



Review

The pancreatic β -cell in ageing: Implications in age-related diabetes

Eva Tudurí^{a,b,c,*}, Sergi Soriano^{a,c}, Lucía Almagro^a, Eduard Montanya^{b,d,e},
Paloma Alonso-Magdalena^{a,b}, Ángel Nadal^{a,b}, Ivan Quesada^{a,b,*}

^a Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universidad Miguel Hernández, Elche, Spain

^b Biomedical Research Center in Diabetes and Associated Metabolic Disorders (CIBERDEM), Madrid, Spain

^c Department of Physiology, Genetics and Microbiology, University of Alicante, Alicante, Spain

^d Department of Clinical Sciences, University of Barcelona, Barcelona, Spain

^e Bellvitge Hospital-IDIBELL, Barcelona, Spain, University of Barcelona, Barcelona, Spain

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ABSTRACT

The prevalence of type 2 diabetes (T2D) and impaired glucose tolerance (IGT) increases with ageing. T2D generally results from progressive impairment of the pancreatic islets to adapt β -cell mass and function in the setting of insulin resistance and increased insulin demand. Several studies have shown an age-related decline in peripheral insulin sensitivity. However, a precise understanding of the pancreatic β -cell response in ageing is still lacking. In this review, we summarize the age-related alterations, adaptations and/or failures of β -cells at the molecular, morphological and functional levels in mouse and human. Age-associated alterations include processes such as β -cell proliferation, apoptosis and cell identity that can influence β -cell mass. Age-related changes also affect β -cell function at distinct steps including electrical activity, Ca^{2+} signaling and insulin secretion, among others. We will consider the potential impact of these alterations and those mediated by senescence pathways on β -cells and their implications in age-related T2D. Finally, given the great diversity of results in the field of β -cell ageing, we will discuss the sources of this heterogeneity. A better understanding of β -cell biology during ageing, particularly at older ages, will improve our insight into the contribution of β -cells to age-associated T2D and may boost new therapeutic strategies.

1. Introduction

The prevalence of diabetes is drastically increasing worldwide. According to the International Diabetes Federation, 536.6 million adult people (20–79 years) suffered from diabetes in 2021, which represents a global prevalence of 10.5%, and these numbers will rise to 783 million by 2045 (IDF, 2021). A similar trend has been projected for impaired glucose tolerance (IGT), with a global estimate of 10.6% in 2021 (IDF, 2021). The prevalence of diabetes increases with ageing, accounting for more than 20% in individuals aged 65–79 years (IDF, 2021). It is well established that ageing along with obesity are among the most important risk factors associated with the development of type 2 diabetes (T2D). Thus, the current ageing of population is expected to further affect the future development of diabetes.

Ageing is a complex phenomenon characterized by a gradual

deterioration of tissues and organs. This functional decline has been related to the alteration of several processes including DNA damage repair, epigenetics homeostasis, mRNA processing and proteostasis, mitochondrial function, inflammatory status, progenitor cell function and cellular senescence, among others (Palmer et al., 2019). Many of these processes are also involved in the etiology of T2D, and thus, both ageing and T2D may share some molecular pathways and disorders (Palmer et al., 2021; Bellary et al., 2021). At the metabolic level, several studies have shown that a common consequence of ageing is a progressive decline in the accurate regulation of glucose homeostasis, which may gradually result in IGT and T2D (Shimokata et al., 1991; Chang et al., 2003, 2006; Bacos et al., 2016; De Jesús Garduno-García et al., 2018; Chia et al., 2018; Bellary et al., 2021). It has been proposed that these metabolic alterations are related to inadequate adaptive responses of pancreatic β -cells to compensate for peripheral insulin

Abbreviations: GSIS, glucose-stimulated insulin secretion, IGT, impaired glucose tolerance; IR, insulin resistance; ISI, insulin secretory index; STZ, streptozotocin; T2D, type 2 diabetes; vs, versus.

* Corresponding authors at: Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universidad Miguel Hernández, Elche, Spain

E-mail addresses: etuduri@umh.es (E. Tudurí), ivanq@umh.es (I. Quesada).

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resistance (IR), which is frequently observed in aged individuals (Barzilai et al., 2012; De Tata, 2014; Walker et al., 2021). In conditions of IR (i.e., obesity, pregnancy or ageing), the endocrine pancreas undergoes several compensatory adaptations that result in increased plasma insulin levels to counterbalance the attenuated insulin action on peripheral tissues, maintaining normal glycaemia (Fig. 1) (Kahn et al., 2006). Inadequate β -cell compensation to IR may favor the progression to IGT and T2D (Alejandro et al., 2015; Chen et al., 2017; Walker et al., 2021).

Abnormal glucose homeostasis and/or IR have been frequently reported in elderly subjects (Rowe et al., 1983; Petersen et al., 2015; Bacos et al., 2016; Ehrhardt et al., 2019). These alterations have been partly attributed to age-related secondary causes such as changes in body composition and overweight (Ferrannini et al., 1996; Chia et al., 2018; Bellary et al., 2021). However, when most of these factors were considered or compensated for, ageing per se was also found to affect insulin sensitivity in the elderly (Rowe et al., 1983; Ehrhardt et al., 2019). Therefore, the specific contribution of the different factors involved in age-related IR remains to be fully elucidated. In mice, age-associated IGT and/or IR have been frequently documented (Yoshino et al., 2011; González-Rodríguez et al., 2012; Li et al., 2014; Almacá et al., 2014; Aguayo-Mazzucato et al., 2017; Bodogai et al., 2018; Xiong et al., 2017; Ehrhardt et al., 2019; Chow et al., 2019), although some studies have found no deleterious effects on glucose tolerance (Gregg et al., 2016; De Leon et al., 2018; Denroche et al., 2021). Some of these divergences may rely on differences in the genetic and metabolic background of the animal models (Pann et al., 2020), and/or the experimental approaches employed in the different studies, suggesting that these factors should be considered when studying metabolism in ageing, as discussed later.

T2D cannot be fully attributed to an IR condition without considering pancreatic β -cell dysfunction and failures in their adaptive potential. T2D cannot be fully attributed to an IR condition without dysfunction of pancreatic β -cells and their adaptive potential. Therefore, understanding the physiology of the endocrine pancreas in ageing is critical to clarify the age-related alterations in glucose homeostasis. In

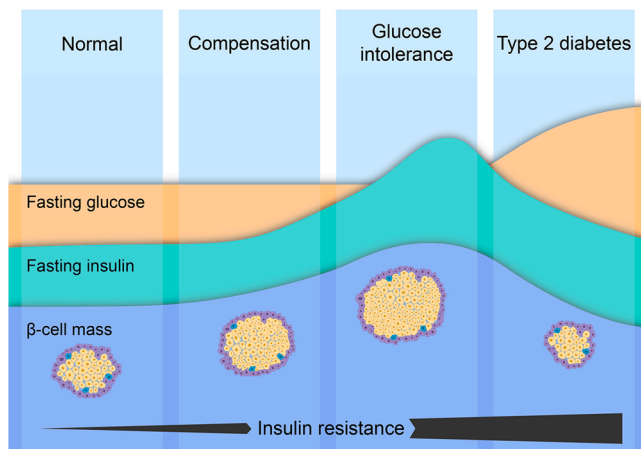


Fig. 1. Schematic representation of the changes in blood glucose, plasma insulin and β -cell mass that occur in the progression to T2D. The normal state is characterized by optimal and physiological levels of blood glucose and plasma insulin. However, in conditions that lead to IR such as obesity and/or ageing, β -cells undergo morphofunctional adaptations to enhance plasma insulin levels in an attempt to compensate for the loss of peripheral insulin sensitivity. This compensatory response allows the maintenance of a normoglycemic state. The progression toward glucose intolerance and T2D occurs when β -cell adaptations are not able to counterbalance the decline in peripheral insulin sensitivity. Although β -cell adaptations can be further enhanced during glucose intolerance, the β -cell secretory responses to glucose challenges are insufficient to maintain normal glucose homeostasis. As glucose levels rise during T2D, detrimental effects on β -cells are exacerbated, leading to cumulative impairment of β -cell function and, finally, β -cell loss.

this regard, several longitudinal studies point to an important contribution of insufficient β -cell compensation to IR in the age-related development of T2D (Morimoto et al., 2013; Ohn et al., 2016). In the present review, we specifically address the current knowledge about the morphofunctional alterations of pancreatic β -cells in ageing and their potential involvement in age-related dysregulation of glucose homeostasis and T2D. Although some important findings in middle-age individuals will be discussed, we have focused particularly on observations in older ages.

2. Ageing and pancreatic β -cell mass

To regulate glucose homeostasis, plasma insulin levels must be adapted to meet the organism's requirements (Fig. 1). Insulin levels and needs may vary throughout life and, particularly, in certain situations such as obesity, pregnancy or ageing, in which changes in peripheral insulin sensitivity take place (Alejandro et al., 2015; Chen et al., 2017; Walker et al., 2021). The high plasticity of the functional β -cell mass allows for the appropriate adaptation to different insulin requirements. Pancreatic β -cell mass is precisely regulated through the dynamic control of processes such as proliferation, neogenesis, cell size, cell death, dedifferentiation and transdifferentiation (Khadra and Schnell, 2015; Chen et al., 2017) (Fig. 2). Some of these processes are altered in aged individuals, since the regenerative capacity and plasticity of organs and tissues are often compromised with advanced age (Chandel et al., 2016). In the next section, we describe how β -cell mass may be altered in ageing in both humans and mice. More details about the studies reporting age-related effects on β -cell mass, which will be discussed in this section, can be found in Supplemental Tables 1 and 2.

2.1. Human β -cell mass

Human β -cell mass may expand up to \sim 30-fold from birth to adulthood (Meier et al., 2008). Such expansion is mainly due to an increased number of β -cells and augmented islet size. The rates of β -cell mass increase were found to be higher within the first two years of postnatal life, gradually declining over the following two decades (Meier et al., 2008). Conversely, fewer morphological changes have been reported in the pancreases from adult subjects. Human β -cell mass and/or volume

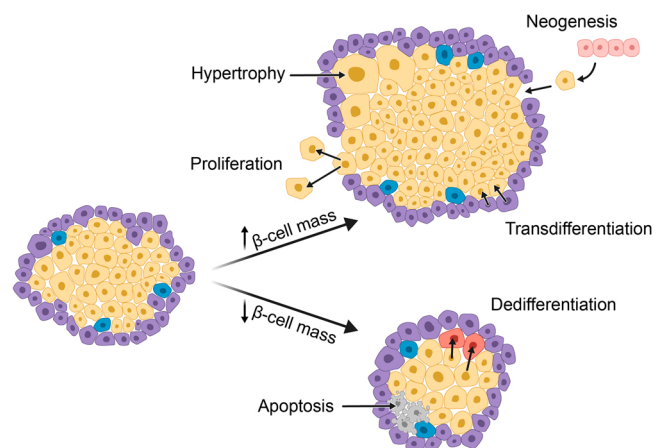


Fig. 2. Dynamic processes that regulate β -cell mass. The upregulation of β -cell mass includes different processes such as hypertrophy (increased cell size), proliferation (increased number of cells resulting from cell division), neogenesis (conversion of a pancreatic progenitor/stem cell into a β -cell) and transdifferentiation (conversion of an islet non- β -cell into a β -cell). The downregulation of β -cell mass results from β -cell apoptosis (programmed cell death) and/or dedifferentiation (conversion of a β -cell into a progenitor-like cell, which involves the loss of β -cell identity and function). Orange: β -cell; purple: α -cell; blue: δ -cell; red: β -cell precursor; pink: ductal cells.

notably increases during adulthood under conditions of high insulin demand such as pregnancy (Butler et al., 2010) or obesity (Butler et al., 2003; Rahier et al., 2008; Saisho et al., 2013). The process of ageing, however, does not substantially affect β -cell mass in adult humans, or in any case, the effect appears to be marginal. According to a study of human autopsies ranging from 20- to 102-year-old subjects, β -cell size and mass remained unchanged over time (Saisho et al., 2013). On the other hand, Rahier et al. (2008) documented a modest age-associated decay in β -cell mass in non-diabetic subjects (39–91 years). This latter finding is in agreement with a marginal decrease in β -cell mass reported in older adults from a Japanese population (0–79 years) (Mizukami et al., 2014). Thus, these observations indicate that β -cell mass is relatively well-preserved during ageing.

