here, for example, human islets preincubated at 11 mmol/l glucose also secreted less insulin in response to 16.7 mmol/l glucose than did mouse islets in static cultures (Fig. 4g, h).

Expression of genes encoding glucose-regulated transcription factors in human islets Since PDX1 and MAFA are critical activators of glucose-responsive insulin gene transcription [21, 24–27], we examined the expression pattern of their genes in human and mouse islets. Our experimental approach featured: (1) species-specific PCR primers; (2) mRNA levels normalised to a number of stably expressed endogenous mRNAs in human and mouse islets; (3) mRNA levels expressed relative to *βGK*/*βgk* (also known as *GCK*/*Gck*), which was expressed at a very similar level in both species (delta Ct: 5.053±0.09 vs 4.987±0.11); and (4) use of several endogenous mRNAs and a stable reference mRNA (*βGK*/*βgk*) to allow comparison of mRNA amount between human and mouse islets. The expression of *MAFA*, *MAFB* and *PDX1* mRNA was greater in human than mouse islets after culture in 5 mmol/l glucose for 48–72 h (human n=15, *MAFA* 1.03±0.11, *MAFB* 8.17±0.79, *PDX1* 2.52±0.29; C57BL/6J n=6, *Mafa* 0.12±0.02, *Mafb* 0.41±0.04, *Pdx1* 1.31±0.12). *MAFA* mRNA was less abundant than *PDX1* and *MAFB* mRNA in human and mouse islets. Furthermore, human islets had relatively more *MAFB* mRNA than *PDX1*, while mouse islets had relatively more *Pdx1* than *Mafb* (Fig. 5a–c).

Fig. 3 Insulin secretion in human and mouse islets. **a** Dynamic glucose-regulated insulin secretory characteristics of perifused human islets (n=24), (**b**) FVB mouse islets (n=9), (**c**) C57BL/6J mouse islets (n=8) and (**d**) shipped C57BL/6J islets. The insulin concentration was determined by radioimmunoassay [19]. Note that the scale for the y-axis is different for human islets to make the insulin secretory pattern more readily visible. The amount of insulin secreted was normalised to IEQ, but it should be realised that islet cell composition in humans is different to that in mice [3]. Based on our experience with mouse and human islet preparations, our rationale for normalising insulin to IEQ is described above (Methods). ESM Fig. 3 shows secreted insulin normalised to islet insulin content. Here (**a**–**d**), the isolated human and mouse islets were precultured in 5 mmol/l glucose for 72 and 48 h, respectively. Human islets (**a**) showed a higher insulin output at basal 5.6 mmol/l glucose (G5.6) than mouse islets (**b**–**d**), and a lower stimulation index at 16.7 mmol/l glucose (G16.7) or 16.7 mmol/l glucose+100 μmol/l IBMX (G16.7+IBMX). The insert (**c**) shows basal secretion by human (black symbols), and FVB and C57BL/6J (white symbols) islets, which in the latter was similar and appears to overlap. An additional 24 h of culture of mouse islets did not change the magnitude of the perifusion response; dynamic GSIS in mouse islets was similar at 48 and 72 h of culture (data not shown). **d** To assess the effects of shipping on islet function, C57BL/6J mouse islets were isolated at Vanderbilt (n=5 islet isolations; three separate shipments) and cultured for 24 h. Identical islet aliquots were either cultured in RPMI + 10% FBS (5 mmol/l glucose) or shipped by overnight courier to the University of Massachusetts using the shipping containers and conditions used by the Islet Cell Resource Centers and the Integrated Islet Distribution Network. Upon arrival at the University of Massachusetts, the shipping container was opened, labelled with a new shipping label and shipped back by the same overnight courier. Upon arrival at Vanderbilt, the cultured (black diamonds) and shipped (white diamonds) islet aliquots were simultaneously evaluated in the perifusion system (i.e. at approximately 72 h after isolation, with approximately 48 h of shipping time). Significantly, control C57BL/6J islets (cultured for approximately 72 h) and shipped islets had similar insulin secretory properties, as assessed by AUC per 100 IEQ; p=0.368 for 16.7 mmol/l glucose peak; p=0.322 for glucose+IBMX peak. **e** Integrated insulin secretion per 100 IEQ for mouse and human islets after stimulation with 16.7 mmol/l glucose (black bars) or 16.7 mmol/l glucose+100 μmol/l IBMX (white bars); ***p<0.001 for comparison with 16.7 mmol/l. One-way ANOVA was used for multiple group comparisons, with p values as follows: 16.7 mmol/l p<0.001 for human vs FVB, p>0.05 for human vs C57 and p<0.001 for FVB vs C57; 16.7 mmol/l glucose+100 μmol/l IBMX p<0.001 for human vs FVB and human vs C57, and p<0.01 for FVB vs C57. **f** Static GSIS was measured in human (n=8) and FVB mouse islets (n=8) exposed to 5 (black bars) or 11 mmol/l (white bars) glucose for an additional 24 h. Human islets showed significantly higher basal insulin secretion than mouse, whereas GSIS in human islets increased only three fold vs 30-fold in mouse islets; *p<0.05 and ***p<0.001 for comparison with 5 mmol/l glucose; $^{\dagger\dagger}p$<0.01 and $^{\dagger\dagger\dagger}p$<0.001 for comparison with human islets



