

Glucose-induced insulin secretion in isolated human islets: Does it truly reflect β -cell function in vivo?



Jean-Claude Henquin

ABSTRACT

Background: Diabetes always involves variable degrees of β -cell demise and malfunction leading to insufficient insulin secretion. Besides clinical investigations, many research projects used rodent islets to study various facets of β -cell pathophysiology. Their important contributions laid the foundations of steadily increasing numbers of experimental studies resorting to isolated human islets.

Scope of review: This review, based on an analysis of data published over 60 years of clinical investigations and results of more recent studies in isolated islets, addresses a question of translational nature. Does the information obtained in vitro with human islets fit with our knowledge of insulin secretion in man? The aims are not to discuss specificities of pathways controlling secretion but to compare qualitative and quantitative features of glucose-induced insulin secretion in isolated human islets and in living human subjects.

Major conclusions: Much of the information gathered in vitro can reliably be translated to the in vivo situation. There is a fairly good, though not complete, qualitative and quantitative coherence between insulin secretion rates measured in vivo and in vitro during stimulation with physiological glucose concentrations, but the concordance fades out under extreme conditions. Perplexing discrepancies also exist between insulin secretion in subjects with Type 2 diabetes and their islets studied in vitro, in particular concerning the kinetics. Future projects should ascertain that the experimental conditions are close to physiological and do not alter the function of normal and diabetic islets.

© 2021 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords Insulin secretion; Human islets; Diabetes; β -cells; Plasma insulin; Glucose homeostasis

1. INTRODUCTION

Perturbations of insulin production by pancreatic β -cells markedly impact metabolic homeostasis. In large excess, insulin causes acute life-threatening hypoglycemia, whereas chronic hypersecretion is a risk factor for progressive development of metabolic dysfunction. Insufficient secretion ineluctably leads to diabetes, the prevalence of which is high and steadily increasing, particularly that of Type 2 diabetes (T2D). Deciphering the mechanisms regulating β -cell secretory function is expected to improve treatment and prevention of these diseases.

The study of the mechanisms controlling insulin secretion began 60 years ago with the development of radioimmunoassays [1]. Initial investigations were performed in humans until techniques were devised to study the endocrine pancreas in vitro. Many laboratories using rat and mouse islets as models have contributed to dissect the stimulus-secretion coupling in β -cells. Several learned reviews have been devoted to specific aspects of that coupling [2–13]. Studies in rodent islets also laid the foundations of understanding the functioning of human β -cells, which is the ultimate goal of that scientific undertaking. In spite of many phenotypic and mechanistic similarities, substantial differences have been identified between species and discussed in topical articles [3,9,10,14,15]. The field keeps

progressing, but it is premature to add yet another contribution to these comparisons.

The present review was written in a different perspective. It is based on a critical, quantitative, and qualitative analysis of the steadily increasing number of studies reporting insulin secretion in isolated human islets. These reports are compared to results of clinical investigations of insulin secretion in humans. The aims are not to discuss species specificities of stimulus-secretion coupling but to examine whether in vitro responses of human islets to glucose, by far their major and most extensively used stimulus, can reliably be translated to the in vivo situation. Except for general reviews, cited references exclusively correspond to original articles providing the actual evidence based on human subjects or islets.

2. MEASUREMENTS OF INSULIN SECRETION IN VIVO AND IN VITRO

2.1. Insulin secretion in vivo

In the first in vivo studies, only peripheral plasma insulin was measured. However, changes in its concentration imperfectly reflect changes in secretion because of insulin clearance in the liver (~50% during the first pass) and peripheral insulin-sensitive tissues [16]. The process varies between individuals and saturates with the duration and

Unit of Endocrinology and Metabolism, Faculty of Medicine, University of Louvain, Brussels, Belgium

E-mail: jean-claude.henquin@uclouvain.be.

Received February 2, 2021 • Revision received March 3, 2021 • Accepted March 9, 2021 • Available online 15 March 2021

<https://doi.org/10.1016/j.molmet.2021.101212>

amplitude of hyperinsulinemia [17]. In vivo, insulin secretion rates (ISR) must be determined indirectly by mathematical analyses such as deconvolution of changes in plasma C-peptide concentration. Under certain stimulatory conditions, increases in plasma insulin may be 2–3 times greater than actual secretion changes [18]. Calculations of ISR are essential for quantitative comparisons. Whenever possible, studies reporting ISR will be referred to rather than those reporting only plasma insulin or C-peptide concentrations. It is also worth emphasizing that only controlled administration of a substance through the intravenous route permits correct evaluation of its effects in β -cells and comparison with responses obtained in isolated islets. Clinical studies based on oral administration of glucose will not be taken into consideration because the stimulation of insulin secretion is then affected by the concomitant secretion of intestinal incretin hormones.

2.2. Insulin secretion in vitro

β -cells reside within the islets of Langerhans scattered throughout the exocrine pancreas. The pancreas of a healthy adult contains on average 1.65 million islets that make up about 1.5% of the organ volume. These islets vary considerably in size and, after isolation, are commonly quantified by normalization to 150- μ m diameter spherical structures (islet equivalent). Such a theoretical islet equivalent contains about 12–14 ng of insulin, for a total of \sim 10.5 mg of insulin in the entire pancreas [19]. Theoretically, the most physiological preparation to study insulin secretion in vitro would be the intact perfused pancreas; however, its major drawbacks are that the number of testable conditions is limited and parallel biochemical measurements are impossible. It has been used by only one group until the early 2000's [20]. A novel technique of superfusion of slices of pancreas shows promising results [21].

Islets can be isolated from the pancreas of organ donors and dispatched to research laboratories for experimental studies. Only exceptional results obtained with freshly-isolated islets have been reported [22]. Culture for a few days is inevitable owing to islet transportation and is often recommended to permit recovery from the trauma of isolation; however, the risk of inducing functional changes should not be overlooked. In vitro studies of insulin secretion are fraught with difficulties and limitations. First, the characteristics of tested islets, such as purity and size, are variable and can influence the results as reviewed recently [23]. Second, because of their limited availability, human islets are often incubated or perfused in small numbers that may not be representative of the entire islet population [24]. Third, all solutions used in vitro substantially differ from plasma. Notably, glucose is most often tested at traditional but non-physiological concentrations, and in the absence of other agents (fuels, hormones, and neurotransmitters) whose influence on insulin secretion by human islets would deserve a closer look. No study ever measured secretion in islets bathed in a medium approaching the plasma composition. These caveats are not meant to invalidate results obtained under conditions sometimes imposed by technical constraints and largely accepted by the scientific community, but to inform the reader that comparisons between in vitro and in vivo studies of insulin secretion are not straightforward.

Quantitative comparisons of insulin secretion data reported by distinct laboratories are also complicated by inconsistent modes of expression [19]. The only useable *common* unit is the “stimulation index” (SI = ratio of insulin secretion in high and low glucose) that is either published or can be recalculated from data presented otherwise. It is only recently that the impact of clinical attributes of the donors on islet function has been taken into consideration [23,25] (see Chapter 5) and

that systematic report of these attributes has been recommended [15]. However, some metabolic features of donors, such as insulin resistance, may also contribute to the heterogeneity of in vitro responses of islets but cannot readily be accounted for.

3. THE TWO PATHWAYS OF GLUCOSE-STIMULATION

The current consensus, based on countless studies in rodent β -cells, attributes glucose-induced insulin secretion to activation of two main pathways: the triggering and amplifying pathways [2]. A few studies have directly verified that the essential features of the model established in rodents also apply to human β -cells (Figure 1).