2.1.1. Human β -cell proliferation

A marked decrease in β -cell proliferation occurs early in life. Double staining of the pancreas for insulin and the proliferation marker Ki67 showed a maximum peak during fetal life (Beamish et al., 2017; Kassem et al., 2000; Bouwens et al., 1997) and a significant decline in β -cell turnover following the first 2 years of life (Meier et al., 2008; Gregg et al., 2012; Beamish et al., 2017; Wang et al., 2016). Afterward, very low β -cell replication rates remain unaltered during childhood, adolescence, adulthood and old ages (Meier et al., 2008; Gregg et al., 2012; Beamish et al., 2017; Saisho et al., 2013; Kassem et al., 2000; Reers et al., 2009; Mizukami et al., 2014). In addition, β -cell nuclei co-stained for the thymidine analogues BrdU/IdU were detected in samples from young (<20 years) but not in adult (>30 years) subjects, and β -cell DNA ^{14}C content measurements further suggested that β -cell proliferation occurs during the first three decades of life (Perl et al., 2010). Recent transcriptomic and epigenetic studies of pancreatic β -cells from juvenile (0.5–9 years) and adult (19–66 years) individuals also support a decline in proliferation along with enhanced β -cell maturation patterns over time (Arda et al., 2016).

Given that β -cell replication plays a key role in the adaptive response of the endocrine pancreas to augmented insulin requirements, the mechanisms involved in the age-related changes in proliferation are of critical interest. The Forkhead Box M1 (*FOXM1*) transcription factor is involved in the proper execution of mitosis, stimulates proliferation by promoting both G1/S and G2/M transitions, and regulates genes involved in cell cycle regulation (Wierstra and Alves, 2007). Concomitant with the age-associated decline in human β -cell turnover, down-regulated expression has been found for *FOXM1* as well as important cell cycle regulators including cyclins (*CCNA1* and *GCNA2*), cyclin-dependent kinases (*CDK1*, *CDK4* and *CDK6*) and proliferation-associated genes such as the platelet-derived growth factor (PDGF) receptors *PDGFR- α* and *PDGFR- β* (Chen et al., 2011; Avrahami et al., 2015; Dai et al., 2017). Additionally, the tumor suppressor protein *p16INK4A* (hereafter referred to as p16), a cell cycle inhibitor and significant effector of cellular senescence, is upregulated in aged human β -cells (Mizukami et al., 2014; Arda et al., 2016; Helman et al., 2016; Kong et al., 2018). Remarkably, genome-wide association studies have related the *CDKN2A/B* locus, which encodes this protein, to T2D risk (Saxena et al., 2007; Kong et al., 2016, 2018).

The response of human β -cells to mitogenic stimuli, such as the glucagon-like peptide 1 (GLP-1) has also been analyzed in ageing. In two different studies, mice with transplanted human islets from juvenile and adult donors were treated with the GLP-1 receptor agonist Exendin-4 (Ex-4) (Tian et al., 2011; 14–22 vs 35–58 years) (Dai et al., 2017; 0.2–9 vs 20–60 years). In both cases, Ex-4 enhanced the β -cell proliferation rate in young but not in old human grafts, indicating a loss of mitogenic potential in aged human β -cells (Tian et al., 2011; Dai et al., 2017). Likewise, PDGF-mediated proliferation was observed in β -cells from juvenile (8 months-6 years) but not from aged (39–56 years) subjects (Chen et al., 2011). Studies with human islets from adult donors (27–76 years) transplanted into streptozotocin (STZ)-induced diabetic NOD-severe combined immunodeficiency (SCID) mice showed that

adult β -cells maintained the capacity to replicate in response to the mitogenic effects induced by high blood glucose levels (Levitt et al., 2011). However, this effect was more potent in grafts from a 7-year-old donor (Levitt et al., 2011), suggesting decreased proliferative capacity with ageing.

The vascular system plays a key role in both producing and spreading mitogenic signals and other proliferative factors within the islet (Walker et al., 2021). In this context, the age-related decline in human β -cell proliferation has been associated with a reduction in the islet blood vessel network (Chen et al., 2021). Other age-related factors such as telomere shortening have also a significant impact on cell proliferation (Liew et al., 2009). In the case of β -cells, an inverse association between telomere length and ageing has been described in human samples ranging from 0 to 100 years (Tamura et al., 2016). Additionally, electron microscopy analysis of islet cells from 1 to 81-year-old individuals revealed a positive correlation between the presence of lipofuscin bodies in β -cells and ageing (Cnop et al., 2010). Given that lipofuscin accumulation is a feature of ageing in post-mitotic cells, these observations reinforce the concept that human β -cells are long-lived and present a low turnover rate during adulthood. In general, the findings described above suggest that human β -cells show a limited proliferative capacity in both basal and mitogen-stimulated conditions in aged adults.

2.1.2. Human β -cell apoptosis

Human β -cell apoptosis is relatively high during the fetal period (~1.5%) (Meier et al., 2010). However, it is quite infrequent afterward and, eventually, not detected in most pancreas samples (Meier et al., 2008). A frequency rate of 1.3% was reported in the perinatal period, dropping to 0.13% by 3 months of age (Kassem et al., 2000), and multiple studies have not observed variations in the low rate of apoptosis with advanced age (Meier et al., 2008; Reers et al., 2009; Saisho et al., 2013).

2.1.3. Human β -cell neogenesis

Neogenesis of human β -cells is a process that commonly occurs in the developing pancreas (Bouwens et al., 1997), although it may also occur at a very low rate after birth as well as in adulthood (Reers et al., 2009; Gregg et al., 2012). Increased β -cell neogenesis from pancreatic ducts has been described during pregnancy (Butler et al., 2010; Dirice et al., 2019), obesity (Butler et al., 2003; Hanley et al., 2010), T2D (Yoneda et al., 2013). However, no association has been reported with ageing (Reers et al., 2009).

2.1.4. Human β -cell identity

A single-cell transcriptomic analysis of human pancreases (1 month-54 years) showed an age-associated pattern of fate drift in α - and β -cells with a subset of cells expressing both insulin and glucagon, suggesting compromised islet cell identity in older subjects. This ageing pattern in islet cells was also characterized by high transcriptional variability as well as mutational signatures likely associated with oxidative stress (Enge et al., 2017). Similarly, in non-human primates, transcriptional noise has been associated with ageing in pancreatic α and β -cells along with an increased unfolding protein response and impaired proteostasis (Li et al., 2021). When comparing β -cells from juvenile (6 months-14 years) and adult (28–64 years) donors, Avrahami et al. (2015) reported a decrease in β -cell maturity and identity genes such as *PDX1*, *NKX6.1*, *FOXA2* and *MAFA*. The expression of the β -cell transcription factor *PDX1* was also found to be downregulated with ageing in the pancreatic tissue of two different groups of donors: 7–66 years (Reers et al., 2009) and 0–79 years (Mizukami et al., 2014). In contrast, another study showed higher expression levels of *MAFA* in β -cells from adult individuals (28–66 years) compared to juvenile subjects (0.5–6 years) (Arda et al., 2016). The interpretation of these findings comparing juvenile and old individuals is complex since juvenile subjects may present metabolic and developmental characteristics of childhood and adolescence that differ from more mature stages in adulthood. Overall,

however, all these results suggest that β -cell identity might be altered in older individuals. The functional implications of these findings and the exploration of older ages remain to be further addressed.

2.2. Mouse β -cell mass

Unlike humans, where β -cell mass does not face substantial changes in elderly subjects, studies conducted in mice generally observe increased β -cell mass with ageing (Rankin and Kushner, 2009; Fan et al., 2011; Talchai et al., 2012; Xin et al., 2016; Xiong et al., 2017; Tudurí et al., 2022). Such progressive expansion in β -cell mass frequently correlates with body weight (Xin et al., 2016). Similar results have been also reported in rats and increased β -cell size seems to be important in this process (Montanya et al., 2000).

2.2.1. Mouse β -cell proliferation

β -cell proliferation dramatically declines with ageing in mouse models (Wong et al., 2009; Rankin and Kushner, 2009; Fan et al., 2011; Stolovich-Rain et al., 2012; Beamish et al., 2017; Kehm et al., 2018; Lam et al., 2019). While proliferation of insulin-positive cells accounted for approximately 1% of β -cells at 3 months of age, it dropped to 0.2% in 25-month-old mice (Stolovich-Rain et al., 2012). Consistent with the idea that pancreatic β -cell proliferation occurs at low rates during adulthood, mapping of the pancreas with high-resolution isotope imaging revealed that aged animals chased for 18 months with the ^{15}N isotope were mainly composed of long-lived β -cells with a quiescent phenotype (Arrojo e Drigo et al., 2019). This age-related decline in β -cell proliferation has been frequently associated with increased expression of the cell cycle inhibitor protein *p16* in mouse islets (Krishnamurthy et al., 2006; Avrahami et al., 2015; Aguayo-Mazzucato et al., 2017; Kehm et al., 2018). Krishnamurthy et al. (2006) elegantly evaluated the role of *p16* in β -cell proliferation using transgenic mice either over-expressing or lacking this protein. An ageing-comparable expression level of *p16* significantly reduced islet cell proliferation in transgenic young mice (26–32 weeks). Conversely, *p16*-deficient islets from middle-aged mice (60–70 weeks) displayed higher cell proliferation than age-matched wild type islets and similar replication rates than 10-week-old wild type mice. Furthermore, following STZ ablation of β -cells, adult *p16*-deficient mice exhibited higher survival and proliferation rates than their wild-type littermates (Krishnamurthy et al., 2006). All these findings indicate that *p16* plays a key role in the diminished replicative capacity of β -cells with ageing. In addition to the action of *p16*, different changes in expression levels have also been demonstrated for other genes involved in cell cycle regulation such as *Pdgfra*, *Pdgfrb*, *Cdk6* and *Cdkn2b* (Wong et al., 2009; Avrahami et al., 2015).

The replicative response of β -cells to mitogenic signals has also been analyzed in mice. Following chronic administration of Ex-4, a proliferation rate of 0.76% per day of β -cells was detected in 2-month-old animals, while minimal effects were observed in 14-month-old mice (0.08% per day) (Rankin and Kushner, 2009). Adaptive β -cell regeneration in response to injury has also been explored in ageing models. Indeed, while 2-month-old mice displayed a notable increase in the percentage of BrdU^+ / insulin^+ cells following either 50% partial pancreatectomy or STZ treatment, mice older than 14 months failed to do so (Rankin and Kushner, 2009). The mode of β -cell injury or the severity of β -cell loss seems to affect the recruitment or activation of proliferative cells, since ablation of ~80% of β -cells by diphtheria toxin was able to induce β -cell replication in 25-month-old mice (Stolovich-Rain et al., 2012), although at lower rates than in young animals. In contrast to these findings, the β -cell regenerative capacity of xenografts from young and old mice (3 vs 20–24 months) transplanted into STZ-treated recipients did not differ under normoglycemic and hyperglycemic conditions (Chen et al., 2009). However, the presence of residual β -cells in the pancreas of the STZ-treated mice, the presence of systemic factors in the young recipients (Salpeter et al., 2013) and/or the potent mitogenic action of hyperglycemia could interfere with the age-related effects on β -cell

replication found in this study.

Interestingly, parabiosis experiments conducted in 1- and 8-month-old mice indicated that a circulating systemic factor regulates β -cell proliferation: when older mice were surgically connected to young animals, they displayed enhanced β -cell replication compared to old mice not parabiosed or parabiosed to old animals (Salpeter et al., 2013). Consistent with this, proliferation was found to be increased in β -cells from 8-month-old mice, when transplanted into young recipients compared to transplanted into aged animals (Salpeter et al., 2013). These findings suggest that age-related decline in β -cell proliferation could be improved by systemic factors from a young host. Along the same lines, transplantation of aged islets into the anterior chamber of the eye of young mice (2 vs 18 months) showed a notable increase in BrdU^+ / insulin^+ cells in the grafts (Almaça et al., 2014). Moreover, islets from aged donors transplanted to young recipients presented new blood vessels with reduced levels of inflammation or fibrosis after a prolonged period (Almaça et al., 2014). It was concluded that the age-dependent decline in islet proliferation was related to impaired vasculature functions rather than direct β -cell alterations. Similarly, decreased blood vessel density within pancreatic islets was found in old mice (2–8 vs 55–70 weeks). Molecular and pharmacological manipulation of a specific subset of endothelial cells augmented islet capillary density, resulting in higher β -cell proliferation rates in aged mice (Chen et al., 2021). The implication of the vascular system in islet ageing seems to involve several processes. For instance, it was reported that age-dependent accumulation of advanced glycation end products occurred in blood vessels within the islets of old mice (from 2.5 to 21 months), an effect that was accompanied by higher levels of inducible nitric oxide synthase and inflammatory markers (Kehm et al., 2018). These changes correlated with decreased Ki67^+ cells within the islets (Kehm et al., 2018). This link between inflammatory events and ageing has also been reported in zebrafish, where age-dependent decrease in beta-cell proliferation was associated with increased chronic inflammation and NF- κ B signaling (Janjuha et al., 2018).