Human and mouse islets were preincubated in 5 mmol/l glucose and then stimulated with 11 mmol/l glucose to examine whether human *MAFA*, *PDX1* or *MAFB* mRNA expression was stimulated by glucose. Under these conditions, glucose stimulated *Mafa* and *Pdx1* mRNA and protein in mouse islets (Fig. 5e, f, ESM Fig. 5). *MAFA* and *PDX1* mRNA and protein levels in human islets were unaffected by these conditions (Fig. 5d and data not shown) or even after prolonged (96 h) incubation (ESM Fig. 4a). *MAFB*/*Mafb* mRNA levels were unaffected by glucose in human and mouse islets. Our data suggest that the inability of human islets to induce expression of transcriptional mediators of the insulin gene such as *MAFA* and *PDX1* may limit their insulin secretory response. We did not note a correlation between insulin secretion or gene expression and BMI of the islet donor.

MAFB is expressed in human islet beta cells *MAFB* mRNA levels were much higher than those of *PDX1* and *MAFA* in human islets (Fig. 5a, b). Since MAFB is exclusively produced in adult mouse alpha cells [26], this might simply reflect the difference in beta:alpha cell ratio in human islets [3, 4]. To address this possibility, islet immunohistochemical studies were performed with antibodies to PDX1, MAFA and MAFB (for details on the biochemical specificity of the recently generated human MAFA and MAFB antibodies, see ESM Table 2 and ESM Fig. 1).

The cellular distribution of MAFA and PDX1 in human and mouse islets was similar, with MAFA only produced in beta cells (Fig. 6a–d), and PDX1 in beta cells (Fig. 6m–p) and in a fraction of delta cells (Fig. 6q–t). In contrast, MAFB was produced in human alpha cells (Fig. 6i–l) and also in a subset of beta cells (Fig. 6e–h). Importantly, the cellular distribution pattern was very similar in whole pancreatic