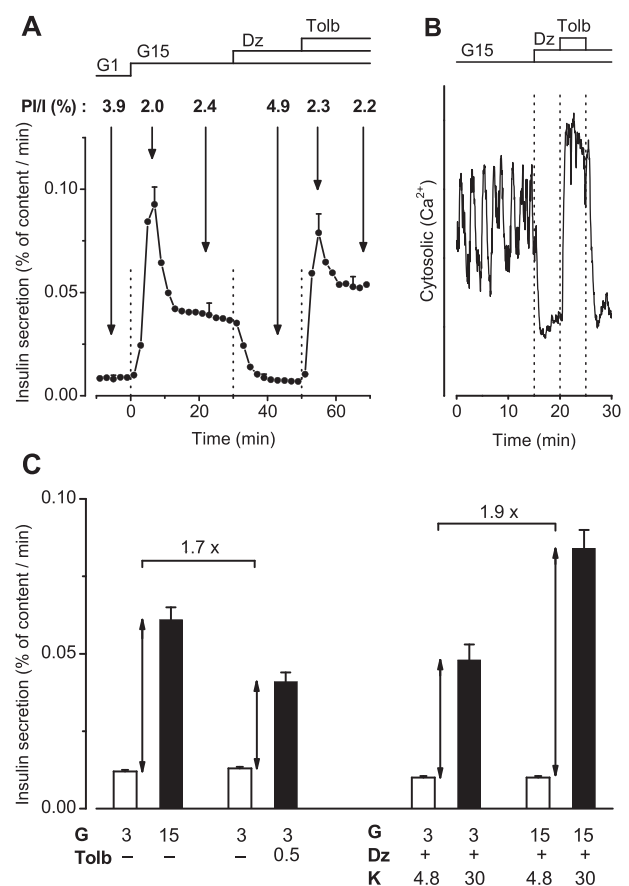


Figure 1: Glucose-induced insulin secretion in human islets is controlled by triggering (A and B) and amplifying (C) pathways. A: Insulin secretion in perfused human islets was stimulated by increasing glucose from 1 mM (G1) to 15 mM (G15). The stimulation was abolished by opening β -cell K_{ATP} channels with diazoxide (Dz 100 μ M) and this inhibition was reversed by closing the channels with tolbutamide (Tolb 100 μ M). At times indicated by vertical arrows, proinsulin was also measured in the effluent and the ratio Proinsulin/Insulin (PI/I) is shown at the top. Values are presented as means \pm standard error of the mean for seven islet preparations. B: Measurements of islet $[Ca^{2+}]_c$ show that the changes in secretion produced by diazoxide and tolbutamide are secondary to changes in the triggering Ca^{2+} signal. C: Schematic illustration of the amplifying pathway. Left panel: stimulation with G15 induced a 1.7-fold greater insulin response (above baseline) than complete closure of K_{ATP} channels with a saturating concentration of tolbutamide (0.5 mM) in G3. Right panel: In the presence of diazoxide, depolarization with 30 mM KCl induced a 1.9-fold greater insulin response in G15 than G3. Results were computed from islet perfusions but similar ones would be provided by incubations. Values are presented as means \pm standard error of the mean for five islet preparations tested in parallel. Data are taken from [37] (A) and [36] (C).

3.1. The triggering pathway

When islets are exposed to low concentrations of glucose (<3 mM), the rate of metabolism in β -cells is slow, and enough K_{ATP} channels are open in the plasma membrane to keep the cells hyperpolarized. The influx of Ca^{2+} is minimal, the concentration of cytosolic Ca^{2+} ($[Ca^{2+}]_c$) is low, and insulin secretion is basal [10,26]. When the concentration of glucose increases, islet cell metabolism accelerates [27–29], and the ratio of cytosolic ATP to ADP augments in a concentration-dependent manner [29,30], which closes K_{ATP} channels in the β -cell membrane [10,31]. The resulting depolarization is followed by opening of several types of voltage-dependent calcium channels which distinctly contribute to acceleration of Ca^{2+} influx into the cell [32]. The ensuing increase in $[Ca^{2+}]_c$ [26,33,34] then activates an effector system that promotes exocytosis of insulin-containing granules. Blockage of L-type calcium channels by micromolar concentrations of dihydropyridines abrogates glucose-induced insulin secretion [35,36]. The key role of K_{ATP} channels to produce the triggering Ca^{2+} signal can be demonstrated with two drugs [37]. Through direct opening of K_{ATP} channels, diazoxide inhibits the effects of glucose on membrane potential, Ca^{2+} influx, $[Ca^{2+}]_c$, and insulin secretion. Conversely, tolbutamide, a sulfonylurea that closes K_{ATP} channels independently of changes in metabolism, reverses these inhibitions (Figure 1A,B). Extensive discussions of this triggering pathway can be found in other reviews [3,5,10,14].

Operation of the K_{ATP} channel-dependent triggering pathway in vivo was first attested by the ability of sulfonylureas and diazoxide to respectively increase and decrease circulating insulin levels. It was further supported by genetic studies showing that inactivating mutations of the channel cause congenital hyperinsulinism [38,39], whereas activating mutations cause insulin-deficient neonatal diabetes [40]. On the other hand, the inhibition of insulin secretion by calcium channel antagonists in vitro is not observed in vivo because their concentration in the plasma of treated patients is at least two orders of magnitude lower [41].

3.2. The amplifying pathway

Generation of the triggering signal is indispensable for the increase in insulin secretion. However, Ca^{2+} would not be fully effective without operation of an amplifying pathway that does not further raise $[Ca^{2+}]_c$ but augments its effectiveness on exocytosis. When islets are stimulated by 15 mM glucose (G15) or by 500 μ M tolbutamide in 3 mM glucose (G3), insulin secretion is greater in response to high glucose (Figure 1C) although tolbutamide is known to induce a greater rise in $[Ca^{2+}]_c$ [26,34]. In the presence of diazoxide insulin secretion is low and similar in G3 and G15, but depolarization with 30 mM KCl induces more insulin secretion in G15 than G3 (Figure 1C) [36,42,43], although the rise in $[Ca^{2+}]_c$ induced by KCl is not augmented by high glucose [44]. The amplifying action of glucose, which also depends on its metabolism, thus augments insulin secretion in response to a given triggering Ca^{2+} signal. It may account for up to 40–50% of the global response. An extensive discussion of the biochemical events implicated in this still incompletely understood pathway can be found in other reviews [2,7,13,45,46].

Direct demonstration that the amplifying pathway contributes to glucose-induced insulin secretion in vivo is still missing because we are currently unable to modulate it selectively in living subjects. Early human studies of insulin secretion distinguished initiating and potentiating actions of glucose [47,48]. The greater response to non-glucose stimuli at elevated blood glucose levels has been attributed to a potentiating action of the sugar, which becomes defective in subjects with T2D [49]. The amplifying action of glucose is most likely

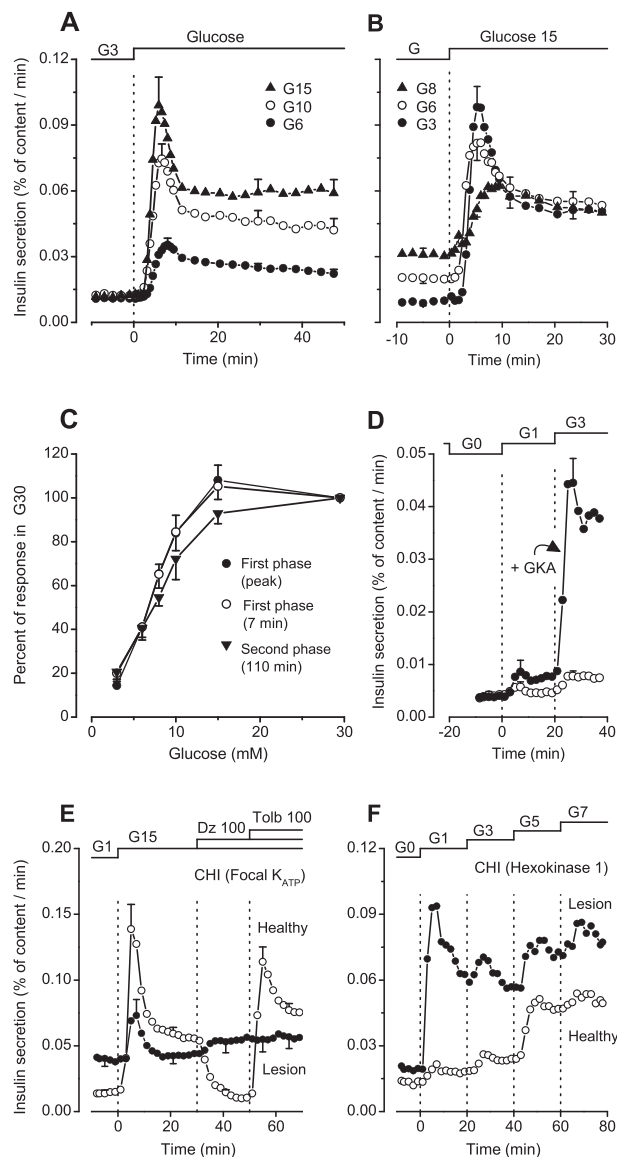


Figure 2: Biphasic dynamics and concentration-dependency of glucose-induced insulin secretion in perfused human islets. A: The concentration of glucose was increased from 3 mM (G3) to G6, G10 or G15 as indicated by different symbols. B: The prestimulatory glucose concentration influences the first phase of the response to high glucose. Islets were initially perfused with G3, G6, or G8 for 60 min before being stimulated with G15. C: Concentration-dependency curves calculated from experiments like those shown in A. Results are expressed as a percentage of responses to G30. D: Threshold of glucose-induced insulin secretion. The increase in secretion already observed at G3 is markedly augmented by a glucokinase activator (GKA). In each panel, all experiments were done in parallel with islets from the same preparations. Values are presented as means \pm standard error of the mean for seven to nine preparations. Data are taken from [37] (A, B and C) and [36] (D). E and F: Abnormal insulin secretion in congenital hyperinsulinism (CHI). E: Insulin secretion by focal lesions containing β -cells with inactivating mutations of K_{ATP} channels is compared to secretion by adjacent healthy pancreases from the same 10 patients (2.5–14 months old). F: Insulin secretion by pancreatic lobules containing β -cells expressing hexokinase-1 is compared to secretion by the adjacent healthy pancreas from the same patient (21 months old). All experiments were done with fragments of tissue. Data are taken from [166] (E) and [112] (F).

implicated in this potentiation, even though changes in the triggering Ca^{2+} signal may also contribute. Interestingly, mathematical modeling of insulin secretion in humans recently incorporated the amplifying pathway and concluded that it is impaired in subjects with T2D [50].