In summary, the majority of studies in mice indicate that, as in humans, the proliferative potential of β -cells decreases with ageing both at basal conditions and in response to mitogenic treatment or injury conditions. However, it would be of key interest to analyze these responses in ageing under other conditions such as metabolic stress and IR, since obesity is an important risk factor for T2D and is highly prevalent among elderly people (Malenfant and Batsis, 2019; Hales et al., 2020). It has been reported that middle-aged mice exposed to high fat diet failed to increase proliferation and further expand β -cell mass, in contrast to young controls (Sone and Kagawa, 2005). However, similar experiments with old mice are scarce. In this regard, old mice (22 months) fed a Western diet for 4 weeks showed expansion of β -cell mass (De Leon et al., 2018). Although proliferation was not specifically analyzed, these findings suggest that aged animals could undergo compensatory β -cell changes in certain conditions. Therefore, further research is required to address the precise role of β -cell proliferation in the context of IR-induced compensatory responses in aged mice.

2.2.2. Mouse β -cell neogenesis

The formation of new β -cells from ductal cell progenitors is the main mechanism to increase β -cell mass in embryonic life (Bonner-Weir et al., 2010). In the postnatal life, rodent models have given conflicting results regarding the presence of neogenesis with positive (Xu et al., 2008) and negative findings (Solar et al., 2009). There is no information supporting an active process of β -cell neogenesis during ageing.

2.2.3. Mouse β -cell identity

Specific pancreatic β -cell genes such as *Pdx-1*, *Nkx6-1*, *NeuroD1*, *Foxa2*, *Isl1*, *MafA* and *Mnx1* have been found to be upregulated in β -cells from aged mice compared with young controls (4–6 weeks vs 16–20 months) (Avrahami et al., 2015). Although these findings suggest a more mature status with ageing, comparison of old adult mice with juvenile

animals could lead to confounding interpretations, as discussed later. Additionally, it has been shown by microarray analysis that the characteristic β -cell gene expression profile changes from juvenile to adult 1-year-old mice but exhibits additional changes in 2-year-old mice (Aguayo-Mazzucato et al., 2017). A specific analysis of senescent β -cells positive for β -galactosidase activity revealed that this group of β -cells exhibit an impaired maturity pattern including decreased expression of *Pdx-1*, *Nkx6-1*, *NeuroD1*, *Foxa2* and *MafA*, among others, along with increased expression of disallowed β -cell genes (Aguayo-Mazzucato et al., 2019). Considering that senescent β -cells are recruited during ageing (Aguayo-Mazzucato et al., 2017, 2019), these findings are consistent with other studies showing decreased *Pdx-1* expression in β -cells from aged mice (Ihm et al., 2007; Kehm et al., 2018). Moreover, while several β -cell transcription factors and functional key genes (*Ins1*, *Ins2*, *Slc2a2*) were down-regulated, the β -cell dedifferentiation markers *Sox9* and *Aldh1a3* were found to be up-regulated in mouse models of ageing and senescence (Murao et al., 2022). Thus, it seems that β -cell identity might be compromised at advanced ages, particularly with the progression of senescence.

Under conditions of genetic manipulation, such as β -cell ablation of FoxO1, β -cells from aged mice (16–20 months) exhibited features of dedifferentiation, which included an increased expression of neurogenin-3 and down-regulation of β -cell markers, suggesting a drift towards a progenitor-like state (Talchai et al., 2012). By employing a mouse model of severe β -cell ablation following diphtheria toxin administration, Herrera's group found that α -to- β -cell conversion can occur following massive β -cell loss in 1.5-year-old mice (Chera et al., 2014). Thus, while genetic models suggest that remodeling of islet cell identity could be induced in some conditions, it remains to be proven whether these processes are active during physiological ageing.

2.2.4. Mouse β -cell apoptosis

Increased levels of β -cell apoptosis were observed when comparing young and old animals (3–4 vs 18–24 months), which may result from the release of the proinflammatory cytokine tumor necrosis factor- α from acinar cells (Xiong et al., 2017). Additionally, β -cells from aged mice exhibited higher sensitivity to endoplasmic reticulum stress and decreased cell viability (6 weeks vs 14 months) (Mihailidou et al., 2017). These effects were associated with augmented unfolded protein response as well as caspase activation (Mihailidou et al., 2017). Consistent with these findings, a higher rate of apoptotic β -cells was also observed in the pancreas from old mice (3 vs 20 months) (Tudurí et al., 2022). Although the link with apoptosis was not analyzed, aged β -cells also exhibited signs of endoplasmic reticulum stress (Tudurí et al., 2022). Overall, it seems that β -cells are more vulnerable to apoptosis in old mice than in young animals.

3. Ageing and pancreatic β -cell function

In addition to the pancreatic β -cell mass, the adequate control of insulin release from pancreatic islets is critical to maintain glucose homeostasis. Pancreatic β -cell secretion is mainly regulated by the coordination and fine coupling of several signal transduction events such as glucose metabolism, electrical activity, Ca^{2+} signaling and exocytosis (Fig. 3) (Rorsman and Ashcroft, 2018). Similar to pancreatic β -cell mass, insulin secretion can be modulated according to the insulin requirements imposed by peripheral insulin sensitivity (Gonzalez et al., 2013) (Fig. 1). In the next section, we describe current evidence about the impact of ageing on β -cell function in both humans and mice. More details about the studies reporting age-related effects on β -cell function can be found at Supplemental Tables 1 and 2.

3.1. Insulin secretion in human islets

In vivo analysis of the effect of ageing on β -cell function, particularly when using glucose clamp studies, has shown either no major changes

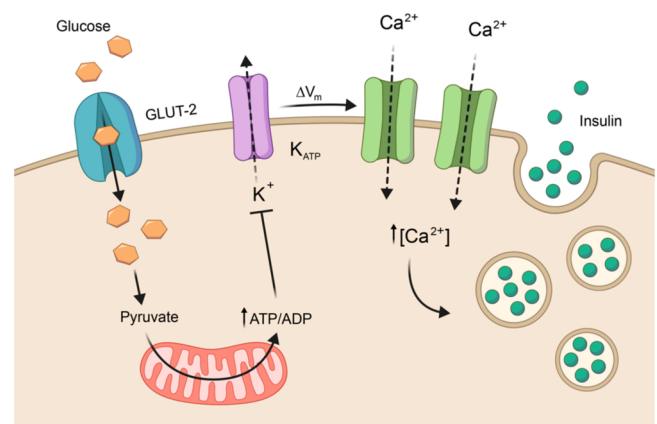


Fig. 3. Glucose-induced signal transduction in pancreatic β -cell insulin secretion. In mouse β -cells, glucose transport from the extracellular space into the cytosol is mediated by facilitated diffusion due to the activity of the glucose transporter GLUT-2. GLUT-1 is the main glucose transporter protein in human β -cells. Mitochondrial metabolism of glucose results in increased intracellular ATP levels, which allow for the closure of ATP-dependent K^+ (K_{ATP}) channels. The blockade of these channels leads to plasma membrane depolarization (ΔV_m), triggering action potential firing because of the activation of voltage-dependent Ca^{2+} channels. The opening of these channels increases intracellular Ca^{2+} concentration, inducing exocytosis of secretory granules and insulin release. A comparable model of stimulus-secretion coupling has been proposed for the human β -cell (Rorsman and Ashcroft, 2018).

(Elahi et al., 1993) or decreased insulin secretion (Iozzo et al., 1999; Fritsche et al., 2002; Basu et al., 2003; Chang and Halter, 2003; Szoke et al., 2008). It has also been reported that *in vivo* β -cell function is impaired in older subjects with IGT but not in those with normal glucose tolerance, who exhibited β -cell responses similar to those of the young controls with normal glucose tolerance (De Jesús Garduno-García et al., 2018). These findings indicate that the glycemic status in older subjects is an important factor to consider in ageing studies. These *in vivo* alterations in the elderly could be associated, at least in part, with age-related impairment of both release and sensitivity to insulinotropic incretins such as GLP-1 and GIP (Ranganath et al., 1998; De Jesús Garduno-García et al., 2018) or changes in proinsulin conversion to insulin (Fritsche et al., 2002), among other factors. In summary, it is generally accepted that human β -cell function *in vivo* may decline with ageing, particularly when the degree of peripheral insulin sensitivity is taken into account (Røder et al., 2000; Fritsche et al., 2002; Basu et al., 2003; Chang and Halter, 2003; Scheen, 2005; Chang et al., 2006; Geloneze et al., 2014; Bacos et al., 2016).

Since *in vivo* insulin release depends on numerous systemic regulatory factors, direct measurement of insulin secretion in pancreatic islets is important to understand pancreatic β -cell function during ageing. Despite the existence of several studies analyzing insulin release from isolated human islets, heterogeneous results have been reported. It has been shown that either glucose-stimulated insulin secretion (GSIS) or the insulin secretory index (ISI; secretion ratio between stimulatory and basal glucose levels) were not affected by donor age in studies using perfusion (Almaça et al., 2014; 17–65 years) or static incubation experiments (Kong et al., 2018; 15–68 years). In contrast, other studies using similar approaches have described an age-related decline in both GSIS and ISI (Lyon et al., 2016; 18–80 years), as well as attenuated secretory responses to the antidiabetic agent tolbutamide, a K_{ATP} channel antagonist that triggers insulin secretion (Henquin, 2018; 20–68 years). Several studies providing mechanistic insights have also shown a detrimental effect of ageing on human insulin secretion. For instance, *in vitro* studies with isolated islets from donors between the ages of 16 and 70 years demonstrated a negative correlation between age and the insulin secretory index, while insulin release at basal glucose concentrations was positively correlated with age (Ihm et al., 2006).

This reduced secretory index was associated with lower intracellular ATP production, suggesting deteriorated β -cell metabolism. Age-associated decreases in GSIS and ISI along with altered mitochondrial function were also described in islets from human donors ranging from 19 to 64 years (Gregg et al., 2016). Similarly, this link between impaired mitochondrial metabolism and insulin secretion capacity has been suggested in a study showing an age-related decrease in mitochondrial DNA copy number in human β -cells (Cree et al., 2008; 17–75 years). In addition, Westacott et al. (2017) found reduced GSIS with ageing, but without changes in ISI, during static incubations with isolated islets from 11 to 62 year-old donors. The authors also reported impaired secretion kinetics with ageing that was related to a decline in both gap-junctional communication and Ca^{2+} signaling coordination among β -cells. Finally, ageing was also associated with islet DNA methylation of several genes, some of which were linked to β -cell and mitochondrial function as well as T2D (Bacos et al., 2016; 26–74 years). These epigenetic changes were correlated with expression of these genes in the pancreatic islets. Moreover, although ageing was not associated with alterations in ISI, silencing of these genes in a pancreatic β -cell line led to different effects on GSIS, indicating that age-related epigenetic changes could affect insulin release from human islets and, also, the susceptibility to T2D in elderly individuals (Bacos et al., 2016). These different alterations in GSIS found in the islets from aged donors would not be related to changes in insulin content, since most studies have not found a correlation with age (Gregg et al., 2016; Henquin et al., 2017; Westacott et al., 2017).

Overall, several pieces of evidence have accumulated indicating that human β -cell function in vitro may decline in older individuals. Although in vivo insulin release depends on different levels of control in addition to intra-islet regulation, in vitro results also support the idea that aged human β -cells might be more vulnerable to fail under stress conditions such as IR, which require compensatory responses for long periods. In this context, elderly subjects would be more susceptible to the progression from normal glucose homeostasis to IGT and T2D.

3.2. Insulin secretion in mouse islets

In vivo experiments have shown either diminished (Santulli et al., 2012; 6 vs 20 months) or enhanced (Gregg et al., 2016; 6 vs >24 months) (Aguayo-Mazzucato et al., 2017; 1.5 vs 24 months) (Murao et al., 2022; 21–24 vs 101–118 weeks) (Tudurí et al., 2022; 3 vs 20 months) plasma insulin levels in response to glucose in aged mice compared with young controls. In a recent study, glucose homeostasis was analyzed in mice matched for body composition to circumvent adiposity-associated effects. In this experimental paradigm, higher plasma insulin levels were measured using glucose tolerance tests and glucose clamp techniques in aged animals (4–5 vs 18–21 months and 8 vs 27 months, respectively) (Ehrhardt et al., 2019), indicating that insulin responses were enhanced in old mice. Additionally, it was reported that in vivo GSIS was augmented in both aged and young mice after short-term exposure to a Western diet (4–6 vs 22 months) (De Leon et al., 2018). Thus, despite some conflicting results, accumulated evidence indicates that ageing per se does not result in a decline in mouse β -cell function in vivo. In any case, additional work is required to fully understand whether old mouse β -cells can efficiently cope with IR and metabolic stress conditions, given that additional factors such as obesity could result in an overload of the β -cell compensatory potential. This aspect will be further discussed later.