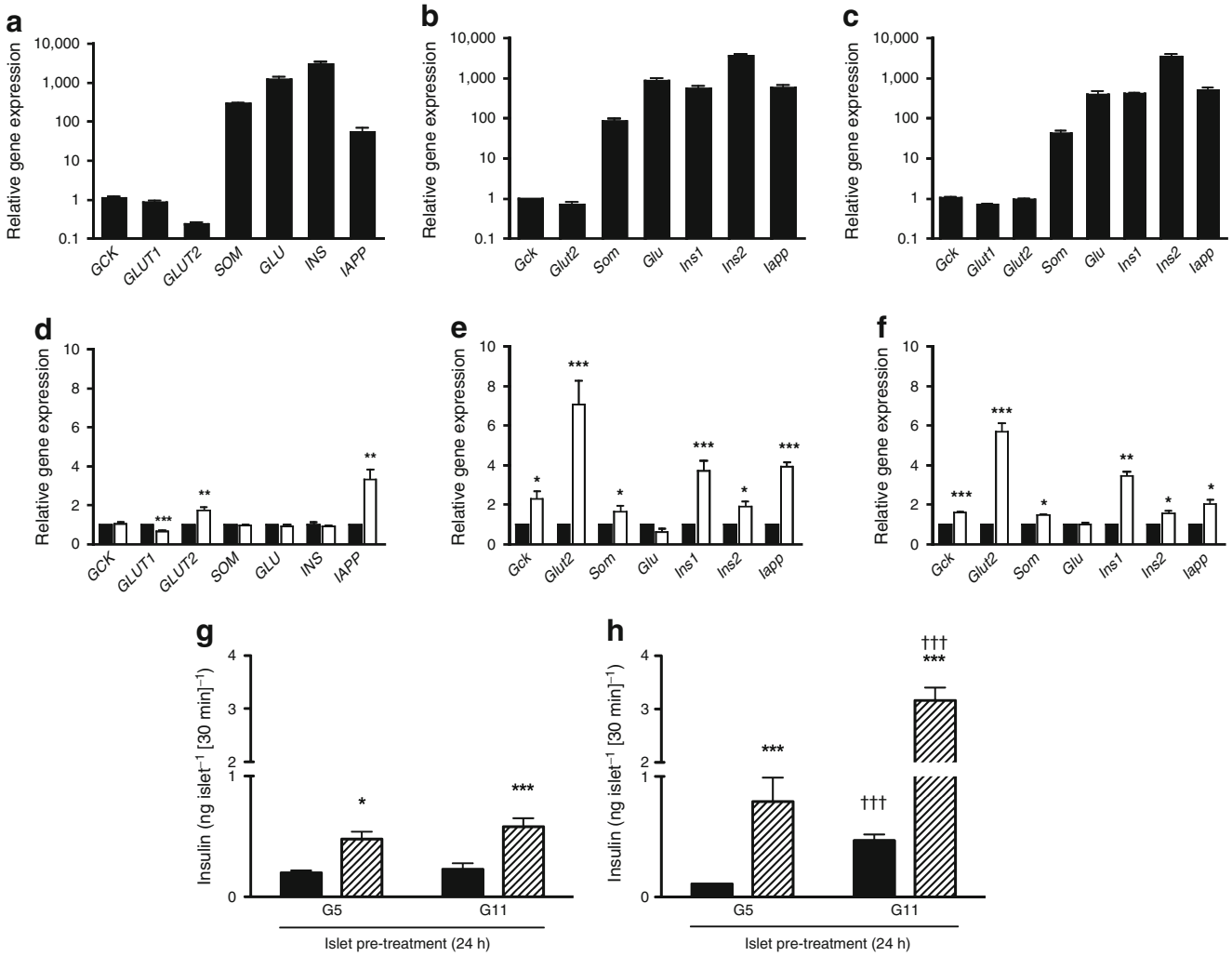


Fig. 4 Human islet beta cell-enriched gene expression is not very responsive to stimulating glucose levels. **a** Human islets (n=11), or FVB (**b**) and C57BL/6J (**c**) mouse islets (n=4 each) were cultured for 72 or 48 h, respectively, after which total RNA was analysed by quantitative RT-PCR. mRNA levels for each of the measured islet-enriched gene products were normalised to four endogenous controls (i.e. 18S, actin, *TFRC*/*Tfrc* and *TBP*/*Tbp* in human and C57BL/6J mice, or 18 s rRNA in FVB mice) and expressed relative to *βGK*/*βgk*, which was set at 1. **d** Human (n=11), (**e**) FVB mouse (n=4) and (**f**) C57BL/6J mouse (n=4) islets were cultured in 5 mmol/l glucose, and then in 5 (black bars) or 11 mmol/l (white bars) glucose for an additional 24 h before RNA analysis. The normalised mRNA level in 11 mmol/l glucose-cultured islets is expressed relative to 5 mmol/l glucose; $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ compared with islets cultured in 5 mmol/l glucose. *SOM*/*Som*, also known as *SST*/*Sst*; *GLU*/*Glu*, also known as *GCG*/*Gcg*. **g** Human or (**h**) FVB mouse islets were cultured for 48 or 24 h, respectively, after isolation in 5 mmol/l glucose. Islets were then cultured in 5 (G5) or 11 mmol/l (G11) glucose for an additional 24 h (pretreatment) and subsequently challenged with 3 mmol/l (black bars) or 16.7 mmol/l (hatched bars) glucose for 30 min. Insulin released into the media was measured by RIA. Gene expression in selected mouse and human islet preparations was similar at 24 and 48 h of culture (data not shown). $^{*}p<0.05$ and $^{***}p<0.001$ compared with response to 3 mmol/l glucose; $^{\dagger\dagger\dagger}p<0.001$ compared with response of islets incubated in 5 mmol/l glucose for 24 h

sections, demonstrating that the presence of MAFB in human beta cells is not an artefact of islet isolation.

Discussion

This study examined a highly selected series of human islet preparations with an intact insulin secretory pattern in an effort to establish their gene expression, and physiological and molecular properties. Two aspects of the human islet preparations are noteworthy: (1) only preparations that had intact glucose-stimulated insulin secretory properties were chosen for subsequent study (representing only 56% of islet preparations received from the human islet distribution programmes supported by the NIH and JDRF); and (2) all human islet preparations analysed were of especially high purity, quality and viability (Figs 1 and 2), with an additional handpicking step to greatly reduce acinar and ductal cell contamination for subsequent gene expression