4. CHARACTERISTICS OF GLUCOSE-INDUCED INSULIN SECRETION

4.1. Kinetics

Physiologically, when glucose is absorbed from the gut, its concentration in blood gradually rises and the corresponding increase in ISR is progressive, following a monophasic time-course [51]. This increase results from the combined action of glucose and intestinal hormones in β -cells. When a similar gradual rise in blood glucose is achieved by intravenous infusion (thus avoiding intestinal signals), ISR also increases progressively and monotonically [52,53]. Only one laboratory used a ramp increase in glucose to stimulate perfused islets and showed that insulin secretion is indeed progressive under these conditions [29,54].

Experimentally, when blood glucose is rapidly raised by an intravenous bolus and subsequently maintained at a steady plateau by sustained infusion (hyperglycemic clamp), the rise in plasma insulin displays two phases: a short first phase peaks after 2–4 min and is followed by a nadir before development of an ascending second phase that lasts as long as glucose is administered [55–58]. The first phase is also commonly studied after a single bolus of glucose (intravenous glucose tolerance test) [59]. Calculation of ISR during hyperglycemic clamps shows that its increase follows a biphasic time-course. Notably, unlike plasma insulin levels, ISR usually remains fairly stable during the second phase when blood glucose is clamped at 6–11 mM [17,57,60,61] but becomes slowly ascending during clamps at higher

glucose levels, particularly in obese subjects [17,62,63]. Although blood glucose concentrations never increase rapidly enough to induce biphasic insulin secretion in real life, this peculiar kinetics is regarded as the most sensitive expression of adequate β -cell function [58]. Impairment of the first phase is an early marker of β -cell dysfunction in the development of type 2 diabetes [55,59,64,65]. That explains the interest paid to the biphasic pattern of glucose-induced insulin secretion in clinical and experimental investigations.

In vitro, biphasic insulin secretion was observed in the first studies testing human islets in dynamic perfusion systems [22,66], and has since been reported by many laboratories. Figure 2A illustrates such a response in islets challenged by a jump from G3 to G6, G10, or G15. A first phase lasting about 7 min was followed by a stable plateau. A non-ascending second phase was consistently observed (also in G20 and G30), not only with isolated islets, but also when islets were still embedded in the exocrine tissue, either in the perfused pancreas [67] or in superfused slices of the organ [21]. Only one exception was found in the literature: islets challenged with G27 immediately after isolation [22].

During stimulation with either G10 or G15 (Figure 2A), insulin secretion was ~ 1.9 -fold higher at the peak of the first phase than during the plateau of the second phase, a ratio that is consistent between laboratories (Table 1). Provided the sampling rate is high enough (fractions collected every minute or less), the ratio ranges between 1.4 and 2.2 for stimulations from G3 to G10–G11 [37,43,68,69], and between 1.3 and 2.5 for stimulations from G3 to G15–G17 [37,69–74]. The two

Table 1 — Dynamics of glucose-induced insulin secretion in perfused human islets.

Refs.	First author and year	Donors (n)	Stimulation		Sampling 1st phase	Ratio 1st/2nd	Stimulation index	
			min	Delta G			1st phase	2nd phase
[37]	Henquin, 2015	8	120	G3-G10	0.7 min	1.9	6.8	3.6
[68]	Schwede, 2015	6	40	G3-G11	0.5 min	2.2	17	7.8
[69]	Alcazar, 2019	34	20	G3-G11	1 min	1.4	5.5	3.8
[43]	Capozzi, 2019	3	20	G3-G10	1 min	2.2	8.3	3.7
Mean						1.93	9.40	4.73
Median						2.05	7.55	3.75
[70]	Davalli, 1991	7	40	G3-G17	1 min	1.4	4.9	3.4
[71]	Bertuzzi, 1998	3	20	G3-G17	1 min	2.5	10	4
[37]	Henquin, 2015	8	120	G3-G15	0.7 min	1.9	8.8	4.7
[72]	Dolai, 2016	4	35	G3-G17	1 min	2	13	6.5
[73]	Kelly, 2019	8	40	G3-G17	1 min	2	8	4
[69]	Alcazar, 2019	9	20	G3-G17	1 min	1.3	10.3	8.4
[74]	Yu, 2020	6	30	G3-G17	1 min	2	15	7.4
Mean						1.87	10.0	5.49
Median						2.00	10.0	4.70
[22]	Warnock, 1988	17	30	G3-G30	2 min	1.8	6.8	3.8
[75]	Squires, 2000	3	20	G2-G20	2 min	1.3	7.2	5.5
[76]	Lehmann, 2007	29	60	G3-G20	2 min	1.2	4.2	3.5
[77]	Zhao, 2007	12	24	G2-G20	2 min	1.6	5.2	3.2
[78]	Johnson, 2009	15	40	G3-G20	2.5 min	1.2	5	4.2
[79]	Butcher, 2014	5	20	G3-G23	2 min	2.4	11	4.3
[80]	Krogvold, 2015	15	48	G2-G20	6 min	1.0	16	16
[81]	Roomp, 2017	6	20	G3-G20	2 min	1.4	6.9	5
[82]	Zuellig, 2017	4	60	G3-G20	3 min	1.4	8.5	6
[83]	Nagao, 2020	49	28	G2-G20	4 min	1.0	4.5	4.3
Mean						1.43	7.53	5.58
Median						1.35	6.85	4.30

Glucose (G) concentrations in mM have been rounded to make the presentation simpler: G2.8 = G3; G16.7 = G17.

Ratios of the 1st/2nd phases were calculated between peak insulin secretion rates (ISR) for the first phase and average ISR for the second phase.

Stimulation index was calculated between peak ISR for the first phase or average ISR for the second phase and average prestimulatory ISR.

(n) = number of islet preparations from distinct donors.

Table 2 — Concentration-dependency of glucose-induced insulin secretion in vivo.

Refs.	First author and year	Method	Subjects (n)	BMI	Insulin Secretion Rate (pmol per min)				
					G5	G6	G8	G10	G14/G16
[85]	Toschi, 2002	Steps	13	32.9	150	230	450	620	840
[99]	Jones, 2000	Graded	38	27.5	160	255	465		
[100]	Brandt, 2001	Graded	10	23.5	120	210	360	500	850
[101]	Wang, 2018	Graded	8	30.4	200	320	490	650	850
[52]	Chang, 2006	Ramp	31	25.5	125	210	430	650	
[53]	Seghieri, 2016	Ramp	13	24.5	120	200	465	550	950
[57]	Natali, 1998	Clamp	15	23.5	175		450		
[102]	Fritsche, 2001	Clamp	27	25.1	110			700	
[103]	Stefan, 2001	Clamp	7	22.2			425		
Mean					145	238	442	612	873
Mean as % of insulin stores per min					0.008	0.014	0.025	0.035	0.050

BMI, Body mass index.

Intravenous infusion of glucose was controlled by the indicated method. Insulin secretion rates measured at the indicated blood glucose levels (G in mM) are expressed as pmol/min, which sometimes required recalculation from data published differently (eg as pmol/min/kg). Values are best estimates read from Figures in the original publications. Means expressed as % of insulin stores were calculated on the basis of 1750 nmol insulin per pancreas [19].

phases are less readily distinguished when sampling rates are lower [22,75–83] (Table 1). The relative amount of insulin secreted during the first phase obviously depends on the periods of integration. For a stimulation lasting 1 h, the first phase does not exceed 20% of total secretion.

It is noteworthy that the first phase of the response to high glucose is acutely influenced by the pre-stimulatory glucose concentration (Figure 2B). Starting from G6 instead of G3 slightly shortened the delay of its onset but reduced its amplitude, and starting from G8 virtually abolished the first phase without impacting the second. The relative contributions of alterations in the Ca^{2+} signal and depletion of granular pools are not known. These secretion changes echo in vivo observations in healthy individuals. Some [84] though not all [48] studies described attenuation of the acute insulin response to glucose after a few hours of moderate elevation of pre-stimulatory glucose. In addition, brief clamping of blood glucose at G8 abolished the first phase response expected from further elevation to G12–G15 [85,86].

In summary, qualitative discrepancies between in vitro and in vivo studies only pertain to the pattern of the second phase during stimulations with G12 and above. The underlying causes are unknown. An extrinsic signal, absent in vitro, could be implicated.