In vitro studies of insulin secretion from mouse pancreatic islets and isolated β -cells have reported heterogeneous findings. An age-related decrease in GSIS has been associated with β -cell mitochondrial defects as well as alterations in Ca^{2+} signaling and the phospholipase C pathway (4 vs 20–24 months) (Li et al., 2014). A reduced insulin secretory index in islets from old mice has been reported along with down-regulated expression of key β -cell genes such as the β -cell transcription factor *Pdx-1* as well as *Slc2a2*, which encodes the glucose transporter 2 (6 vs 20

months) (Santulli et al., 2012). Similarly, the insulin secretory index as well as the expression of β -cell specific genes such as *Pdx-1*, *Slc2a2*, *Ins2* and *Pcsk1* were found to be diminished in islets from old mice (2 vs 22 months) (Ihm et al., 2007). Aguayo-Mazzucato et al. (2017) did not find differences in islet GSIS between young and old animals (3 vs 18 months). However, insulin release at basal glucose concentrations was significantly elevated in the islets from aged mice. Moreover, the recruitment capacity of glucose to induce insulin secretion in isolated islet cells was decreased in the aged group, indicating a lower individual responsiveness to glucose (Aguayo-Mazzucato et al., 2017).

In contrast, several other studies support the notion that GSIS is preserved or increased in islets from aged mice. For instance, static incubations using islets from adult mice (6, 11 and 27 months) showed an augmented GSIS compared with juvenile animals (1 month) (Helman et al., 2016), but no differences were found among the islet secretory response in the adult ages. This enhanced GSIS with respect to young mice was associated with an age-related increase in the activity of the cell cycle inhibitor p16, which would be playing a dual effect on proliferation and secretion. Increased β -cell function with age has also been suggested by experiments showing enhanced GSIS in isolated islets and improved Ca^{2+} responsiveness in individual β -cells from old mice (4–6 weeks vs 16–20 months) (Avrahami et al., 2015). Although these two latter studies indicate that ageing is not necessarily linked to deteriorated β -cell function, their conclusions are difficult to interpret because of the use of juvenile mice as controls instead of young mature adults. In any case, augmented GSIS has also been described when comparing aged mice to young adults. This is the case of the studies of Leiter et al. (1988) and Almaça et al., who reported enhanced GSIS in the islets of aged animals during perfusion experiments (4–5 vs 21–25 months) (Almaça et al., 2014; 2 vs 18 months). Consistent with this, similar observations were described using static incubations with isolated islets in the presence of stimulatory glucose concentrations or depolarizing concentrations of K^{+} (3 vs 24 months) (Denroche et al., 2021). Moreover, Gregg et al. (2016) described that both basal and stimulated islet secretion were augmented in old mice (6 vs >24 months). These studies showed that, although β -cells from old mice exhibited reduced mitochondrial function, this response was compensated by increased plasma membrane excitability due to lower K_{ATP} channel conductance, and subsequent improvement of Ca^{2+} signaling. Similar to these findings, altered mitochondrial function and glucose-regulated K_{ATP} channel activity were compensated by enhanced voltage-dependent Ca^{2+} currents and Ca^{2+} signals in aged β -cells, leading to improved GSIS (3 vs 20 months) (Tudurí et al., 2022). This study also showed that the majority of these changes occurred at post-transcriptional levels, with minimal changes in the expression of genes involved in these processes. A recent article in young, middle-aged and old mice (18, 59 and 110 weeks) also found augmented secretory responsiveness to glucose in the islets from aged animals, an effect that was linked to hyperactivity of glycolysis due to the increased expression of the enzyme nicotinamide mononucleotide adenylyl transferase 2 (Murao et al., 2022). Similar results in senescence-accelerated prone and resistant mice, an animal model of premature senescence, further supported these findings (Murao et al., 2022). Changes in insulin protein synthesis are unlikely to be involved in the age-related alterations in GSIS since the majority of reports did not find significant differences (Gregg et al., 2016; Denroche et al., 2021; Tudurí et al., 2022). Only one study identified an age-related decrease in islet insulin content (Murao et al., 2022). Taken as a whole, and despite some heterogeneous results, numerous studies indicate that ageing is not necessarily associated with a decline in GSIS in mouse islets, and even, that β -cell function might increase to compensate for potential changes in glucose homeostasis in old mice.

4. Ageing and non- β -cells in mice and humans

In addition to the central role of β -cells in the regulation of glucose homeostasis, pancreatic α -cells also play a key function by secreting the

hyperglycemic hormone glucagon (Gilon, 2020). Moreover, pancreatic δ -cells act as important paracrine modulators of α - and β -cells via somatostatin secretion (Briant et al., 2018; Rorsman and Huising, 2018). Due to the very limited number of studies in non- β -cells, our knowledge about pancreatic α - and δ -cells during ageing is scarce. To date, the analysis of human pancreas from non-diabetic subjects (39–91 years) has shown a slight decrease in α -cell mass, while no age-associated changes were observed in T2D individuals (50–86 years) (Henquin and Rahier, 2011). Similarly, a recent analysis in non-diabetic individuals (30–102 years) indicated that the α -cell mass is well preserved over time (Moin et al., 2020). In the case of δ -cells, there is even less information in humans. The replicative capacity of human non- β -cells is also affected by ageing. Indeed, diminished α - and δ -cell proliferation and/or cell numbers have been documented in several studies in human pancreas sections (Gregg et al., 2012; Wang et al., 2016; Arda et al., 2016; Lam et al., 2018) or in human islets transplanted under the kidney capsule of mice (Dai et al., 2017).

In mice, data on α - and δ -cells in ageing are also scarce. Augmented α -cell mass has been shown in old animals (3 vs 16–20 months) (Talchai et al., 2012). It has also been described that basal α -cell proliferation is very high (15%) in 3-day-old newborn pups, dropping to very low rates (0.08%) by 15 months of age (Lam et al., 2019). Given the limited information available, further research on α - and δ -cells is required to elucidate the effects of ageing on these islet cell types.

5. Senescence and the endocrine pancreas

Cellular senescence is a process characterized by growth arrest (Gasek et al., 2021). Given that reduced proliferation is a hallmark of β -cell ageing, the study of senescence in islet cells and its involvement in diabetes is a current focus of intense research. It has been shown that, during ageing, mouse β -cells with senescence features form heterogeneous subpopulations and become more frequent (from ~1 month to 2 years) (Aguayo-Mazzucato et al., 2017). Several ageing and senescence markers such as p16, IGF1R and p53BP1 among others were identified in old β -cells, and their expression was increased during IR and metabolic stress. Remarkably, β -cells expressing IGF1R presented abnormal GSIS (Aguayo-Mazzucato et al., 2017). Additionally, analysis of senescent β -cells from middle-aged mice (7–8 months) revealed a reduced expression in β -cell identity genes along with increased expression of age and senescence-associated genes (Aguayo-Mazzucato et al., 2019; Midha et al., 2021). This β -cell senescence profile was upregulated under conditions of IR. The application of senolytic strategies to remove senescent β -cells in mouse models of IR and ageing alleviated the senescence features and improved β -cell function and glucose homeostasis. Moreover, analysis of human samples indicated that the percentage of β -cells with senescence features augmented with age and in T2D, suggesting that the observations in animal models could be clinically relevant (Aguayo-Mazzucato et al., 2019). Consistent with these findings, immunohistochemical analysis of human pancreases revealed higher expression of both senescence markers p16 and γ H2AX in 60–79 year-old subjects (Mizukami et al., 2014). Furthermore, the accumulation of senescent p16⁺ β -cells with ageing has also been identified in mice and humans (Helman et al., 2016). Therefore, all these findings indicate that the presence of senescent β -cells may play an important role in the pathophysiology of age-related diabetes. In this regard, the development of senolytic therapies could be beneficial for the treatment of this pathology (Aguayo-Mazzucato et al., 2019; Palmer et al., 2019). Nonetheless, more translational research is required to test whether senolytic strategies used in mouse models can be efficiently implemented in humans (Gasek et al., 2021).

The pancreatic islet, particularly in mice, works as a β -cell functional unit due to cell-to-cell communication through gap junctions as well as paracrine communication. These characteristics are crucial for optimal insulin secretion (Cigliola et al., 2013; Hodson et al., 2013; Johnston et al., 2016). It is noteworthy that senescent β -cells show a high degree

of heterogeneity in the expression of age markers (Aguayo-Mazzucato et al., 2017). Although this complexity could point to the existence of diverse subpopulations of senescent β -cells within the islet (Benninger and Hodson, 2018), it has been proposed that this heterogeneity relies on the individual ageing state of each β -cell (Aguayo-Mazzucato et al., 2017). Given that most of these senescence-related findings come from studies with dissociated islet cells, it would be interesting to know how this β -cell heterogeneity is functionally integrated within the whole islet during ageing.

6. Limitations in the research of the pancreatic β -cell in ageing

Numerous studies in humans and mice have documented age-associated alterations, adaptations and/or failures in pancreatic β -cell mass and function both in vivo and in vitro. However, in certain areas of this research, some studies have resulted in heterogeneous observations, leading to conclusions that are difficult to interpret. It is important to consider the potential sources of this variability to minimize its occurrence in future studies.

Among the key factors contributing to data variability, the age ranges are an important aspect to consider. Analysis of maturation patterns indicates that the range of 3–6 months is the most appropriate to establish young adulthood in mice (Flurkey et al., 2007; Hagan, 2017; Jackson et al., 2017), and thus, this period should be considered the most adequate as the reference/control group in ageing studies. Analysis of animals at younger periods, however, would include physiological and developmental features of adolescence or childhood. On the other hand, studies of aged mice should be performed in animals older than 18 months, when age-specific senescence markers and processes are extensively observed (Flurkey et al., 2007; Hagan, 2017; Jackson et al., 2017). Unfortunately, these age criteria have not been systematically considered throughout the scientific literature. Likewise, several human studies included individuals in juvenile and adolescent stages, or adults that do not reach the age-associated period of senescence. Furthermore, studies including individuals at older ages (> 65 years) are scarce, and thus, less information is available at this period, particularly regarding β -cell function.

Another important issue is that ageing has been associated with changes in peripheral insulin sensitivity (Chang and Halter, 2003; Barzilai et al., 2012; Chia et al., 2018). Given that β -cell mass and function adapts to the degree of insulin sensitivity to maintain normoglycemia, the presence of IR in elderly individuals and aged mice should impact their β -cell responses and, also, their glucose homeostasis (De Tata, 2014; Alejandro et al., 2015; Chen et al., 2017; Walker et al., 2021), making it more difficult to analyze the specific effect of ageing. Thus, it would be desirable to analyze β -cell ageing considering the glycemic status of the old individuals (Mezza et al., 2014, 2019), particularly in *ex vivo* and *in vitro* studies with isolated islets or β -cells. Indeed, it has also been reported that β -cells exhibit several *ex vivo* alterations with ageing, with these changes being more pronounced in mice with lower insulin sensitivity (Tudurí et al., 2022).

Other causes of potential variability include the use of different experimental procedures, cell models (i.e. whole pancreas, isolated islets or dispersed β -cells) and animal strains with diverse genetic backgrounds. For instance, Pann et al. (2020) identified significant variability in the metabolome when different mouse strains were compared. Data representation is also a valuable issue for the appropriate interpretation of the results. Several studies, particularly in humans, have described their results only in terms of the insulin secretory index. This data description without further information about the secretory process makes it difficult to fully assess the impact of ageing on basal and stimulated insulin secretion, which are central elements when considering β -cell function. Gender variability could be another potential source of data heterogeneity, since β -cell physiological responses and glucose homeostasis may have a sex-related component. Age-associated differences between males and females in GSIS, glucose homeostasis and

metabolism have been reported (Yoshino et al., 2011; Xiong et al., 2017; Pann et al., 2020; Moreno-Fernandez et al., 2021). Yoshino et al. (2011) described significant interindividual and gender variability in their studies: while a fraction (~15%) of males fed a normal chow naturally developed age-related T2D (15–26 months), females required a high-fat diet to acquire a diabetic phenotype at these ages. In future studies, it would be important to consider and, when possible, to limit the aforementioned sources of variability to minimize the heterogeneity of results and the occurrence of possible discrepancies. This methodical approach would probably contribute to a better understanding of pancreatic β -cell dynamics in ageing and its role in the changes in glucose homeostasis in elderly individuals.