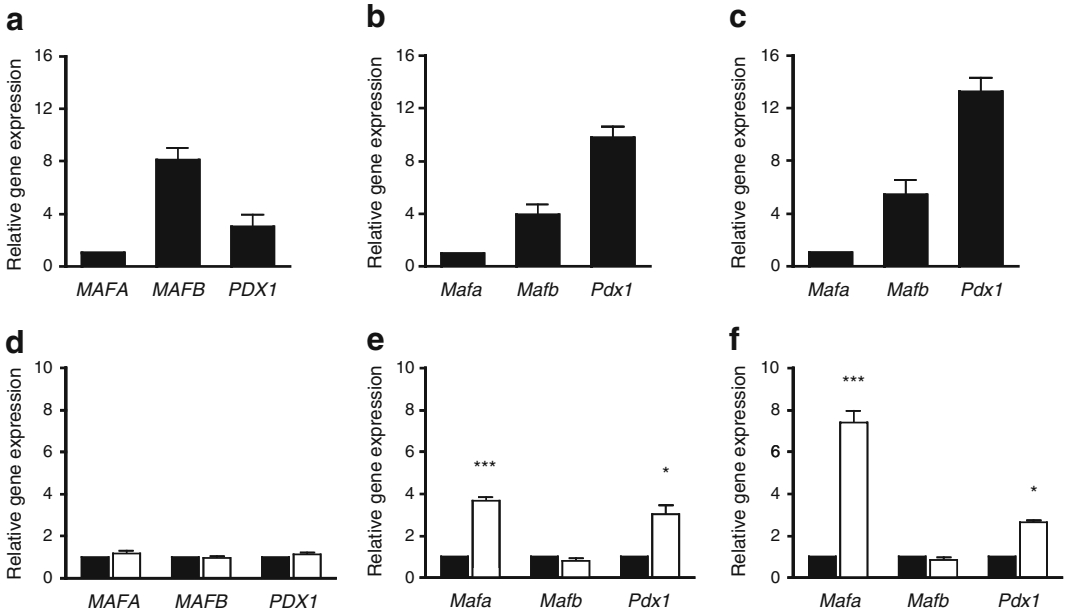


Fig. 5 *MAFA* and *PDX1* transcription factor mRNA levels are not glucose-regulated in cultured human islets. **a** Human (n=15), (**b**) FVB (n=4) and (**c**) C57BL/6J (n=4) mouse islets were cultured for 72 h (human) or 48 h (mice), respectively. *MAFA*/*Mafa*, *MAFB*/*Mafb* and *PDX1*/*Pdx1* mRNA levels were then quantified by RT-PCR and expressed relative to *MAFA*/*Mafa*. **d** Human (n=11), (**e**) FVB (n=4) and (**f**) C57BL/6J (n=4) mouse islets were first cultured for 48 h (human) or 24 h (mouse) in 5 mmol/l glucose, and then for 24 h in 5 (black bars) or 11 mmol/l (white bars) glucose before RNA analysis. The normalised amount of mRNA in 11 mmol/l glucose-cultured islets is expressed relative to 5 mmol/l glucose-cultured islets; *p<0.05 and ***p<0.001 compared with islets cultured in 5 mmol/l glucose

studies. Parallel studies were performed in islets from two mouse strains. The human islets secreted more insulin at baseline 5.6 mmol/l glucose, but less insulin upon stimulation with 16.7 or 16.7 mmol/l glucose+IBMX. Human islets also expressed more *MAFB* than *PDX1* mRNA, with MAFB protein being produced in human islet alpha cells and beta cells. Stimulation by glucose did not induce the production of beta cell-enriched gene products in human islets, including that of the glucose-regulated MAFA and PDX1 transcriptional mediators of insulin secretion and beta cell replication in rodents [27, 40, 41]

In recent years, human islets for research have become more available to a wide range of investigators, thanks to distribution programmes supported by the NIH and the Juvenile Diabetes Research Foundation [33]. Several issues surrounding isolated human islets create considerable challenges for the provision of islets for transplantation or research, including donor age and sex, the pre-morbid condition of the pancreas donor, the isolation procedure and shipping conditions. While many assays have been used to assess islet function, we chose the glucose-stimulated insulin secretory profile in a dynamic cell perifusion system as our 'gold standard' for selection of human islets for subsequent study. We did this because this method represents the integration of molecular processes such as glucose metabolism, Ca^{2+} ion channel activity, and secretory vesicle formation and movement. Most previous studies have not reported the function of the human islet preparations or the criteria for selecting preparations for study. The human islets reported on here were chosen from preparations similar to those distributed to other investigators and reported on in a number of publications, but our results highlight the fact that independent human islet preparations cannot be considered to be equivalent. Since these preparations were selected on the basis of their insulin secretory pattern, this is likely to have introduced a bias, and thus the gene expression pattern described by us may not reflect that of islet preparations with a lower insulin secretory capacity. Similarly, the insulin secretory pattern of the selected islet preparations may not be representative of the situation in humans, where the fold increase in insulin secretion in vivo is much greater.

These results not only provide important baseline information on the function of human islets as currently used for clinical transplantation and made available for research, but also outline approaches that could ensure the standardisation of conditions for selecting human islets for research or transplantation, or for improving the techniques used for human islet isolation. Furthermore, the insulin secretory profile of the human islets used here suggests that improved islet isolation procedures (including pre-isolation preparation) are needed and could well improve the function of isolated islets. While it is possible that our observations in human islets were affected by islet isolation protocol variables or shipping