4.2. Pulsatility

Small oscillations of plasma insulin have been observed in peripheral blood [87] and greater ones in portal blood [88,89], in both the fasting and postprandial states. By analogy with experimental data, they were ascribed to pulsatile secretion of insulin. This pulsatility was unlikely to be entrained only by the tiny oscillations of glucose that also exist, even though the amplitude of insulin oscillations increased with blood glucose while the period (5–8 min) remained unaffected [90,91]. In vitro, pulsatile insulin secretion was detected during perfusion of single islets [92,93] as well as in batches of islets [93–96]. Oscillations were present at low glucose (G3–G4) [92,93] and increased in amplitude without change in frequency at high glucose [94,97]. Their period usually ranged between 4.5 and 7 min, in keeping with in vivo observations. The causes of pulsatile insulin secretion are not entirely clear. Possible mechanisms generating insulin pulses in individual β -cells and synchronizing β -cells within individual islets, islets within the pancreas, and isolated islets in vitro have been expertly discussed elsewhere [90,91,98].

4.3. Concentration-dependency

Four approaches permit evaluation of the concentration-dependency of glucose-induced insulin secretion in vivo: step-wise increases of blood glucose to successive plateaus [85], graded glucose infusion tests [99–101], regular ramp increases [52,53], and separate hyperglycemic clamps at different levels [57,102,103]. In most recent studies, tested glucose concentrations ranged from G5 to G15. As shown in Table 2, results are very consistent between laboratories and might suggest that half-maximal stimulation occurs around G8. They should however be interpreted with caution because it remains uncertain whether the maximum response was reached at G15. In rarer studies where glucose levels were raised above G15, plasma insulin or C-peptide concentrations [56,104] and calculated ISR [53] kept increasing up to G25, suggesting that half-maximal stimulation is closer to G11. Incidentally, the maximum response to glucose should not be equated with the maximum possible rate of secretion since non-glucose stimuli such as arginine remain effective at G15 [49,105]. Another limitation of in vivo studies starting from G5 is that the threshold of stimulation by glucose cannot be established. However, there is no doubt that insulin secretion is already stimulated in the fasting state as shown by the lowering of plasma C-peptide induced by diazoxide [106] or insulin-mediated hypoglycemia [107]. Furthermore, ISR regularly decreased during stepped insulin-induced hypoglycemic clamps between G5.5 and G3 [108].

In vitro, four methods were used to characterize the concentration-dependency of glucose-induced insulin secretion: parallel incubations of islets in different glucose concentrations [27,109]; parallel perfusions with a jump to a single glucose concentration [37,69]; single perfusions with sequential, stepwise increases in glucose concentration [29,110]; and single perfusions with a slow ramp increase in glucose [54]. Key findings of studies comparing islets from the same donors at 5 to 9 glucose concentrations spanning a range of at least 20 mM are summarized in Table 3, and complete results of one of these studies are shown in Figure 2C. The relationship was similar for the two phases of secretion, with maximal stimulation around G15 and half-maximal stimulation around G7. Insulin synthesis in cultured islets is characterized by a somewhat greater sensitivity to glucose

Table 3 — Concentration-dependency of glucose-induced insulin secretion in isolated human islets.

Refs.	First author and year	Method	Donors (n)	Number [G]	Range [G]	Time at each [G]	Threshold mM	Km mM	Max mM
[27]	Harrison, 1985	Incubation	2	8	G0-G20	1 h	4	5.5	15
[109]	Walker, 2011	Incubation	4	5	G0-G20	1 h	3	7.5	15
[37]	Henquin, 2015	Peri/parallel	8	6	G3-G30	2 h	?	7	15
[69]	Alcazar, 2019	Peri/parallel	4–9	7	G3-G30	20 min	?	7.9	17
[110]	Henquin, 2006	Peri/steps	8	9	G0-G30	30 min	3	6.5	15
[29]	Doliba, 2012	Peri/steps	3	5	G0-G24	40 min	3	5.2	12
[54]	Li, 2017	Peri/ramp	3		G0-G25		4	7	12
Mean							3.4	6.7	14.4
Peri: Perfusion. [G]: glucose concentration in mM. (n) = number of islet preparations from distinct donors.									

with half-maximum and maximum stimulations at G5 and G10, respectively [111].

The threshold of glucose-induced insulin secretion corresponds to the concentration at which metabolism sufficiently increases the ATP/ADP ratio to depolarize the β -cell membrane and allow influx of Ca^{2+} . Studies that also measured the response of islets in G0 (Table 3) consistently detected a low threshold close to G3, as illustrated in Figure 2D: insulin secretion was 1.7-fold higher in G3 than G0. The key role of glucokinase in the setting of this threshold is demonstrated by a lowering to G1 in β -cells with an activating mutation of the enzyme [112] or during its pharmacological activation [29,36] (Figure 2D). A threshold at G3 implies that quantification of insulin secretion in terms of SI is influenced by the low glucose concentration (G1 or G3) used as reference.

In summary, while *in vitro* and *in vivo* studies agree that the sensitivity of β -cells to changes in glucose is greatest between G5 and G11, doubts persist concerning maximally and half-maximally effective concentrations that appear to be higher *in vivo* than *in vitro*. This difference is likely linked to distinct time courses of the second phase (ascending *in vivo* and flat *in vitro*). Comparing dose–response curves in islets challenged in the absence and presence of physiological mixtures of non-glucose stimuli would be a first easy step to tackle the issue.

4.4. Insulin secretion rates *in vivo* and *in vitro*

Comparisons of ISR measured *in vitro* and *in vivo* are possible if both are expressed relative to the available insulin stores (*fractional* ISR). The insulin content of tested islets can be measured, but the insulin content of the pancreas of tested subjects must be estimated from literature data. The average value of 1750 nmol insulin per pancreas will be used [19]. In cohorts including both lean and obese non-diabetic (ND) subjects, intravenous glucose injection rapidly increased ISR to peaks around 1500 pmol/min at G11 [61,102] and 2100 pmol/min at G16 [51,113,114]. Relative to total pancreatic stores, these rates correspond to ~ 0.09 and 0.12% per min. During steady state elevation of blood glucose to G6, G8, G10, and G15, measured ISR correspond to about 0.014, 0.025, 0.035, and 0.050% of pancreatic insulin per min (Table 2). Fractional ISR measured in perfused islets, both at the peak of first phase and during the plateau of second phase (Figure 2A,B), are close to those calculated in clinical investigations. An ISR of 0.05% per min in G15, as observed *in vivo* and during perfusions, corresponds to 3.0% of insulin stores per hour. Values between 2.1 and 3.7% per hour in G15 have also been reported for incubated islets [25,72,115,116]. Expressing results as fractional insulin secretion is simple and

informative, and should be standardized to facilitate comparisons of different studies [19].

The dynamics of triggering Ca^{2+} and the participation of distinct pools of insulin granules are both involved in the generation of biphasic insulin secretion [10,117,118]. Within the frame of this review, only some quantitative features of exocytosis deserve discussion. On the basis of 10,000 insulin granules per β -cell [10], one can calculate that, in response to G15, each β -cell secretes ~ 11 and 6 granules per min at the peak of the first phase and during the plateau of the second phase, with no more than ~ 50 granules during the whole first phase [37]. Admittedly, these average calculations are based on the unlikely assumption that all islets and all β -cells within each islet are functionally homogenous [119], but the proportion of non-contributing cells is not known. Notwithstanding these concerns, the take home message is that normal islets secrete only a small fraction of their insulin stores even when they are challenged by a high concentration of glucose for 1 h. This simple calculation reinforces a notion that is still often overlooked in human physiology: insulin synthesis is not necessary for second phase secretion. In healthy individuals, pancreatic stores of insulin could cover the needs for 5–7 days [19].

4.5. Proinsulin secretion

Proinsulin conversion to insulin is incomplete and small amounts of the pro-hormone are secreted. In the plasma of healthy subjects, basal proinsulin to insulin ratio is above 10% because the clearance of proinsulin is much slower than that of insulin. During stimulation with glucose, the ratio decreases by dilution, because proinsulin is secreted in lower amounts than insulin [60,120–122]. The proinsulin concentration does not exceed 3.0% of insulin in the whole pancreas and in isolated islets [81,123,124], and acute stimulation with glucose induces a smaller relative rise of proinsulin than insulin in the portal blood of healthy subjects [120], and a smaller increase in proinsulin than insulin secretion in normal islets [124–126]. Figure 1A shows that the proportion of proinsulin to insulin secreted by perfused islets rapidly decreased during stimulation with glucose or tolbutamide [37]. It is unknown whether such rapid changes correspond to exocytosis of granules containing different proportions of the prohormone in different islets, in different β -cells or in each β -cell (pools of young and aged granules).

5. ANTHROPOMETRICS OF TEST SUBJECTS AND ISLET DONORS

Whereas sex, age and body weight of test subjects are always taken into consideration in the analysis of clinical investigations, only few

studies have examined in some detail how the donor attributes influence the insulin-secreting properties of isolated islets [23,25]. However, the information is important from a physiological point of view and to identify possible biases in cohorts used to address specific questions [15].