7. Conclusions and future perspectives

It is widely documented that ageing is associated with numerous alterations in the regulation of β -cell mass and function, which might have an important effect on the regulation of glucose homeostasis. Based on the overall body of evidence reported to date, pancreatic β -cell mass seems to be relatively preserved in older human adults, and is increased in aged mice. Among the processes involved in the regulation of β -cell mass, apoptosis appears to have a minor role in human ageing. In mice, although old β -cells seem to be more susceptible to apoptosis than β -cells from young individuals, the contribution of this process to the regulation of islet cell mass in ageing is unclear. Additionally, recent studies show that ageing may involve changes in cell identity, dedifferentiation and loss of maturation in β -cells, aspects that should be thoroughly explored in the future. The most consistent observation found in both human and mouse β -cell ageing is the decline of the proliferative capacity. Considering that the rates of proliferation and apoptosis are low during adulthood, both processes should have a limited impact on the overall regulation of adult β -cell mass, particularly in ageing. Therefore, the age-associated increase in β -cell mass documented in mice should be related with other processes. In this regard, a progressive rise in β -cell size has been described in rodents during ageing (Montanya et al., 2000). This is also consistent with the observation that ageing in the endocrine pancreas involves the recruitment of β -cells into a senescent state, which are characterized by increased cell size (Helman et al., 2016; Aguayo-Mazzucato et al., 2017).

Furthermore, studies in humans and mice have also documented age-associated alterations in GSIS and/or in key steps of its regulation such as mitochondrial function, electrical activity, Ca^{2+} signaling and cell-to-cell communication, among others. These alterations have been generally related to a β -cell functional decline in humans. In mice, although data are less consistent, ageing is not necessarily linked to β -cell dysfunction. Indeed, several studies have described improved β -cell function in old mice. In general, human pancreatic β -cells appear to be more vulnerable to ageing than mice, particularly at the functional level. This may be due to the longer lifespan of human β -cells (Cnop et al., 2010), and thus, longer periods of exposure to metabolic challenges and stress compared with mouse β -cells. Additionally, interspecies features of metabolism could also result in different outcomes during ageing. For instance, age-dependent lipid droplet accumulation was detected in human β -cells and found to be increased in T2D patients, but not in mice (Tong et al., 2020).

Although pancreatic β -cells may exhibit some resilience to ageing, particularly in mice, numerous studies have reported from subtle to marked alterations that may predispose β -cells to fail over the long term or when they face an additional metabolic challenge. As described in the previous sections, these age-related alterations may include limited proliferation potential, compromised cellular identity, increased proportion of senescent β -cells and several alterations in the cellular events that regulate GSIS, among others. Additionally, aged individuals with impaired glucose homeostasis exhibit more pronounced alterations in insulin secretion and its regulation (Chang et al., 2006; Tudurí et al., 2022). Thus, a limited adaptive potential of β -cells could be detrimental

in aged individuals to maintain adequate compensatory responses in additional conditions of metabolic stress, such as IR and obesity. This is of particular interest, given the link between IR and obesity (Kahn et al., 2006), and the high prevalence of overweight and obesity in the elderly population (Malenfant and Batsis, 2019; Hales et al., 2020). In this regard, the impact of diet-induced obesity was analyzed in young and middle-aged mice that were exposed to high-fat diet for 4 or 12 months starting at 6 weeks of age (Sone and Kagawa, 2005). Although a lipotoxic effect derived from the long dietary treatment could also affect glucose homeostasis in these animals, the findings indicated that compensatory β -cell responses could be attenuated with age. However, this is in contrast with some recent evidence showing that old mice (22 months) subjected to diet-induced obesity were protected against the deleterious effects of these conditions, enhancing β -cell mass and function (De Leon et al., 2018). Therefore, future research should further explore the ability of aged β -cells to maintain their performance over time and also, to cope with conditions of metabolic stress such as IR and obesity. As discussed earlier, the potential sources of data variability, such as age analysis periods, should also be considered in future studies. Finally, given the promising potential of senolytic strategies, more basic and clinical research should be implemented to pave the way for the translational development of these therapies in age-related T2D.

In summary, ageing has been associated with alterations, adaptations and/or failures of the pancreatic β -cell mass and function. These β -cell alterations might have an important role in the higher prevalence of IGT and T2D reported in the elderly (Chia et al., 2018). A better understanding of all the factors that alter the proper regulation of glucose metabolism at advanced ages will facilitate the design of therapies that allow for a better management of glycaemia in the elderly.

Declarations of interests

The authors have no conflicts of interest to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.arr.2022.101674](https://doi.org/10.1016/j.arr.2022.101674).

References

- Aguayo-Mazzucato, C., van Haaren, M., Mruk, M., Lee, T.B., Crawford, C., Hollister-Lock, J., Sullivan, B.A., Johnson, J.W., Ebrahimi, A., Dreyfuss, J.M., Van Deursen, J., Weir, G.C., Bonner-Weir, S., 2017. β cell aging markers have heterogeneous distribution and are induced by insulin resistance. *Cell Metab.* 25, 898–910. <https://doi.org/10.1016/j.cmet.2017.03.015>.
- Aguayo-Mazzucato, C., Andle, J., Lee, T.B., Midha, A., Talemal, L., Chipashvili, V., Hollister-Lock, J., van Deursen, J., Weir, G., Bonner-Weir, S., 2019. Acceleration of β cell aging determines diabetes and senolysis improves disease outcomes. *Cell Metab.* 30, 129–142. <https://doi.org/10.1016/j.cmet.2019.05.006>.
- Alejandro, E.U., Gregg, B., Blandino-Rosano, M., Cras-Méneur, C., Bernal-Mizrachi, E., 2015. Natural history of β -cell adaptation and failure in type 2 diabetes. *Mol. Asp. Med.* 42, 19–41. <https://doi.org/10.1016/j.mam.2014.12.002>.
- Almaça, J., Molina, J., Arrojo e Drigo, R., Abdulreda, M.H., Jeon, W.B., Berggren, P.-O., Caicedo, A., Nam, H.G., 2014. Young capillary vessels rejuvenate aged pancreatic islets. *Proc. Natl. Acad. Sci.* 111, 17612–17617. <https://doi.org/10.1073/pnas.1414053111>.
- Arda, H.E., Li, L., Tsai, J., Torre, E.A., Rosli, Y., Peiris, H., Spitale, R.C., Dai, C., Gu, X., Qu, K., Wang, P., Wang, J., Grompe, M., Scharfmann, R., Snyder, M.S., Bottino, R., Powers, A.C., Chang, H.Y., Kim, S.K., 2016. Age-dependent pancreatic gene