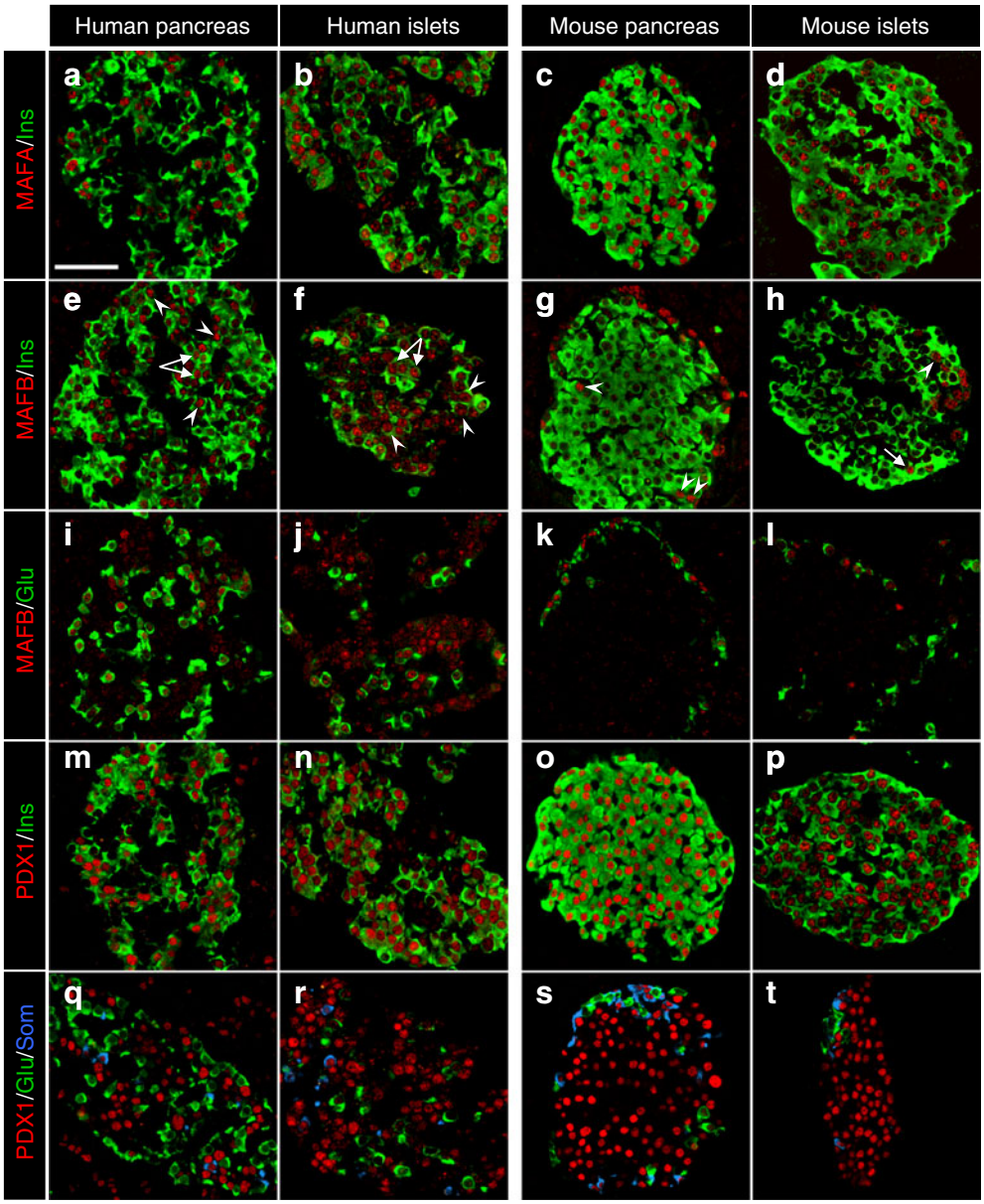


Fig. 6 MAFA, PDX1 and MAFB are present in the human islet beta cell population. The percentage of MAFA-, MAFB- or PDX1-producing alpha (glucagon, Glu), beta (insulin, Ins) and delta (somatostatin, Som) cells was determined from the immunohistochemical staining pattern. The intact human pancreas index (**a**, **e**, **i**, **m**, **q**) was 55% $MAFA^+$ beta cells (n=2,935 [total number of analysed cells]), 80% $PDX1^+$ beta cells (n=1,700), 9% $MAFB^+$ beta cells (n=1,567) and 40% $MAFB^+$ alpha cells (n=1,150). MAFA and PDX1 were produced in a larger fraction of beta cells in the mouse pancreas (**c**, **g**, **k**, **o**, **s**): 95% $MAFA^+$ beta cells (n=2,470), 100% $PDX1^+$ beta cells (n=1,516), 0.4% $MAFB^+$ beta cells (n=670) and 73% $MAFB^+$ alpha cells (n=327). **e**–**h** Arrows point to $Ins^+/MAFB^+$ cells, arrowheads indicate $Ins^-/MAFB^+$ cells. Scale bar (**a**) 50 μm applies to all other panels (**b**–**t**). Because, in human islets, beta and non-beta cells are more intermingled, two criteria were used for cell-counting purposes to ensure cell identity. First, an islet cell was deemed positive for nuclear factor or TUNEL only when at least 75% of the nucleus was surrounded by the cytoplasm labelled for a given hormone. Second, if the first criterion was met and there was a gap between the nucleus and the cell cytoplasm, this cell was excluded from the analysis. Thus, it is possible that due to the stringency of our counting procedure, counts of cells doubly positive for a hormone and a transcription factor (especially in human islets) may have been slightly underestimated

conditions, we believe that the unique features we observed are not due to the viability, purity or quality of our islets, which were all comparable in their preparation. For example, the cellular distribution of MAFB was similar in isolated human islets and pancreatic sections (Fig. 6), suggesting that this is a distinctive property of human islets.