5.1. Influence of sex

Subtle differences in glucose homeostasis between normal or prediabetic men and women have been attributed to differences in body composition, insulin action, nutrient absorption, and hormonal milieu [127–129]. There is no solid evidence for sex-specific, intrinsic traits of the insulin-secretion capacity of β -cells [130–132]. Four comparisons of insulin secretion in islets isolated from large cohorts of male and female donors have been published. Using static incubations, one study reported a 20% greater SI of glucose in female than male islets [133], whereas two others found no difference [25,134]. In perfusions, both the dynamics and amplitude of insulin secretion were similar in islets from male and female donors [23].

5.2. Influence of age

It is known that glucose homeostasis progressively deteriorates with aging [135,136]; however, the relative contribution of intrinsic β -cell defects remains uncertain. Clinical investigations based on hyperglycemic clamps or bolus glucose injections disclosed decreases in ISR with aging. These decreases variably affected basal secretion, the first phase or the second phase of the response to high glucose [52,137–140]. It is generally accepted that even when absolute insulin secretion appears normal in aged subjects, it may prove insufficient after correction for insulin resistance. Such a deficit cannot be attributed to insufficient insulin stores because the insulin content of the pancreas does not fall with age (28–87 years) [141].

There is also consensus that the insulin content of isolated islets does not decrease with the age of the donor. It was modestly increased (by 20% after 60 years) in one series [25] and was independent of age (16–68 years) in three others [23,142,143]. There is less agreement concerning insulin secretion. In four studies based on static incubations, a negative correlation was found between age of the donor and SI of glucose [25,142–144], whereas no link was observed in two others [134,145]. Furthermore, four studies using perfused islets found no impact of age on the first and second phases of glucose-induced insulin secretion [23,142,146,147]. These somewhat contradictory results cannot be explained by differences in cohort characteristics or size. Nevertheless, the balance favors the conclusion that aging is not accompanied by major deterioration of β -cell function when it is studied in vitro. Extrinsic factors such as changes in vascularization of the endocrine pancreas [147] might explain a greater impact of aging on insulin secretion in vivo than in vitro.

5.3. Influence of body mass index

Basal hyperinsulinemia characteristically observed in obese subjects largely results from increased ISR [62,148]. During hyperglycemic clamps [62] or graded glucose infusions [99,149], ISR increases more in obese than lean subjects. Both basal and stimulated ISR are approximately doubled in ND obese individuals. The magnitude of this functional change largely exceeds the increase in β -cell mass (from 0 to 50% depending on body mass index [BMI]) [150–152]. The insulin content of the pancreas moderately augments with BMI [141]. It is thus evident that β -cells are hyperactive in obese subjects. The picture is not so clear in isolated islets. When insulin secretion was measured in static incubations, the SI of glucose was independent of donor BMI in three studies [25,142,153] and positively correlated with

BMI in a fourth one [134]. In perfusion experiments, both phases of insulin secretion were slightly higher in islets from obese than lean donors but the SI was unchanged [23,83]. However, the total response increased with BMI [23]. The incomplete consensus between these studies cannot be ascribed to the insufficient size of individual cohorts (>40 preparations each) or differences in islet size and insulin content that have been taken into consideration. The discrepancy between the major impact of obesity on insulin secretion in vivo and the inconstant changes observed in vitro is disturbing. One possible confounding factor could be the delay between islet isolation and testing if the hyperactivity of β -cells in vivo is maintained by extrinsic factors and therefore progressively fades out after isolation.

5.4. Islets from children

Premature newborns only showed a small rise in plasma insulin during glucose infusion over 30 min [154,155]. In full-term newborns, a faster and greater increase in blood glucose was followed by a rapid rise in plasma insulin that displayed a biphasic time course [156,157]. No such effect of glucose was observed in vitro. In islet-like cell clusters from two neonates (two and five weeks of age), glucose only transiently increased insulin secretion [158]. A single study characterized insulin secretion in islets isolated from a five-day-old newborn [159]. Glucose alone was virtually inactive but induced a biphasic and concentration-dependent increase in insulin secretion when islet cAMP was increased. Two other features of the immaturity of these neonatal islets were the lack of amplifying action of glucose and the elevation of basal insulin secretion [159].

In children, the rapid increase in plasma insulin induced by intravenous glucose augmented with body-weight and age between 1 and 10 years [160–162], and displayed a biphasic time course during hyperglycemic clamps at the age of 9–10 years [163,164]. Islets isolated from the pancreas of two infants (2.5 and 4 months of age) showed biphasic insulin responses to glucose; however, the SI was smaller than that in adult islets [165]. A larger study was performed with fragments (not isolated islets) of healthy pancreas resected during surgical treatment of 12 infants (2–11 months old) suffering from focal congenital hyperinsulinism [166]. No differences with adult islets were noted with regard to dynamics and amplitude of the insulin response to glucose (Figure 2E). The concentration-dependency however showed maximum stimulation already at 7–10 mM glucose [166]. Islets from 5 toddlers (11–36 months old) were also tested in vitro [167]. They behaved qualitatively like adult islets, with a maximum effect of glucose at 15 mM. Quantitatively, they secreted lower proportions of their insulin stores, but the SI was not reduced because unstimulated secretion rates were also lower [167].

The global message is that β -cells are immature just after birth and that the transition to functional maturity occurs during the first year of life. The secretion of smaller proportions of insulin reserves is in keeping with lower in vivo needs during infancy.

6. DYSFUNCTIONAL ISLETS

Insufficient insulin secretion is a prerequisite for the development of diabetes. Type 1 diabetes (T1D) results from autoimmune β -cell destruction leading to a virtually complete insulin deficiency. T2D results from a relative insulin deficiency often in the context of insulin resistance in target tissues. Recent years have witnessed significant headway in the identification of genetic, metabolic, and inflammatory factors implicated in the pathogenesis of β -cell dysfunction in both forms of the disease [168–171]. In contrast to these in-depth investigations based on “multi-omics” approaches, qualitative, and

quantitative characteristics of insulin secretion by dysfunctional human islets have only been sketchily outlined.

6.1. Type 2 diabetes

Several decades of clinical investigation have identified the features and progressivity of the insulin deficit in T2D. There is no doubt that this deficit involves functional impairment of β -cells owing to its greater magnitude than the decrease in β -cell mass and its partial reversibility after adequate treatment. These issues have been extensively discussed in learned review articles [59,64,172–174]. For comparisons with in vitro studies described below, it is sufficient to recall the main characteristics of insulin secretion in T2D. Impairment of the first phase response to glucose is an early and predictive marker of β -cell dysfunction which is already detectable while fasting blood glucose is barely increased [51,175–177]. The second phase is also blunted in subjects with impaired glucose tolerance and more so in patients with overt diabetes [49,176–179]. Even when their acute response to glucose is lost, subjects with T2D still secrete insulin upon acute stimulation with sulfonylureas or arginine [59,180].

Studying islets from subjects with T2D in vitro is challenging for investigators because the availability of such islets is very limited. Unfortunately, correct appreciation of published results may also be problematic. From a literature survey, it has been calculated that the insulin content of isolated T2D islets is on average 65% that of ND islets, but this parameter is not consistently measured and taken into consideration [19]. Moreover, duration, severity, and type of treatment of diabetes in donors inevitably contribute to the high variability between preparations as already noted in the first study of T2D islets [181]. Finally, it is difficult to exclude that the (dys)-functional phenotype of T2D islets has not changed before testing. A few days of culture in G5-G6, as for control islets, could reverse some of the

defects that were present in vivo when β -cells were exposed to hyperglycemia. Consequently, while the efforts of implicated laboratories are commendable, it is fair to conclude that the current information remains fragmentary and sometimes fragile.

Several groups have reported the dynamics of insulin secretion in perfused T2D islets. It is noteworthy that all experiments followed the classic protocol including an initial period in G0–G3 before stimulation with high glucose. Unfortunately, samples were sometimes collected at an insufficient rate for precise analysis of kinetics, so that the only possible conclusion was that insulin secretion was not more blunted during the first 10 min than the subsequent 40 min of stimulation [83,182]. In three other studies, a first phase was identified in the response of T2D islets, and its onset was as rapid [183] or slower than in ND islets [29,184]. In the most detailed investigation, a first phase was present in most preparations of T2D islets with an average ratio of first to second phase of 1.6 vs. 2.4 in ND islets [79]. Another study mentions persistence of a first phase in 4/6 preparations of T2D islets [185]. A single report suggesting that glucose no longer has a rapid effect on insulin secretion in T2D islets is undermined by the lack of a second phase in both T2D and ND islets [186]. Most in vitro experiments therefore agree that an acute challenge of T2D islets with glucose triggers a rapid increase in insulin secretion that is, at the most, of slightly smaller amplitude and slower onset than the first phase occurring in ND islets. The reasons for such a discrepancy with the rapid disappearance of first phase in vivo are unclear and deserve careful investigation, with particular attention at pre-test culture periods and pre-stimulatory glucose concentration.