- regulation reveals mechanisms governing human β cell function. *Cell Metab.* 23, 909–920. <https://doi.org/10.1016/j.cmet.2016.04.002>.
- Arrojo e Drigo, R., Lev-Ram, V., Tyagi, S., Ramachandra, R., Deerinck, T., Bushong, E., Phan, S., Orphan, V., Lechene, C., Ellisman, M.H., Hetzer, M.W., 2019. Age mosaicism across multiple scales in adult tissues. *Cell Metab.* 30, 343–351. <https://doi.org/10.1016/j.cmet.2019.05.010>.
- Avrahami, D., Li, C., Zhang, J., Schug, J., Avrahami, R., Rao, S., Stadler, M.B., Burger, L., Schübeler, D., Glaser, B., Kaestner, K.H., 2015. Aging-dependent demethylation of regulatory elements correlates with chromatin state and improved β cell function. *Cell Metab.* 22, 619–632. <https://doi.org/10.1016/j.cmet.2015.07.025>.
- Bacos, K., Gillberg, L., Volkov, P., Olsson, A.H., Hansen, T., Pedersen, O., Gjesing, A.P., Eiberg, H., Tuomi, T., Almgren, P., Groop, L., Eliasson, L., Vaag, A., Dayeh, T., Ling, C., 2016. Blood-based biomarkers of age-associated epigenetic changes in human islets associate with insulin secretion and diabetes. *Nat. Commun.* 7, 11089. <https://doi.org/10.1038/ncomms11089>.
- Barzilai, N., Huffman, D.M., Muzumdar, R.H., Bartke, A., 2012. The critical role of metabolic pathways in aging. *Diabetes* 61, 1315–1322. <https://doi.org/10.2337/db11-1300>.
- Basu, R., Breda, E., Oberg, A.L., Powell, C.C., Man, C.D., Basu, A., Vittone, J.L., Klee, G., Arora, P., Jensen, M.D., Toffolo, G., Cobelli, C., Rizza, R.A., 2003. Mechanisms of the age-associated deterioration in glucose tolerance: Contribution of alterations in insulin secretion, action, and clearance. *Diabetes* 52, 1738–1748. <https://doi.org/10.2337/diabetes.52.7.1738>.
- Beamish, C.A., Mehta, S., Strutt, B.J., Chakrabarti, S., Hara, M., Hill, D.J., 2017. Decrease in Ins+ Glut2 LO β -cells with advancing age in mouse and human pancreas. *J. Endocrinol.* 233, 229–241. <https://doi.org/10.1530/JOE-16-0475>.
- Bellary, S., Kyrou, I., Brown, J.E., Bailey, C.J., 2021. Type 2 diabetes mellitus in older adults: clinical considerations and management. *Nat. Rev. Endocrinol.* 17, 534–548. <https://doi.org/10.1038/s41574-021-00512-2>.
- Benninger, R.K.P., Hodson, D.J., 2018. New understanding of β -cell heterogeneity and in situ islet function. *Diabetes* 67, 537–547. <https://doi.org/10.2337/db17-0040>.
- Bodogai, M., O'Connell, J., Kim, K., Kim, Y., Moritoh, K., Chen, C., Gusev, F., Vaughan, K., Shulzhenko, N., Mattison, J.A., Lee-Chang, C., Chen, W., Carlson, O., Becker, K.G., Gurusu, M., Morgun, A., White, J., Meade, T., Perdue, K., Mack, M., Ferrucci, L., Trinchieri, G., de Cabo, R., Rogae, E., Egan, J., Wu, J., Biragyn, A., 2018. Commensal bacteria contribute to insulin resistance in aging by activating innate B1a cells. *Sci. Transl. Med.* 10. <https://doi.org/10.1126/scitranslmed.aat4271>.
- Bonner-Weir, S., Li, W.C., Ouziel-Yahalom, L., Guo, L., Weir, G.C., Sharma, A., 2010. Beta-cell growth and regeneration: replication is only part of the story. *Diabetes* 59, 2340–2348. <https://doi.org/10.2337/db10-0084>.
- Bouwens, L., Lu, W.G., De Krijger, R., 1997. Proliferation and differentiation in the human fetal endocrine pancreas. *Diabetologia* 40, 398–404. <https://doi.org/10.1007/s001250050693>.
- Briant, L.J.B., Reinbothe, T.M., Spiliotis, I., Miranda, C., Rodríguez, B., Rorsman, P., 2018. δ -cells and β -cells are electrically coupled and regulate α -cell activity via somatostatin. *J. Physiol.* 596, 197–215. <https://doi.org/10.1113/JP274581>.
- Butler, A.E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R.A., Butler, P.C., 2003. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2. *Diabetes* 52, 102–110. <https://doi.org/10.2337/diabetes.52.1.102>.
- Butler, A.E., Cao-Minh, L., Galasso, R., Rizza, R.A., Corradin, A., Cobelli, C., Butler, P.C., 2010. Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. *Diabetologia* 53, 2167–2176. <https://doi.org/10.1007/s00125-010-1809-6>.
- Chandel, N.S., Jasper, H., Ho, T.T., Passetgué, E., 2016. Metabolic regulation of stem cell function in tissue homeostasis and organismal ageing. *Nat. Cell Biol.* <https://doi.org/10.1038/ncb3385>.
- Chang, A.M., Halter, J.B., 2003. Aging and insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* 284, E7–E12. <https://doi.org/10.2337/db11-130010.1152/ajpendo.00366.2002>.
- Chang, A.M., Smith, M.J., Galecki, A.T., Bloem, C.J., Halter, J.B., 2006. Impaired β -cell function in human aging: Response to nicotinic acid-induced insulin resistance. *J. Clin. Endocrinol. Metab.* 91, 3303–3309. <https://doi.org/10.1210/jc.2006-0913>.
- Chen, C., Cohrs, C.M., Stertmann, J., Bozsak, R., Speier, S., 2017. Human beta cell mass and function in diabetes: Recent advances in knowledge and technologies to understand disease pathogenesis. *Mol. Metab.* 6, 943–957. <https://doi.org/10.1016/j.molmet.2017.06.019>.
- Chen, H., Gu, X., Liu, Y., Wang, J., Wirt, S.E., Bottino, R., Schorle, H., Sage, J., Kim, S.K., 2011. PDGF signalling controls age-dependent proliferation in pancreatic β -cells. *Nature* 478, 349–355. <https://doi.org/10.1038/nature10502>.
- Chen, J., Lippo, L., Labella, R., Tan, S.L., Marsden, B.D., Dustin, M.L., Ramasamy, S.K., Kusumbe, A.P., 2021. Decreased blood vessel density and endothelial cell subset dynamics during ageing of the endocrine system. *EMBO J.* 40, e105242. <https://doi.org/10.15252/embo.2020105242>.
- Chen, X., Zhang, X., Chen, F., Larson, C.S., Wang, L.J., Kaufman, D.B., 2009. Comparative study of regenerative potential of β cells from young and aged donor mice using a novel islet transplantation model. *Transplantation* 88, 496–503. <https://doi.org/10.1097/TP.0b013e3181b0d2ee>.
- Chera, S., Baronnier, D., Ghila, L., Cigliola, V., Jensen, J.N., Gu, G., Furuyama, K., Thorel, F., Gribble, F.M., Reimann, F., Herrera, P.L., 2014. Diabetes recovery by age-dependent conversion of pancreatic δ -cells into insulin producers. *Nature* 514, 503–507. <https://doi.org/10.1038/nature13633>.
- Chia, C.W., Egan, J.M., Ferrucci, L., 2018. Age-related changes in glucose metabolism, hyperglycemia, and cardiovascular risk. *Circ. Res.* 123, 886–904. <https://doi.org/10.1161/CIRCRESAHA.118.312806>.
- Chow, H.M., Shi, M., Cheng, A., Gao, Y., Chen, G., Song, X., So, R.W.L., Zhang, J., Herrup, K., 2019. Age-related hyperinsulinemia leads to insulin resistance in neurons and cell-cycle-induced senescence. *Nat. Neurosci.* 22, 1806–1819. <https://doi.org/10.1038/s41593-019-0505-1>.
- Cigliola, V., Chellakudam, V., Arabieter, W., Meda, P., 2013. Connexins and β -cell functions. *Diabetes Res. Clin. Pract.* 99, 250–259. <https://doi.org/10.1016/j.diabres.2012.10.016>.
- Cnop, M., Hughes, S.J., Igoillo-Esteve, M., Hoppa, M.B., Sayyed, F., Van De Laar, L., Gunter, J.H., De Koning, E.J.P., Walls, G.V., Gray, D.W.G., Johnson, P.R.V., Hansen, B.C., Morris, J.F., Pipeleers-Marichal, M., Cnop, I., Clark, A., 2010. The long lifespan and low turnover of human islet beta cells estimated by mathematical modeling of lipofuscin accumulation. *Diabetologia* 53, 321–330. <https://doi.org/10.1007/s00125-009-1562-x>.
- Cree, L.M., Patel, S.K., Pyle, A., Lynn, S., Turnbull, D.M., Chinnery, P.F., Walker, M., 2008. Age-related decline in mitochondrial DNA copy number in isolated human pancreatic islets. *Diabetologia* 51, 1440–1443. <https://doi.org/10.1007/s00125-008-1054-4>.
- Dai, C., Hang, Y., Shostak, A., Poffenberger, G., Hart, N., Prasad, N., Phillips, N., Levy, S. E., Greiner, D.L., Shultz, L.D., Bottino, R., Kim, S.K., Powers, A.C., 2017. Age-dependent human β cell proliferation induced by glucagon-like peptide 1 and calcineurin signaling. *J. Clin. Invest.* 127, 3835–3844. <https://doi.org/10.1172/JCI91761>.
- De Jesús Garduno-García, J., Gastaldelli, A., Defronzo, R.A., Lertwattanarak, R., Holst, J. J., Musi, N., 2018. Older subjects with β -cell dysfunction have an accentuated incretin release. *J. Clin. Endocrinol. Metab.* 103, 2613–2619. <https://doi.org/10.1210/je.2018-00260>.
- De Leon, E.R., Brinkman, J.A., Fenske, R.J., Gregg, T., Schmidt, B.A., Sherman, D.S., Cummings, N.E., Peter, D.C., Kimple, M.E., Lamming, D.W., Merrins, M.J., 2018. Age-dependent protection of insulin secretion in diet induced obese mice. *Sci. Rep.* 8, 1–11. <https://doi.org/10.1038/s41598-018-36289-0>.
- De Tata, V., 2014. Age-related impairment of pancreatic beta-cell function: pathophysiological and cellular mechanisms. *Front. Endocrinol. (Lausanne)* 5, 1–8. <https://doi.org/10.3389/fendo.2014.00138>.
- Denroche, H.C., Miard, S., Sallé-Lefort, S., Picard, F., Verchere, C.B., 2021. T cells accumulate in non-diabetic islets during ageing. *Immun. Ageing* 18, 8. <https://doi.org/10.1186/s12979-021-00221-4>.
- Dirice, E., De Jesus, D.F., Kahraman, S., Basile, G., Ng, R.W., El Ouaamari, A., Teo, A.K. K., Bhatt, S., Hu, J., Kulkarni, R.N., 2019. Human duct cells contribute to β cell compensation in insulin resistance. *JCI Insight* 4, e99576. <https://doi.org/10.1172/jci.insight.99576>.
- Ehrhardt, N., Cui, J., Dagdeviren, S., Saengnipantkul, S., Goodridge, H.S., Kim, J.K., Lantier, L., Guo, X., Chen, Y.-D.I., Raffel, L.J., Buchanan, T.A., Hsueh, W.A., Rotter, J.L., Goodarzi, M.O., Péterfy, M., 2019. Adiposity-independent effects of aging on insulin sensitivity and clearance in mice and humans. *Obes. (Silver Spring)* 27, 434–443. <https://doi.org/10.1002/oby.22418>.
- Elahi, D., Muller, D.C., McAloon-Dyke, M., Tobin, J.D., Andres, R., 1993. The effect of age on insulin response and glucose utilization during four hyperglycemic plateaus. *Exp. Gerontol.* 28, 393–409. [https://doi.org/10.1016/0531-5565\(93\)90066-m](https://doi.org/10.1016/0531-5565(93)90066-m).
- Engel, M., Arda, H.E., Mignardi, M., Beausang, J., Bottino, R., Kim, S.K., Quake, S.R., 2017. Single-cell analysis of human pancreas reveals transcriptional signatures of aging and somatic mutation patterns. *Cell* 171, 321–330. <https://doi.org/10.1016/j.cell.2017.09.004>.
- Fan, R., Kang, Z., He, L., Chan, J., Xu, G., 2011. Exendin-4 improves blood glucose control in both young and aging normal non-diabetic mice, possible contribution of beta cell independent effects. *PLoS One* 6, 1–9. <https://doi.org/10.1371/journal.pone.0020443>.
- Ferrannini, E., Vichi, S., Beck-Nielsen, H., Laakso, M., Paolisso, L., Smith, G., 1996. Insulin action and age: european group for the study of insulin resistance (EGIR). *Diabetes* 45, 947–953. <https://doi.org/10.2337/diab.45.7.947>.
- Flurkey, K., Currier, J.M., Harrison, D.E., 2007. Mouse models in aging research. In: Fox, J.G., Barthold, S.W., Davisson, M.T., Newcomer, C.E., Quimby, F.W., Smith, A. L. (Eds.), *The Mouse in Biomedical Research*, 2nd edition., Academic Press, Burlington, pp. 637–672.
- Fritsche, A., Madaus, A., Stefan, N., Tschrirter, O., Maerker, E., Teigeler, A., Häring, H., Stumvoll, M., 2002. Relationships among age, proinsulin conversion, and beta-cell function in nondiabetic humans. *Diabetes* 51 (Suppl 1), S234–S239. <https://doi.org/10.2337/diabetes.51.2007.s234>.
- Gasek, N.S., Kuchel, G.A., Kirkland, J.L., Xu, M., 2021. Strategies for targeting senescent cells in human disease. *Nat. Aging* 1, 870–879. <https://doi.org/10.1038/s43587-021-00121-8>.
- Geloneze, B., de Oliveira, Mda, S., Vasques, A.C., Novaes, F.S., Pareja, J.C., Tambascia, M.A., 2014. Impaired incretin secretion and pancreatic dysfunction with older age and diabetes. *Metabolism* 63, 922–929. <https://doi.org/10.1016/j.metabol.2014.04.004>.
- Gilon, P., 2020. The role of α -cells in islet function and glucose homeostasis in health and type 2 diabetes. *J. Mol. Biol.* <https://doi.org/10.1016/j.jmb.2020.01.004>.
- Gonzalez, A., Merino, B., Marroquí, L., Neco, P., Alonso-Magdalena, P., Caballero-Garrido, E., Vieira, E., Soriano, S., Gomis, R., Nadal, A., Quesada, I., 2013. Insulin hypersecretion in islets from diet-induced hyperinsulinemic obese female mice is associated with several functional adaptations in individual β -cells. *Endocrinology* 154, 3515–3524. <https://doi.org/10.1210/en.2013-1424>.
- González-Rodríguez, Á., Más-Gutiérrez, J.A., Mirasierra, M., Fernández-Pérez, A., Lee, Y. J., Ko, H.J., Kim, J.K., Romanos, E., Carrascosa, J.M., Ros, M., Vallejo, M., Rondinone, C.M., Valverde, Á.M., 2012. Essential role of protein tyrosine phosphatase 1B in obesity-induced inflammation and peripheral insulin resistance