Any comparison of mouse and human islets would ideally use the same isolation procedures, and mouse

and human donors of similar age, sex, BMI and pre-death status. However, human islets are often isolated using a continuous isolation procedure and separated in a refrigerated centrifuge and gradient, a process that is not feasible based on mouse pancreas size and the volumes of media needed for the refrigerated apparatus. Thus while it would be desirable to have mouse islets isolated in the same fashion from similar donors, we do not believe it is possible to control for all isolation and donor variables. Consequently, human islets cannot be directly compared with mouse islets. Instead, our findings should be viewed as a characterisation of human islets with an intact insulin secretory capacity and not as a direct comparison of mouse and human islets. Nevertheless, the distinctive features of human islets (such as MAFB production in islet alpha and beta cells) suggest important biological differences between mouse and human islets.

Human islets secreted more insulin at basal glucose concentrations than mouse islets. These results support the concept that human islets have a different set-point for glucose sensing [5, 6, 8, 42]. This hypothesis is also reinforced by observations showing that human islets have a lower glucose threshold for Ca^{2+} transport [4] and secrete insulin at lower glucose concentrations than mouse islets [5, 6, 8, 42, 43]. The current report and previous studies indicate that the threshold for insulin secretion is not only higher in rodent islets, but that the magnitude of insulin secretion and range of glucose sensitivity in rodents are greater than in human islets [5]. Another observation supporting a different glucose-sensing set-point is that xenotransplants of human islets into mice establish a lower blood glucose baseline [44]. The shift in insulin secretion by human islets to lower glucose concentrations could be attributed, at least in part, to their preferential production of high-affinity glucose transporters, compared with rodent islet beta cells, which mainly produce a low-affinity transporter [37, 38]. Other potential mechanisms may involve differences in sensitivity or number of ion channels downstream from glucokinase [5, 45, 46]. While the current report and the work of Henquin and colleagues [5] found a difference in the threshold between mouse and human islets, the difference in nutrient-stimulated secretion was greater in our studies. Despite the notable differences in insulin secretion between human and mouse islets, there are also many similarities, including the importance of ATP-sensitive potassium channels, a sustained second phase of insulin secretion and the effects of interactions between glucose and amino acids on insulin secretion [5, 43].

Interestingly, we also found that the production of many beta cell-enriched gene products was only responsive to glucose in mouse islets and that mouse, but not human islets were capable of increasing beta cell insulin production and insulin secretory output following acute and overnight glucose stimulation. In experiments seeking mechanistic insights into the difference in glucose-responsive gene expression between mouse and human islets, we found that the production of transcription factors important to glucose-responsive gene transcription in mice, and specifically of PDX1 and MAFA [21, 23–27], was selectively stimulated by glucose in mouse islets. Moreover, human islets were found to produce relatively more *MAFA*, *MAFB* and *PDX1* mRNA than mouse islets.

The abundance and/or activity of these islet-enriched transcription factors in human islets may limit their insulin secretion to high glucose challenge. The reason why *MAFA* and *PDX1* expression is unresponsive to glucose stimulation in human islets is unclear, but may reflect differences in transcription (MAFA [47]) and/or nuclear transport (PDX1 [48–50]) mechanisms associated with activation in rodent islets. Alternatively, there may be differences in molecular events connecting glucose transport and metabolism with *MAFA*/*Mafa* and *PDX1*/*Pdx1* regulation in human and mouse beta cells. Additional work is needed to determine whether these differences in *MAFA, MAFB* and *PDX1* expression or regulation actually influence human beta cell activity.

These results emphasise the importance of translating and integrating discoveries from rodent islets into our knowledge of human islets in order to expand our understanding of the regulatory processes involved in normal human beta cell physiology and islet dysfunction. Importantly, the question of how to define baseline human beta cell function in terms of gene expression and protein production patterns is critical to the development of beta cells or beta cell surrogates from alternative cell types for the treatment of type 1 diabetes and to the provision of insight into islet dysfunction in type 2 diabetes.