The most consistent and solid observation (independent of islet insulin content) is a decrease, on average by 47%, of the SI of glucose in T2D compared to ND islets during the second phase of perfusions or whole incubations [25,29,79,83,182–184,187–196] (Table 4). Such a

Table 4 — Insulin secretion in isolated islets from subjects with T2D.

Refs.	First author and year	Donors ND/T2D	Method	Test	SI Glucose		ISR T2D/ND (%)		Ins content T2D/ND (%)
					ND	T2D	Basal	Stim	
Results as % insulin content									
[183]	Campbell, 2020	6/3	Peri	G11/G3	3.4	1.7	260	130	58
[184]	Liang, 2020	4/4	Peri	G17/G3	4.4	1.4	130	50	?
[187]	Anello, 2005	11/7	Incub	G17/G3	2.4	1.2	140	70	66
[188]	Ehehalt, 2010	16/8	Incub	G25/G0	3.9	2.7	90	60	?
[25]	Lyon, 2016	65/19	Incub	G17/G1	6.4	3.8	110	70	65
[192]	Daneshpajooh, 2018	3/3	Incub	G17/G3	3.0	1.8	100	60	49
Mean							138	73	
Results per islet									
[193]	Deng, 2004	5/5	Peri ramp	G17/G0	5.0	2.6	100	50	
[29]	Doliba, 2012	3/3	Peri	G12/G0	7.0	3.2	140	70	
[79]	Butcher, 2014	5/12	Peri	G23/G3	6.2	3.3			
[182]	Lundberg, 2018	7/7	Peri	G20/G2	11	5.2	150	70	
[83]	Nagao, 2020	49/26	Peri	G20/G2	4.3	3.7	45	50	
[188]	Ostenson, 2006	4/4	Incub	G17/G3	3.9	1.5	90	30	72
[190]	Rosengren, 2012	42/17	Incub	G20/G3	5.1	2.7			71
[191]	Locke, 2014	10/10	Incub	G28/G3	4.2	2.6			
[194]	Batchuluun, 2018	5/5	Incub	G11/G2	3.9	2.4	70	40	
[195]	Solimena, 2018	61/19	Incub	G17/G3	3.4	1.8	100	50	
[196]	Taneera, 2019	6/6	Incub	G17/G1	10	3.0	100	30	
Mean							99	49	
Mean corrected for 65% of insulin content							152	75	
Global mean					5.1	2.6			64

ND: non diabetic. T2D: Type2 diabetic. ISR: Insulin secretion rate. Pre-testing culture of T2D islets was rarely defined and varied from 1 to 9 days in G5-G6, except in Ehehalt 2010 (2 days in G11).

Table 5 — Insulin secretion in isolated islets from subjects with T1D.

Refs.	First author and year	Donors ND/T1D	Duration diabetes	Culture (days)	SI Glucose		ISR T1D/ND (%)		Ins content T1D/ND (%)
					ND	T1D	Basal	Stim	
Results as % insulin content									
[209]	Marchetti, 2000	1/1	0.7 y	5	2.6	1.3	120	65	?
[210]	Lupi, 2004	3/2	1 y	3	2.2	1.1	110	55	50
[212]	Brissova, 2018	7/4	2–7 y	2–4	3.8	2.8	95	80	25
Results per islet									
[80]	Krogvold, 2015	15/6	3–9 w	3	16	7.0	50	25	?
[208]	Conget, 1993	7/1	recent	?	2.1	1.0	10	5	40
[211]	Walker, 2011	5/1	13 y	?	3.9	2.9	100	75	55

ND: non diabetic. T1D: Type1 diabetic. ISR: Insulin secretion rate. When a pre-testing culture period was mentioned, the medium always contained G5.5.

decrease resulted sometimes from higher basal sometimes from lower stimulated secretion. Basal insulin secretion was similar in T2D and ND islets when results were expressed per islet, but was increased in T2D islets after normalization for the measured or estimated lowering of insulin content. Glucose-stimulated insulin secretion was consistently decreased with and without correction for insulin content. From the results of 14 studies, one can estimate that *fractional insulin secretion* in T2D islets averaged ~140% (basal conditions) and ~75% (glucose stimulation) of control islets (Table 4).

Of course, what matters for blood glucose control is absolute not fractional ISR. Knowing that insulin stores in subjects with T2D are ~60% those of ND subjects [19], one can extrapolate that subjects with T2D should achieve ~45% ($100\% \times 0.75 \times 0.60$) of stimulated ISR measured in ND individuals. During clamps at G10–G15, the increase in plasma C-peptide in subjects with T2D was ~55% that of ND controls [178,179]. In other investigations, ISR was calculated during hyperglycemic clamps [64], graded glucose infusions [149,197] and ramp glucose infusions [54]. The increase measured in subjects with T2D reached 40–50% that of controls. There is thus excellent agreement with the 45% roughly predicted from in vitro experiments. In summary, in vivo and vitro results show good quantitative coherence for the second phase but are discordant regarding the first phase. One should however bear in mind that the ISR referred to above were measured during isolated glucose stimulation in vivo and in vitro. Greater differences in ISR between ND controls and subjects with T2D or between their islets could be observed during multifactorial stimulation of β -cells following oral glucose or meals [63].

To parallel clinical investigations, non-glucose stimuli were also tested in T2D islets. Stimulation by arginine (20 mM in G3) induced immediate insulin secretion during perfusions [186] and the total response was similar to ND controls over 1 h of incubation [195]. At the high concentration of 2 μ M, glibenclamide was ineffective in T2D islets [188]. At the very high concentration of 100 μ M in G3, it showed the same effect in T2D than in ND islets during perfusions [186] but was 40% less effective during incubations [195]. Acutely, 100 nM glucagon-like peptide-1 (GLP-1) completely restored the responsiveness of T2D islets to glucose [188], but the effect was rather small at 10 nM [198,199]. One study compared the effects of GLP-1 and GIP in T2D islets and found the two incretins similarly effective in T2D islets, producing an increase in insulin secretion that was not different from that in ND islets [198]. This contrasts sharply with clinical studies showing that, unlike GLP-1, GIP loses its incretin properties in subjects with T2D [200,201]. Objectively, the picture that emerges from these in vitro studies of T2D islets remains blurry.

Finally, it is instructive to compare results obtained with isolated islets and pancreatic slices. In superfused slices from T2D pancreas

fractional insulin secretion was higher than normal in G3, whereas G17 was virtually unable to induce any further increase [21]. This striking difference with the persisting effectiveness of glucose in isolated islets could have two explanations. First, the insulin content of islets within T2D slices was very low (20% of controls) whereas the insulin content of isolated T2D islets averaged 65% that of ND islets. Second, in contrast to isolated islets, slices were tested freshly, without prior culture and possible recovery period.

6.2. Type 1 diabetes

Although T1D is a disease of absolute insulin deficiency caused by autoimmune destruction of β -cells, many patients retain low rates of insulin secretion as assessed by C-peptide measurements [202], and their pancreas still contains a small number of islets with insulin-positive cells [202–204]. Stimulation of plasma C-peptide after a mixed meal permits clinical detection of residual β -cell function in patients with T1D [205]. The proportion of subjects with a positive response is lower in those with young age at diagnosis, and decreases with time from clinical onset [206]. Patients with the greatest C-peptide response to a meal also respond to intravenous glucose [207]. Only few in vitro studies have been done with islets isolated from whole pancreases [208–212] or pancreatic biopsies [80] obtained from subjects with T1D. Although the β -cell mass is decreased by 90–95% in subjects with long-term T1D [204], the insulin content of tested islets ranged between 25 and 55% that of control islets (Table 5). That discrepancy likely reflects selective handpicking of healthy-looking islets from donors with relatively short duration of diabetes. It should first be noted that some preparations were glucose-insensitive [80,208]. When insulin secretion was measurable, basal secretion was close to that in ND islets whereas stimulated secretion was consistently lower even when differences in islet insulin content were taken into account (Table 5). Although the SI was about 50% lower, the response was biphasic in some preparations [80,209,212]. In three studies, the islet responsiveness to glucose improved with duration of the culture prior to testing [80,209,210]. Insulin secretion was also measured in slices of pancreases within 12 h of tissue reception [213]. In recent T1D (0 and 1.5 years), insulin secretion was very low, but high glucose evoked a biphasic response with a SI of 2.2 vs. 9 in ND pancreas. In T1D of longer duration (4 and 10 years), no insulin secretion was measurable [213].

The extreme rarity of islets from T1D donors and variations in the age of onset and duration of the disease largely explain the incompleteness of these studies and their somewhat discrepant results. They share the merit of showing that persisting islets in the pancreas of T1D subjects retain some normal functional features. Expansion of such studies promises to be difficult but is feasible. Conversely, in vitro

characterization of the abnormalities of β -cell secretory function that pre-exist clinical manifestations of T1D [214] currently appears illusive.