- during aging. *Aging Cell* 11, 284–296. <https://doi.org/10.1111/j.1474-9726.2011.00786.x>.
- Gregg, B.E., Moore, P.C., Demozay, D., Hall, B.A., Li, M., Husain, A., Wright, A.J., Atkinson, M.A., Rhodes, C.J., 2012. Formation of a human β -cell population within pancreatic islets is set early in life. *J. Clin. Endocrinol. Metab.* 97, 3197–3206. <https://doi.org/10.1210/jc.2012-1206>.
- Gregg, T., Poudel, C., Schmidt, B.A., Dhillon, R.S., Sdao, S.M., Truchan, N.A., Baar, E.L., Fernandez, L.A., Denu, J.M., Eliceiri, K.W., Rogers, J.D., Kimple, M.E., Lamming, D. W., Merrins, M.J., 2016. Pancreatic β -cells from mice offset age-associated mitochondrial deficiency with reduced KATP channel activity. *Diabetes* 65, 2700–2710. <https://doi.org/10.2337/db16-0432>.
- Hagan, C., 2017. When Are Mice Considered Old? Jackson Labs. (<https://www.jax.org/news-and-insights/jax-blog/2017/november/when-are-mice-considered-old>).
- Hales, C.M., Carroll, M.D., Fryar, C.D., Ogden, C.L., 2020. Prevalence of obesity and severe obesity among adults: United States, 2017–2018. *NCHS Data Brief* 360, 1–8. (<https://www.cdc.gov/nchs/products/index.htm>).
- Hanley, S.C., Austin, E., Assouline-Thomas, B., Kapeluto, J., Blaichman, J., Moosavi, M., Petropavlovskaja, M., Rosenberg, L., 2010. Beta-Cell mass dynamics and islet cell plasticity in human type 2 diabetes. *Endocrinology* 151, 1462–1472. <https://doi.org/10.1210/en.2009-1277>.
- Helman, A., Klochendler, A., Azazmeh, N., Gabai, Y., Horwitz, E., Anzi, S., Swisa, A., Condiotti, R., Granit, R.Z., Nevo, Y., Fixler, Y., Shreibman, D., Zamir, A., Tornovsky-Babeay, S., Dai, C., Glaser, B., Powers, A.C., Shapiro, A.M.J., Magnuson, M.A., Dor, Y., Ben-Porath, I., 2016. p16Ink4a-induced senescence of pancreatic beta cells enhances insulin secretion. *Nat. Med.* 22, 412–420. <https://doi.org/10.1038/nm.4054>.
- Henquin, J.C., 2018. Influence of organ donor attributes and preparation characteristics on the dynamics of insulin secretion in isolated human islets. *Physiol. Rep.* 6, e13646. <https://doi.org/10.14814/phy2.13646>.
- Henquin, J.C., Rahier, J., 2011. Pancreatic alpha cell mass in European subjects with type 2 diabetes. *Diabetologia* 54, 1720–1725. <https://doi.org/10.1007/s00125-011-2118-4>.
- Henquin, J.C., Ibrahim, M.M., Rahier, J., 2017. Insulin, glucagon and somatostatin stores in the pancreas of subjects with type-2 diabetes and their lean and obese non-diabetic controls. *Sci. Rep.* 7, 11015. <https://doi.org/10.1038/s41598-017-10296-z>.
- Hodson, D.J., Mitchell, R.K., Bellomo, E.A., Sun, G., Vinet, L., Meda, P., Li, D., Li, W.-H., Bugliani, M., Marchetti, P., Bosco, D., Piemonti, L., Johnson, P., Hughes, S.J., Rutter, G.A., 2013. Lipotoxicity disrupts incretin-regulated human β cell connectivity. *J. Clin. Invest.* 123, 4182–4194. <https://doi.org/10.1172/JCI68459>.
- IDF, 2021. *International Diabetes Federation Diabetes Atlas*, 10th Ed. (<http://www.diabetesatlas.org>).
- Ihm, S.H., Matsumoto, I., Sawada, T., Nakano, M., Zhang, H.J., Ansit, J.D., Sutherland, D.E.R., Hering, B.J., 2006. Effect of donor age on function of isolated human islets. *Diabetes* 55, 1361–1368. <https://doi.org/10.2337/db05-1333>.
- Ihm, S.H., Moon, H.J., Kang, J.G., Park, C.Y., Oh, K.W., Jeong, I.K., Oh, Y.S., Park, S.W., 2007. Effect of aging on insulin secretory function and expression of beta cell function-related genes of islets. *Diabetes Res. Clin. Pract.* 77, 150–154. <https://doi.org/10.1016/j.diabres.2007.01.049>.
- Iozzo, P., Beck-Nielsen, H., Laakso, M., Smith, U., Yki-Järvinen, H., Ferrannini, E., 1999. Independent influence of age on basal insulin secretion in nondiabetic humans. *European Group for the Study of Insulin Resistance. J. Clin. Endocrinol. Metab.* 84, 863–868. <https://doi.org/10.1210/jcem.84.3.5542>.
- Jackson, S.J., Andrews, N., Ball, D., Bellantuono, I., Gray, J., Hachoumi, L., Holmes, A., Latham, J., Petrie, A., Potter, P., Rice, A., Ritchie, A., Stewart, M., Strepka, C., Yeoman, M., Chapman, K., 2017. Does age matter? The impact of rodent age on study outcomes. *Lab. Anim.* 51, 160–169. <https://doi.org/10.1177/0023677216653984>.
- Janjuha, S., Singh, S.P., Tsakmaki, A., Mousavy Gharavy, S.N., Murawala, P., Konantz, J., Birke, S., Hodson, D.J., Rutter, G.A., Bewick, G.A., Ninov, N., 2018. Age-related islet inflammation marks the proliferative decline of pancreatic beta-cells in zebrafish. *Elife* 7, e32965. <https://doi.org/10.7554/eLife.32965>.
- Johnston, N.R., Mitchell, R.K., Haythorne, E., Pessoa, M.P., Semplici, F., Ferrer, J., Piemonti, L., Marchetti, P., Bugliani, M., Bosco, D., Berishvili, E., Duncanson, P., Watkinson, M., Broichhagen, J., Trauner, D., Rutter, G.A., Hodson, D.J., 2016. Beta cell hubs dictate pancreatic islet responses to glucose. *Cell Metab.* 24, 389–401. <https://doi.org/10.1016/j.cmet.2016.06.020>.
- Kahn, S.E., Hull, R.L., Utzschneider, K.M., 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444, 840–846. <https://doi.org/10.1038/nature05482>.
- Kassem, S.A., Ariel, I., Thornton, P.S., Scheimberg, I., Glaser, B., 2000. β -Cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. *Diabetes* 49, 1325–1333. <https://doi.org/10.2337/diabetes.49.8.1325>.
- Kehm, R., König, J., Nowotny, K., Jung, T., Deubel, S., Gohlke, S., Schulz, T.J., Höhn, A., 2018. Age-related oxidative changes in pancreatic islets are predominantly located in the vascular system. *Redox Biol.* 15, 387–393. <https://doi.org/10.1016/j.redox.2017.12.015>.
- Khadra, A., Schnell, S., 2015. Development, growth and maintenance of β -cell mass: models are also part of the story. *Mol. Asp. Med.* 42, 78–90. <https://doi.org/10.1016/j.mam.2015.01.005>.
- Kong, Y., Sharma, R.B., Nwosu, B.U., Alonso, L.C., 2016. Islet biology, the CDKN2A/B locus and type 2 diabetes risk. *Diabetologia* 59, 1579–1593. <https://doi.org/10.1007/s00125-016-3967-7>.
- Kong, Y., Sharma, R.B., Ly, S., Stamateris, R.E., Jesdale, W.M., Alonso, L.C., 2018. CDKN2A/B T2D genome-wide association study risk SNPs impact locus gene expression and proliferation in human islets. *Diabetes* 67, 872–884. <https://doi.org/10.2337/db17-1055>.
- Krishnamurthy, J., Ramsey, M.R., Ligon, K.L., Torrice, C., Koh, A., Bonner-Weir, S., Sharpless, N.E., 2006. p16INK4a induces an age-dependent decline in islet regenerative potential. *Nature* 443, 453–457. <https://doi.org/10.1038/nature05092>.
- Lam, C.J., Cox, A.R., Jacobson, D.R., Rankin, M.M., Kushner, J.A., 2018. Highly proliferative α -cell-related islet endocrine cells in human pancreata. *Diabetes* 67, 674–686. <https://doi.org/10.2337/db17-1114>.
- Lam, C.J., Rankin, M.M., King, K.B., Wang, M.C., Shook, B.C., Kushner, J.A., 2019. Glucagon receptor antagonist-stimulated α -cell proliferation is severely restricted with advanced age. *Diabetes* 68, 963–974. <https://doi.org/10.2337/db18-1293>.
- Leiter, E.H., Premdas, F., Harrison, D.E., Lipson, L.G., 1988. Aging and glucose homeostasis in C57BL/6J male mice. *FASEB J.* 2, 2807–2811. <https://doi.org/10.1096/fasebj.2.12.3044905>.
- Levitt, H.E., Cyphert, T.J., Pascoe, J.L., Hollern, D.A., Abraham, N., Lundell, R.J., Rosa, T., Romano, L.C., Zou, B., O'Donnell, C.P., Stewart, A.F., Garcia-Ocaña, A., Alonso, L.C., 2011. Glucose stimulates human beta cell replication in vivo in islets transplanted into NOD-severe combined immunodeficiency (SCID) mice. *Diabetologia* 54, 572–582. <https://doi.org/10.1007/s00125-010-1919-1>.
- Li, L., Trifunovic, A., Kohler, M., Wang, Y., Petrovic Berglund, J., Illies, C., Juntti-Berggren, L., Larsson, N.-G., Berggren, P.-O., 2014. Defects in beta-cell Ca²⁺ dynamics in age-induced. *Diabetes* 63, 4100–4114. <https://doi.org/10.2337/db13-1855>.
- Li, J., Zheng, Y., Yan, P., Song, M., Wang, S., Sun, L., Liu, Z., Ma, S., Izpisua Belmonte, J. C., Chan, P., Zhou, Q., Zhang, W., Liu, G.H., Tang, F., Qu, J., 2021. A single-cell transcriptomic atlas of primate pancreatic islet aging. *Natl. Sci. Rev.* 8, nwaal127. <https://doi.org/10.1093/nsr/nwaa127>.
- Liew, C.W., Holman, A., Kulkarni, R.N., 2009. The roles of telomeres and telomerase in beta-cell regeneration. *Diabetes Obes. Metab.* 11 (Suppl 4), 21–29. <https://doi.org/10.1111/j.1463-1326.2009.01103.x>.
- Lyon, J., Manning Fox, J.E., Spiegelman, A.F., Kim, R., Smith, N., O'Gorman, D., Kin, T., Shapiro, A.M., Rajotte, R.V., MacDonald, P.E., 2016. Research-focused isolation of human islets from donors with and without diabetes at the alberta diabetes institute isletcore. *Endocrinology* 157, 560–569. <https://doi.org/10.1210/en.2015-1562>.
- Malenfant, J.H., Batsis, J.A., 2019. Obesity in the geriatric population - a global health perspective. *J. Glob. Health Rep.* 3, e2019045. <https://doi.org/10.29392/joghr.3.e2019045>.
- Meier, J.J., Butler, A.E., Saisho, Y., Monchamp, T., Galasso, R., Bhushan, A., Rizza, R.A., Butler, P.C., 2008. B-cell replication is the primary mechanism subserving the postnatal expansion of B-cell mass in humans. *Diabetes* 57, 1584–1594. <https://doi.org/10.2337/db07-1369>.
- Meier, J.J., Köhler, C.U., Alkhatib, B., Sergi, C., Junker, T., Klein, H.H., Schmidt, W.E., Fritsch, H., 2010. B-cell development and turnover during prenatal life in humans. *Eur. J. Endocrinol.* 162, 559–568. <https://doi.org/10.1530/EJE-09-1053>.
- Mezza, T., Muscogiuri, G., Sorice, G.P., Clemente, G., Hu, J., Pontecorvi, A., Holst, J.J., Giaccari, A., Kulkarni, R.N., 2014. Insulin resistance alters islet morphology in nondiabetic humans. *Diabetes* 63, 994–1007. <https://doi.org/10.2337/db13-1013>.
- Mezza, T., Cinti, F., Cefalo, C.M.A., Pontecorvi, A., Kulkarni, R.N., Giaccari, A., 2019. B-cell fate in human insulin resistance and type 2 diabetes: a perspective on islet plasticity. *Diabetes* 68, 1121–1129. <https://doi.org/10.2337/db18-0856>.
- Midha, A., Pan, H., Abarca, C., Andle, J., Carapeto, P., Bonner-Weir, S., Aguayo-Mazzucato, C., 2021. Unique human and mouse β -cell senescence-associated secretory phenotype (SASP) reveal conserved signaling pathways and heterogeneous factors. *Diabetes* 70, 1098–1116. <https://doi.org/10.2337/db20-0553>.
- Mihalidou, C., Chatzistamou, I., Papavassiliou, A.G., Kiaris, H., 2017. Modulation of pancreatic islets' function and survival during aging involves the differential regulation of endoplasmic reticulum stress by p21 and CHOP. *Antioxid. Redox Signal* 27, 185–200. <https://doi.org/10.1089/ars.2016.6671>.
- Mizukami, H., Takahashi, K., Inaba, W., Osonoi, S., Kamata, K., Tsuboi, K., Yagihashi, S., 2014. Age-associated changes of islet endocrine cells and the effects of body mass index in Japanese. *J. Diabetes Investig.* 5, 38–47. <https://doi.org/10.1111/jdi.12118>.
- Moin, A.S.M., Cory, M., Gurlo, T., Saisho, Y., Rizza, R.A., Butler, P.C., Butler, A.E., 2020. Pancreatic alpha-cell mass across adult human lifespan. *Eur. J. Endocrinol.* 182, 219–231. <https://doi.org/10.1530/EJE-19-0844>.
- Montanya, E., Nacher, V., Biarnes, M., Soler, J., 2000. Linear correlation between β -cell mass and body weight throughout the lifespan in Lewis rats. Role of β -cell hyperplasia and hypertrophy. *Diabetes* 49, 1341–1346. <https://doi.org/10.2337/diabetes.49.8.1341>.
- Moreno-Fernandez, M.E., Sharma, V., Stankiewicz, T.E., Oates, J.R., Doll, J.R., Damen, M.S.M.A., Almanan, M.A.T.A., Choungnet, C.A., Hildeman, D.A., Divanovic, S., 2021. Aging mitigates the severity of obesity-associated metabolic sequelae in a gender independent manner. *Nutr. Diabetes* 11, 15. <https://doi.org/10.1038/s41387-021-00157-0>.
- Morimoto, A., Tatsumi, Y., Deura, K., Mizuno, S., Ohno, Y., Miyamoto, N., Watanabe, S., 2013. Impact of impaired insulin secretion and insulin resistance on the incidence of type 2 diabetes mellitus in a Japanese population: The Saku study. *Diabetologia* 56, 1671–1679. <https://doi.org/10.1007/s00125-013-2932-y>.
- Murao, N., Yokoi, N., Takahashi, H., Hayami, T., Minami, Y., Seino, S., 2022. Increased glycolysis affects β -cell function and identity in aging and diabetes. *Mol. Metab.* 55, 101414. <https://doi.org/10.1016/j.molmet.2021.101414>.
- Ohn, J.H., Kwak, S.H., Cho, Y.M., Lim, S., Jang, H.C., Park, K.S., Cho, N.H., 2016. 10-year trajectory of β -cell function and insulin sensitivity in the development of type 2 diabetes: a community-based prospective cohort study. *Lancet Diabetes Endocrinol.* 4, 27–34. [https://doi.org/10.1016/S2213-8587\(15\)00336-8](https://doi.org/10.1016/S2213-8587(15)00336-8).