Acknowledgements We thank M. Jewel for technical assistance with the islet perifusions, and P. diIorio and D. Greiner at the University of Massachusetts for assistance with the islet shipping experiments. This work was supported by grants from the Juvenile Diabetes Research Foundation International (JDRF), the VA Research Service, the NIH (DK42502, DK66636, DK69603, DK63439, DK62641, DK68751, DK68854), the Beta Cell Biology Consortium (DK72473; DK89572), the Vanderbilt Mouse Metabolic Phenotyping Center (DK59637), and the Vanderbilt Diabetes Research and Training Center (DK20593; Islet Procurement and Analysis Core, Hormone Assay and Analytical Services Core, and Cell Imaging Shared Resource). Human islets were provided by NIH- and JDRF-supported islet isolation centres, and the Islet Cell Resource Centers and the Integrated Islet Distribution Network (http://iidp.coh.org/). Human pancreatic samples were provided by the JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD). Organ procurement organisations partnering with nPOD are listed at www.jdrfnpod.org/our-partners.php.

Contribution statement In accordance with ICMJE requirements, all authors were involved with the conception and design or analysis and interpretation of data, drafted or revised the manuscript, and approved the final version.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

1. Groop L, Lyssenko V (2008) Genes and type 2 diabetes mellitus. Curr Diab Rep 8:192–197
2. Bonnefond A, Froguel P, Vaxillaire M (2010) The emerging genetics of type 2 diabetes. Trends Mol Med 16:407–416
3. Brissova M, Fowler MJ, Nicholson WE et al (2005) Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. J Histochem Cytochem 53:1087–1097
4. Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A (2006) The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci U S A 103:2334–2339
5. Henquin JC, Dufrane D, Nenquin M (2006) Nutrient control of insulin secretion in isolated normal human islets. Diabetes 55:3470–3477
6. Henquin JC, Nenquin M, Stiernet P, Ahren B (2006) In vivo and in vitro glucose-induced biphasic insulin secretion in the mouse: pattern and role of cytoplasmic Ca2+ and amplification signals in beta-cells. Diabetes 55:441–451
7. Matschinsky FM (1996) A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. Diabetes 45:223–241
8. Matschinsky FM, Glaser B, Magnuson MA (1998) Pancreatic beta cell glucokinase. Closing the gap between theoretical concepts and experimental realities. Diabetes 47:307–315
9. 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
10. Tyrberg B, Andersson A, Borg LA (2001) Species differences in susceptibility of transplanted and cultured pancreatic islets to the beta-cell toxin alloxan. Gen Comp Endocrinol 122:238–251
11. Eizirik DL, Pipeleers DG, Ling Z, Welsh N, Hellerstrom C, Andersson A (1994) Major species differences between humans and rodents in the susceptibility to pancreatic beta-cell injury. Proc Natl Acad Sci U S A 91:9253–9256
12. Butler PC, Meier JJ, Butler AE, Bhushan A (2007) The replication of beta cells in normal physiology, in disease and for therapy. Nat Clin Pract Endocrinol Metab 3:758–768
13. Bosco D, Armanet M, Morel P et al (2010) Unique arrangement of alpha- and beta-cells in human islets of Langerhans. Diabetes 59:1202–1210
14. Ackermann AM, Gannon M (2007) Molecular regulation of pancreatic beta-cell mass development, maintenance, and expansion. J Mol Endocrinol 38:193–206
15. Oliver-Krasinski JM, Stoffers DA (2008) On the origin of the beta cell. Genes Dev 22:1998–2021
16. Vaxillaire M, Froguel P (2008) Monogenic diabetes in the young, pharmacogenetics and relevance to multifactorial forms of type 2 diabetes. Endocr Rev 29:254–264
17. Stoffers DA, Ferrer J, Clarke WL, Habener JF (1997) Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. Nat Genet 17:138–139
18. Clocquet AR, Egan JM, Stoffers DA et al (2000) Impaired insulin secretion and increased insulin sensitivity in familial Maturity-onset diabetes of the young 4 (insulin promoter factor 1 gene). Diabetes 49:1856–1864
19. Brissova M, Shiota M, Nicholson W et al (2002) Reduction in transcription factor pdx-1 impairs normal glucose sensing and insulin secretion by pancreatic islets. J Biol Chem 277:11225–11232
20. Johnson JD, Ahmed NT, Luciani DS et al (2003) Increased islet apoptosis in Pdx1+/− mice. J Clin Invest 111:1147–1160
21. Zhang C, Moriguchi T, Kajihara M et al (2005) MafA is a key regulator of glucose-stimulated insulin secretion. Mol Cell Biol 25:4969–4976
22. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF (1997) Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF-1 gene coding sequence. Nat Genet 15:106–110
23. Artner I, Blanchi B, Raum JC et al (2007) MafB is required for islet beta cell maturation. Proc Natl Acad Sci U S A 104:3853–3858
24. Marshak S, Totary H, Cerasi E, Melloul D (1996) Purification of the beta-cell glucose-sensitive factor that transactivates the insulin gene differentially in normal and transformed islet cells. Proc Natl Acad Sci U S A 93:15057–15062
25. Olbrot M, Rud J, Moss LG, Sharma A (2002) Identification of beta-cell-specific insulin gene transcription factor RIPE3b1 as mammalian MafA. Proc Natl Acad Sci U S A 99:6737–6742
26. Artner I, Le Lay J, Hang Y et al (2006) MafB: an activator of the glucagon gene expressed in developing islet alpha- and beta-cells. Diabetes 55:297–304
27. Nishimura W, Kondo T, Salameh T et al (2006) A switch from MafB to MafA expression accompanies differentiation to pancreatic beta-cells. Dev Biol 293:526–539
28. Offield MF, Jetton TL, Labosky PA et al (1996) PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 122:983–995
29. Jonsson J, Carlsson L, Edlund T, Edlund H (1994) Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 371:606–609
30. Kataoka K, Han SI, Shioda S, Hirai M, Nishizawa M, Handa H (2002) MafA is a glucose-regulated and pancreatic beta-cell-specific transcriptional activator for the insulin gene. J Biol Chem 277:49903–49910
31. Matsuoka TA, Artner I, Henderson E, Means A, Sander M, Stein R (2004) The MafA transcription factor appears to be responsible for tissue-specific expression of insulin. Proc Natl Acad Sci U S A 101:2930–2933
32. Brissova M, Fowler MJ, Wiebe P et al (2004) Intra-islet endothelial cells contribute to revascularization of transplanted pancreatic islets. Diabetes 53:1318–1325
33. Kaddis JS, Olack BJ, Sowinski J, Cravens J, Contreras JL, Niland JC (2009) Human pancreatic islets and diabetes research. JAMA 301:1580–1587
34. Ricordi C, Gray DWR, Hering BJ et al (1990) Islet isolation assessment in man and large animals. Acta Diabetol 27:185–195
35. Bustin SA, Benes V, Garson JA et al (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55:611–622
36. Zhao L, Guo M, Matsuoka TA et al (2005) The islet beta cell-enriched MafA activator is a key regulator of insulin gene transcription. J Biol Chem 280:11887–11894
37. De Vos A, Heimberg H, Quartier E et al (1995) Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. J Clin Invest 96:2489–2495
38. Ferrer J, Benito C, Gomis R (1995) Pancreatic islet GLUT2 glucose transporter mRNA and protein expression in humans with and without NIDDM. Diabetes 44:1369–1374