6.3. Congenital hyperinsulinism

Congenital hyperinsulinism (CHI), the major cause of persistent hypoglycemia in newborns and infants, can result from mutations impacting the functioning of ionic channels or metabolic enzymes in β -cells [215,216]. Certain forms are treated by surgery [217] and the resected portion of the pancreas has been studied in vitro. The most severe cases are caused by inactivating mutations in the genes encoding the two subunits of K_{ATP} channels, which result in continuous closure of the channels and persistent depolarization of β -cells [38,39].

In focal forms of the disease, a localized hyperplasia of abnormal β -cells is present in an otherwise normal pancreas and its selective resection cures the patient [218]. Parallel perfusions of fragments from both pathological and healthy regions of the pancreas showed completely normal behavior of unaffected regions whereas lesions exhibited high basal insulin secretion, small and transient effects of glucose, and lack of effects of diazoxide and tolbutamide [166] (Figure 2E), in agreement with clinical observations [218].

In diffuse forms of the disease, all β -cells are affected and extensive pancreatectomy is usually required. Perfusion of fragments from these pancreases evidenced the same anomalies as those in focal lesions [166]. Other groups isolated islets from the pancreas of these patients and observed a clear stimulation [219] or no effect of glucose [54]. They also disclosed a greater insulin-secreting action of amino acids [54,220], a characteristic that may explain the protein-induced hypoglycemia in some of these infants with CHI caused by K_{ATP} channel mutations. Globally, the in vitro findings agree well with clinical observations.

Rarer cases of CHI are characterized by nuclear enlargement in β -cells within hyperactive islets that are confined in one or in a few adjacent lobules of the pancreas [221]. As they are not caused by mutations in K_{ATP} channels, these mosaic forms may benefit from long-term treatment with diazoxide but can be cured by resection of the affected region [217,221]. Perfusing fragments of these pancreases indeed confirmed the expected efficacy of diazoxide and tolbutamide but uncovered an abnormal response to glucose. A large peak of insulin secretion was induced by G1 (Figure 2F), which could be ascribed to expression of the low-Km hexokinase-1 in these β -cells [112]. The behavior of these pathological β -cells illustrates why blood glucose control would be impossible if hexokinase-1 was expressed together with glucokinase in normal β -cells.

7. CONCLUSIONS

Since it has become evident that malfunction of pancreatic β -cells plays a causal role in all types of diabetes, the perspective of contributing to the prevention or cure of the disease is laying behind most research projects using isolated islets. The increasing resort to human islets raises a question of translational nature, which has been addressed in this review. Does the information obtained from in vitro studies of human islets reliably reflect the characteristics of insulin secretion in living subjects? Analysis of data published over 50 years revealed a fairly good, though not complete, qualitative and quantitative coherence between glucose-induced insulin secretion in vivo and in vitro. These similarities are reassuring on the validity of the model and provide criteria to evaluate the quality of individual islet preparations. However, there are shadows on the picture. Our ability to

detect effects being limited we tend to oversize stimulations to make differences statistically significant, in particular when the number of repeats with heterogeneous islet preparations is small. The extreme conditions, often imposed to isolated islets deprived of their complex nutrient, hormonal and neural influences, could distort some responses measured in artificial experimental milieus. This might explain the perplexing discrepancies noted between insulin secretion in subjects with T2D and their islets studied in vitro, in particular concerning the kinetics. A must in future projects will be to ascertain that the experimental conditions do not modify the function of these ailing β -cells and their normal controls.

In vitro studies of human islets are necessary and very promising but remain challenging [15,19,222,223]. The “islet research community” can look back with pride at what has been achieved but the way ahead is still long before we untangle the complex regulation of insulin secretion in human β -cells and identify the causes of its anomalies. I hope that this review will be felt constructive and useful by my colleagues in the field.

ACKNOWLEDGMENTS

I am most grateful to Erol Cerasi, Ele Ferrannini and Andrea Mari for their critical and constructive comments on an earlier version of this review. Special thanks are due to Myriam Nenquin, who did all experiments with human islets in my former laboratory. These human islets were provided by D. Dufrane (Brussels) from 2002 to 2012, and by V. Gmyr, J. Kerr-Conte and F. Pattou (Lille) from 2013 to 2016.

CONFLICT OF INTEREST

I have no conflicts of interest relevant to this study to declare.

FINANCIAL SUPPORT

No specific funds were dedicated to the writing of this review.

REFERENCES

- [1] Yalow, R.S., Berson, S.A., 1960. Immunoassay of endogenous plasma insulin in man. *Journal of Clinical Investigation* 39:1157–1175.
- [2] Henquin, J.C., Nenquin, M., Ravier, M.A., Szollosi, A., 2009. Shortcomings of current models of glucose-induced insulin secretion. *Diabetes, Obesity and Metabolism* 11(Suppl 4):168–179.
- [3] Fridlyand, L.E., Jacobson, D.A., Philipson, L.H., 2013. Ion channels and regulation of insulin secretion in human β -cells: a computational systems analysis. *Islets* 5:1–15.
- [4] Prentki, M., Matschinsky, F.M., Madiraju, S.R., 2013. Metabolic signaling in fuel-induced insulin secretion. *Cell Metabolism* 18(2):162–185.
- [5] Gilon, P., Chae, H.Y., Rutter, G.A., Ravier, M.A., 2014. Calcium signaling in pancreatic β -cells in health and in Type 2 diabetes. *Cell Calcium* 56(5):340–361.
- [6] Rutter, G.A., Pullen, T.J., Hodson, D.J., Martinez-Sanchez, A., 2015. Pancreatic β -cell identity, glucose sensing and the control of insulin secretion. *Biochemical Journal* 466(2):203–218.
- [7] Kalwat, M.A., Cobb, M.H., 2017. Mechanisms of the amplifying pathway of insulin secretion in the β -cell. *Pharmacology & Therapeutics* 179:17–30.
- [8] Boland, B.B., Rhodes, C.J., Grimsby, J.S., 2017. The dynamic plasticity of insulin production in β -cells. *Molecular Metabolism* 6(9):958–973.
- [9] Amisten, S., Atanes, P., Hawkes, R., Ruz-Maldonado, I., Liu, B., Parandeh, F., et al., 2017. A comparative analysis of human and mouse islet G-protein coupled receptor expression. *Scientific Reports* 7:46600.