- Palmer, A.K., Gustafson, B., Kirkland, J.L., Smith, U., 2019. Cellular senescence: at the nexus between ageing and diabetes. *Diabetologia* 62, 1835–1841. <https://doi.org/10.1007/s00125-019-4934-x>.
- Palmer, A.K., Tchkonja, T., Kirkland, J.L., 2021. Senolytics: potential for alleviating diabetes and its complications. *Endocrinology* 162. <https://doi.org/10.1210/endoqr/bqab058>.
- Pann, P., de Angelis, M.H., Prehn, C., Adamski, J., 2020. Mouse age matters: how age affects the murine plasma metabolome. *Metabolites* 10, 472. <https://doi.org/10.3390/metabo10110472>.
- Perl, S.Y., Kushner, J.A., Buchholz, B.A., Meeker, A.K., Stein, G.M., Hsieh, M., Kirby, M., Pechhold, S., Liu, E.H., Harlan, D.M., Tisdale, J.F., 2010. Significant human β -cell turnover is limited to the first three decades of life as determined by in vivo thymidine analog incorporation and radiocarbon dating. *J. Clin. Endocrinol. Metab.* 95, 234–239. <https://doi.org/10.1210/jc.2010-0932>.
- Petersen, K.F., Morino, K., Alves, T.C., Kibbey, R.G., Dufour, S., Sono, S., Yoo, P.S., Cline, G.W., Shulman, G.I., 2015. Effect of aging on muscle mitochondrial substrate utilization in humans. *Proc. Natl. Acad. Sci. U. S. A* 112, 11330–11334. <https://doi.org/10.1073/pnas.1514844112>.
- Rahli, J., Guiot, Y., Goebbels, R.M., Sempoux, C., Henquin, J.C., 2008. Pancreatic beta-cell mass in European subjects with type 2 diabetes. *Diabetes Obes. Metab.* 10 (Suppl 4), 32–42. <https://doi.org/10.1111/j.1463-1326.2008.00969.x>.
- Ranganath, L., Sedgwick, I., Morgan, L., Wright, J., Marks, V., 1998. The ageing entero-insular axis. *Diabetologia* 41, 1309–1313. <https://doi.org/10.1007/s001250051070>.
- Rankin, M.M., Kushner, J.A., 2009. Adaptive β -cell proliferation is severely restricted with advanced age. *Diabetes* 58, 1365–1372. <https://doi.org/10.2337/db08-1198>.
- Reers, C., Erbel, S., Esposito, I., Schmied, B., Büchler, M.W., Nawroth, P.P., Ritzel, R.A., 2009. Impaired insulin turnover in human donor pancreata with aging. *Eur. J. Endocrinol.* 160, 185–191. <https://doi.org/10.1530/EJE-08-0596>.
- Röder, M.E., Schwartz, R.S., Prigeon, R.L., Kahn, S.E., 2000. Reduced pancreatic B cell compensation to the insulin resistance of aging: impact on proinsulin and insulin levels. *J. Clin. Endocrinol. Metab.* 85, 2275–2280. <https://doi.org/10.1210/jcem.85.6.6635>.
- Rorsman, P., Ashcroft, F.M., 2018. Pancreatic β -cell electrical activity and insulin secretion: of mice and men. *Physiol. Rev.* 98, 117–214. <https://doi.org/10.1152/physrev.00008.2017>.
- Rorsman, P., Huisling, M.O., 2018. The somatostatin-secreting pancreatic δ -cell in health and disease. *Nat. Rev. Endocrinol.* 14, 404–414. <https://doi.org/10.1038/s41574-018-0020-6>.
- Rowe, J.W., Minaker, K.L., Pallotta, J.A., Flier, J.S., 1983. Characterization of the insulin resistance of aging. *J. Clin. Invest.* 71, 1581–1587. <https://doi.org/10.1172/JCI110914>.
- Saisho, Y., Butler, A.E., Manesso, E., Elashoff, D., Rizza, R.A., Butler, P.C., 2013. β -Cell mass and turnover in humans: effects of obesity and aging. *Diabetes Care* 36, 111–117. <https://doi.org/10.2337/dc12-0421>.
- Salpeter, S.J., Khalalileh, A., Weinberg-Corem, N., Ziv, O., Glaser, B., Dor, Y., 2013. Systemic regulation of the age-related decline of pancreatic β -cell replication. *Diabetes* 62, 2843–2848. <https://doi.org/10.2337/db13-0160>.
- Santulli, G., Lombardi, A., Sorriento, D., Anastasio, A., Del Giudice, C., Formisano, P., Béguinot, F., Trimarco, B., Miele, C., Iaccarino, G., 2012. Age-related impairment in insulin release: the essential role of $\beta(2)$ -adrenergic receptor. *Diabetes* 61, 692–701. <https://doi.org/10.2337/db11-1027>.
- Saxena, R., Voight, B.F., Lyssenko, V., Burt, N.P., de Bakker, P.I., Chen, H., Roix, J.J., Kathiresan, S., Hirschhorn, J.N., et al., 2007. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316, 1331–1336. <https://doi.org/10.1126/science.1142358>.
- Scheen, A.J., 2005. Diabetes mellitus in the elderly: insulin resistance and/or impaired insulin secretion? *Diabetes Metab.* 31, 5S27–5S34. [https://doi.org/10.1016/s1262-3636\(05\)73649-1](https://doi.org/10.1016/s1262-3636(05)73649-1).
- Shimokata, H., Muller, D.C., Fleg, J.L., Sorkin, J., Ziemba, A.W., Andres, R., 1991. Age as independent determinant of glucose tolerance. *Diabetes* 40, 44–51. <https://doi.org/10.2337/diab.40.1.44>.
- Solar, M., Cardalda, C., Houbracken, I., Martín, M., Maestro, M.A., De Medts, N., Xu, X., Grau, V., Heimberg, H., Bouwens, L., Ferrer, J., 2009. Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. *Dev. Cell.* 17, 849–860. <https://doi.org/10.1016/j.devcel.2009.11.003>.
- Sone, H., Kagawa, Y., 2005. Pancreatic beta cell senescence contributes to the pathogenesis of type 2 diabetes in high-fat diet-induced diabetic mice. *Diabetologia* 48, 58–67. <https://doi.org/10.1007/s00125-004-1605-2>.
- Stolovich-Rain, M., Hija, A., Grimsby, J., Glaser, B., Dor, Y., 2012. Pancreatic beta cells in very old mice retain capacity for compensatory proliferation. *J. Biol. Chem.* 287, 27407–27414. <https://doi.org/10.1074/jbc.M112.350736>.
- Szoke, E., Shrayyef, M.Z., Messing, S., Woerle, H.J., van Haefen, T.W., Meyer, C., Mitrakou, A., Pimenta, W., Gerich, J.E., 2008. Effect of aging on glucose homeostasis. *Diabetes Care* 31, 539–543. <https://doi.org/10.2337/dc07-1443>.
- Abbreviations.**
- Talchai, C., Xuan, S., Lin, H.V., Sussel, L., Accili, D., 2012. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. *Cell* 150, 1223–1234. <https://doi.org/10.1016/j.cell.2012.07.029>.
- Tamura, Y., Izumiya-Shimomura, N., Kimbara, Y., Nakamura, K. ichi, Ishikawa, N., Aida, J., Chiba, Y., Matsuda, Y., Mori, S., Arai, T., Fujiwara, M., Poon, S.S.S., Ishizaki, T., Araki, A., Takubo, K., Ito, H., 2016. Telomere attrition in beta and alpha cells with age. *Age* 38. <https://doi.org/10.1007/s11357-016-9923-0>.
- Tian, L., Gao, J., Weng, G., Yi, H., Tian, B., Brien, T.D.O., 2011. Comparison of exendin-4 on beta-cell replication in mouse and human islet grafts. *Transpl. Int* 24, 856–864. <https://doi.org/10.1111/j.1432-2277.2011.01275.x>.
- Tong, X., Dai, C., Walker, J.T., Nair, G.G., Kennedy, A., Carr, R.M., Hebrok, M., Powers, A.C., Stein, R., 2020. Lipid droplet accumulation in human pancreatic islets is dependent on both donor age and health. *Diabetes* 69, 342–354. <https://doi.org/10.2337/db19-0281>.
- Tudurí, E., Soriano, S., Almagro, L., García-Heredia, A., Rafacho, A., Alonso-Magdalena, P., Nadal, Á., Quesada, I., 2022. The effects of aging on male mouse pancreatic β -cell function involve multiple events in the regulation of secretion: influence of insulin sensitivity. *J. Gerontol. A Biol. Sci. Med. Sci.* 77, 405–415. <https://doi.org/10.1093/gerona/glab276>.
- Walker, J.T., Saunders, D.C., Brissova, M., Powers, A.C., 2021. The human islet: mini-organ with mega-impact. *Endocr. Rev.* 42, 605–657. <https://doi.org/10.1210/endoevr/bnab010>.
- Wang, Y.J., Golson, M.L., Schug, J., Traum, D., Liu, C., Vivek, K., Dorrell, C., Naji, A., Powers, A.C., Chang, K.-M., Grompe, M., Kaestner, K.H., 2016. Single-cell mass cytometry analysis of the human endocrine pancreas. *Cell Metab.* 24, 616–626. <https://doi.org/10.1016/j.cmet.2016.09.007>.
- Westacott, M.J., Farnsworth St, N.L., Clair, J.R., Poffenberger, G., Heintz, A., Ludin, N. W., Hart, N.J., Powers, A.C., Benninger, R.K.P., 2017. Age-dependent decline in the coordinated $[Ca^{2+}]$ and insulin secretory dynamics in human pancreatic islets. *Diabetes* 66, 2436–2445. <https://doi.org/10.2337/db17-0137>.
- Wierstra, I., Alves, J., 2007. FOXM1, a typical proliferation-associated transcription factor. *Biol. Chem.* 388, 1257–1274. <https://doi.org/10.1515/BC.2007.159>.
- Wong, E.S., Le Guezennec, X., Demidov, O.N., Marshall, N.T., Wang, S.T., Krishnamurthy, J., Sharpless, N.E., Dunn, N.R., Bulavin, D.V., 2009. p38MAPK controls expression of multiple cell cycle inhibitors and islet proliferation with advancing age. *Dev. Cell.* 17, 142–149. <https://doi.org/10.1016/j.devcel.2009.05.009>.
- Xin, Y., Okamoto, H., Kim, J., Ni, M., Adler, C., Cavino, K., Na, E., Murphy, A.J., Yancopoulos, G.D., Lin, C., Gromada, J., 2016. Single-Cell RNAseq reveals that pancreatic β -cells from very old male mice have a young gene signature. *Endocrinology* 157, 3431–3438. <https://doi.org/10.1210/en.2016-1235>.
- Xiong, Y., Yepuri, G., Necetin, S., Montani, J.P., Ming, X.F., Yang, Z., 2017. Arginase-II promotes tumor necrosis factor- α release from pancreatic acinar cells causing β -cell apoptosis in aging. *Diabetes* 66, 1636–1649. <https://doi.org/10.2337/db16-1190>.
- Xu, X., D'Hoker, J., Stangé, G., Bonné, S., De Leu, N., Xiao, X., Van de Castele, M., Mellitzer, G., Ling, Z., Pipeleers, D., Bouwens, L., Scharfmann, R., Gradwohl, G., Heimberg, H., 2008. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* 132, 197–207. <https://doi.org/10.1016/j.cell.2007.12.015>.
- Yoneda, S., Uno, S., Iwahashi, H., Fujita, Y., Yoshikawa, A., Kozawa, J., Okita, K., Takiuchi, D., Eguchi, H., Nagano, H., Imagawa, A., Shimomura, I., 2013. Predominance of β -cell neogenesis rather than replication in humans with an impaired glucose tolerance and newly diagnosed diabetes. *J. Clin. Endocrinol. Metab.* 98, 2053–2061. <https://doi.org/10.1210/jc.2012-3832>.
- Yoshino, J., Mills, K.F., Yoon, M.J., Imai, S., 2011. Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. *Cell Metab.* 14, 528–536. <https://doi.org/10.1016/j.cmet.2011.08.014>.