39. Hou X, Ling Z, Quartier E et al (1999) Prolonged exposure of pancreatic beta cells to raised glucose concentrations results in increased cellular content of islet amyloid polypeptide precursors. Diabetologia 42:188–194
40. Keller DM, McWeeney S, Arsenlis A et al (2007) Characterization of pancreatic transcription factor Pdx-1 binding sites using promoter microarray and serial analysis of chromatin occupancy. J Biol Chem 282:32084–32092
41. Artner I, Hang Y, Mazur M et al (2010) MafA and MafB regulate genes critical to beta-cells in a unique temporal manner. Diabetes 59:2530–2539
42. Ling Z, Pipeleers DG (1996) Prolonged exposure of human beta cells to elevated glucose levels results in sustained cellular activation leading to a loss of glucose regulation. J Clin Invest 98:2805–2812
43. Matschinsky FM, Magnuson MA, Zelent D et al (2006) The network of glucokinase-expressing cells in glucose homeostasis and the potential of glucokinase activators for diabetes therapy. Diabetes 55:1–12
44. Davalli AM, Ogawa Y, Scaglia L et al (1995) Function, mass, and replication of porcine and rat islets transplanted into diabetic nude mice. Diabetes 44:104–111
45. Braun M, Ramracheya R, Bengtsson M et al (2008) Voltage-gated ion channels in human pancreatic beta-cells: electrophysiological characterization and role in insulin secretion. Diabetes 57:1618–1628
46. Serre-Beinier V, Bosco D, Zulianello L et al (2009) Cx36 makes channels coupling human pancreatic beta-cells, and correlates with insulin expression. Hum Mol Genet 18:428–439
47. Raum JC, Hunter CS, Artner I et al (2010) Islet beta-cell-specific MafA transcription requires the 5′-flanking conserved region 3 control domain. Mol Cell Biol 30:4234–4244
48. Elrick LJ, Docherty K (2001) Phosphorylation-dependent nucleocytoplasmic shuttling of pancreatic duodenal homeobox-1. Diabetes 50:2244–2252
49. Rafiq I, Kennedy HJ, Rutter GA (1998) Glucose-dependent translocation of insulin promoter factor-1 (IPF-1) between the nuclear periphery and the nucleoplasm of single MIN6 beta-cells. J Biol Chem 273:23241–23247
50. Macfarlane WM, McKinnon CM, Felton-Edkins ZA, Cragg H, James RF, Docherty K (1999) Glucose stimulates translocation of the homeodomain transcription factor PDX1 from the cytoplasm to the nucleus in pancreatic beta-cells. J Biol Chem 274:1011–1016