- [10] Rorsman, P., Ashcroft, F.M., 2018. Pancreatic β -cell electrical activity and insulin secretion: of mice and men. *Physiological Reviews* 98(1):117–214.
- [11] Spegel, P., Mulder, H., 2020. Metabolomics analysis of nutrient metabolism in β -cells. *Journal of Molecular Biology* 432(5):1429–1445.
- [12] Jacobson, D.A., Shyng, S.L., 2020. Ion channels of the islets in type 2 diabetes. *Journal of Molecular Biology* 432(5):1326–1346.
- [13] Campbell, J.E., Newgard, C.B., 2021. Mechanisms controlling pancreatic islet cell function in insulin secretion. *Nature Reviews Molecular Cell Biology* 22(2): 142–158.
- [14] Skelin Klemen, M., Dolensšek, J., Slak Rupnik, M., Stožer, A., 2017. The triggering pathway to insulin secretion: functional similarities and differences between the human and the mouse β cells and their translational relevance. *Islets* 9(6):109–139.
- [15] Hart, N.J., Powers, A.C., 2019. Use of human islets to understand islet biology and diabetes: progress, challenges and suggestions. *Diabetologia* 62(2):212–222.
- [16] Bergman, R.N., Piccinini, F., Kabir, M., Kolka, C.M., Ader, M., 2019. Hypothesis: role of reduced hepatic insulin clearance in the pathogenesis of type 2 diabetes. *Diabetes* 68(9):1709–1716.
- [17] Tillil, H., Shapiro, E.T., Rubenstein, A.H., Galloway, J.A., Polonsky, K.S., 1988. Reduction of insulin clearance during hyperglycemic clamp. Dose-response study in normal humans. *Diabetes* 37(10):1351–1357.
- [18] Polonsky, K.S., 2000. Dynamics of insulin secretion in obesity and diabetes. *International Journal of Obesity and Related Metabolic Disorders* 24(Suppl 2): S29–S31.
- [19] Henquin, J.C., 2019. The challenge of correctly reporting hormones content and secretion in isolated human islets. *Molecular Metabolism* 30:230–239.
- [20] Brunicardi, F.C., Atiya, A., Moldovan, S., Lee, T.C., Fagan, S.P., Kleinman, R.M., et al., 2003. Activation of somatostatin receptor subtype 2 inhibits insulin secretion in the isolated perfused human pancreas. *Pancreas* 27(4):e84–e89.
- [21] Cohrs, C.M., Panzer, J.K., Drotar, D.M., Enos, S.J., Kipke, N., Chen, C., et al., 2020. Dysfunction of persisting β cells is a key feature of early type 2 diabetes pathogenesis. *Cell Reports* 31(1):107469.
- [22] Warnock, G.L., Ellis, D., Rajotte, R.V., Dawidson, I., Baekkeskov, S., Egebjerg, J., 1988. Studies of the isolation and viability of human islets of Langerhans. *Transplantation* 45(5):957–963.
- [23] Henquin, J.C., 2018. Influence of organ donor attributes and preparation characteristics on the dynamics of insulin secretion in isolated human islets. *Physiological Reports* 6:13646.
- [24] Dybala, M.P., Hara, M., 2019. Heterogeneity of the human pancreatic islet. *Diabetes* 68(6):1230–1239.
- [25] Lyon, J., Manning Fox, J.E., Spigelman, A.F., Kim, R., Smith, N., O’Gorman, D., et al., 2016. Research-focused isolation of human islets from donors with and without diabetes at the Alberta Diabetes Institute IsletCore. *Endocrinology* 157(2):560–569.
- [26] Mislser, S., Barnett, D.W., Pressel, D.M., Gillis, K.D., Scharp, D.W., Falke, L.C., 1992. Stimulus-secretion coupling in β -cells of transplantable human islets of Langerhans. Evidence for a critical role for Ca^{2+} entry. *Diabetes* 41(6): 662–670.
- [27] Harrison, D.E., Christie, M.R., Gray, D.W., 1985. Properties of isolated human islets of Langerhans: insulin secretion, glucose oxidation and protein phosphorylation. *Diabetologia* 28(2):99–103.
- [28] Sweet, I.R., Gilbert, M., Jensen, R., Sabek, O., Fraga, D.W., Gaber, A.O., et al., 2005. Glucose stimulation of cytochrome C reduction and oxygen consumption as assessment of human islet quality. *Transplantation* 80(8): 1003–1011.
- [29] Doliba, N.M., Qin, W., Najafi, H., Liu, C., Buettger, C.W., Sotiris, J., et al., 2012. Glucokinase activation repairs defective bioenergetics of islets of Langerhans isolated from type 2 diabetics. *American Journal of Physiology. Endocrinology and Metabolism* 302(1):E87–E102.
- [30] Detimary, P., Dejonghe, S., Ling, Z., Pipeleers, D., Schuit, F., Henquin, J.C., 1998. The changes in adenine nucleotides measured in glucose-stimulated rodent islets occur in beta cells but not in alpha cells and are also observed in human islets. *Journal of Biological Chemistry* 273(51):33905–33908.
- [31] Mislser, S., Gee, W.M., Gillis, K.D., Scharp, D.W., Falke, L.C., 1989. Metabolite-regulated ATP-sensitive K^+ channel in human pancreatic islet cells. *Diabetes* 38(4):422–427.
- [32] Braun, M., Ramracheya, R., Bengtsson, M., Zhang, Q., Karanauskaite, J., Partridge, C., et al., 2008. Voltage-gated ion channels in human pancreatic β -cells: electrophysiological characterization and role in insulin secretion. *Diabetes* 57(6):1618–1628.
- [33] Hellman, B., Gylfe, E., Bergsten, P., Grapengiesser, E., Lund, P.E., Berts, A., et al., 1994. Glucose induces oscillatory Ca^{2+} signalling and insulin release in human pancreatic beta cells. *Diabetologia* 37(Suppl 2):S11–S20.
- [34] Martin, F., Soria, B., 1996. Glucose-induced $[\text{Ca}^{2+}]_i$ oscillations in single human pancreatic islets. *Cell Calcium* 20(5):409–414.
- [35] Davalli, A.M., Biancardi, E., Pollo, A., Soggi, C., Pontiroli, A.E., Pozza, G., et al., 1996. Dihydropyridine-sensitive and -insensitive voltage-operated calcium channels participate in the control of glucose-induced insulin release from human pancreatic beta cells. *Journal of Endocrinology* 150(2): 195–203.
- [36] Henquin, J.C., Dufrane, D., Gmyr, V., Kerr-Conte, J., Nenquin, M., 2017. Pharmacological approach to understanding the control of insulin secretion in human islets. *Diabetes, Obesity and Metabolism* 19:1061–1070.
- [37] Henquin, J.C., Dufrane, D., Kerr-Conte, J., Nenquin, M., 2015. Dynamics of glucose-induced insulin secretion in normal human islets. *American Journal of Physiology. Endocrinology and Metabolism* 309:E640–E650.
- [38] Aguilar-Bryan, L., Bryan, J., 1999. Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocrine Reviews* 20(2):101–135.
- [39] Dunne, M.J., Cosgrove, K.E., Shepherd, R.M., Aynsley-Green, A., Lindley, K.J., 2004. Hyperinsulinism in infancy: from basic science to clinical disease. *Physiological Reviews* 84(1):239–275.
- [40] Ashcroft, F.M., Puljung, M.C., Vedovato, N., 2017. Neonatal diabetes and the KATP channel: from mutation to therapy. *Trends in Endocrinology and Metabolism* 28(5):377–387.
- [41] Sarafidis, P.A., McFarlane, S.I., Bakris, G.L., 2007. Antihypertensive agents, insulin sensitivity, and new-onset diabetes. *Current Diabetes Reports* 7(3): 191–199.
- [42] Straub, S.G., James, R.F., Dunne, M.J., Sharp, G.W., 1998. Glucose activates both K_{ATP} channel-dependent and K_{ATP} channel-independent signaling pathways in human islets. *Diabetes* 47(5):758–763.
- [43] Capozzi, M.E., Svendsen, B., Encisco, S.E., Lewandowski, S.L., Martin, M.D., Lin, H., et al., 2019. β -Cell tone is defined by proglucagon peptides through cAMP signaling. *JCI Insight* 4:e126742.
- [44] Rojas, E., Carroll, P.B., Ricordi, C., Boschero, A.C., Stojilkovic, S.S., Atwater, I., 1994. Control of cytosolic free calcium in cultured human pancreatic beta-cells occurs by external calcium-dependent and independent mechanisms. *Endocrinology* 134(4):1771–1781.
- [45] Ferdaoussi, M., Fu, J., Dai, X., Manning Fox, J.E., Suzuki, K., Smith, N., et al., 2017. SUMOylation and calcium control syntaxin-1A and secretagogin sequestration by tomosyn to regulate insulin exocytosis in human β cells. *Scientific Reports* 7(1):248.
- [46] Prentki, M., Corkey, B.E., Madiraju, S.R.M., 2020. Lipid-associated metabolic signalling networks in pancreatic beta cell function. *Diabetologia* 63(1):10–20.
- [47] Cerasi, E., 1975. Mechanisms of glucose stimulated insulin secretion in health and in diabetes: some re-evaluations and proposals. *Diabetologia* 11(1):1–13.

- [48] Pfeifer, M.A., Graf, R.J., Halter, J.B., Porte Jr., D., 1981. The regulation of glucose-induced insulin secretion by pre-stimulus glucose level and tolbutamide in normal man. *Diabetologia* 21(3):198–205.
- [49] Ward, W.K., Bolgiano, D.C., McKnight, B., Halter, J.B., Porte Jr., D., 1984. Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *Journal of Clinical Investigation* 74:1318–1328.
- [50] Grespan, E., Giorgino, T., Arslanian, S., Natali, A., Ferrannini, E., Mari, A., 2018. Defective amplifying pathway of β -cell secretory response to glucose in type 2 diabetes: integrated modeling of in vitro and in vivo evidence. *Diabetes* 67(3):496–506.
- [51] Mari, A., Tura, A., Pacini, G., Kautzky-Willer, A., Ferrannini, E., 2008. Relationships between insulin secretion after intravenous and oral glucose administration in subjects with glucose tolerance ranging from normal to overt diabetes. *Diabetic Medicine* 25(6):671–677.
- [52] Chang, A.M., Smith, M.J., Galecki, A.T., Bloem, C.J., Halter, J.B., 2006. Impaired beta-cell function in human aging: response to nicotinic acid-induced insulin resistance. *Journal of Clinical Endocrinology & Metabolism* 91:3303–3309.
- [53] Seghieri, M., Rebelos, E., Astiarraga, B.D., Baldi, S., Mari, A., Ferrannini, E., 2016. Impact of a mild decrease in fasting plasma glucose on β -cell function in healthy subjects and patients with type 2 diabetes. *American Journal of Physiology. Endocrinology and Metabolism* 310:E919–E924.
- [54] Li, C., Ackermann, A.M., Boodhansingh, K.E., Bhatti, T.R., Liu, C., Schug, J., et al., 2017. Functional and metabolomic consequences of ATP-dependent potassium channel inactivation in human islets. *Diabetes* 66(7):1901–1913.
- [55] Cerasi, E., Luft, R., 1967. The plasma insulin response to glucose infusion in healthy subjects and in diabetes mellitus. *Acta Endocrinologica* 55(2):278–304.
- [56] van Haefen, T.W., Boonstra, E., Veneman, T.F., Gerich, J.E., van der Veen, E.A., 1990. Dose-response characteristics for glucose-stimulated insulin release in man and assessment of influence of glucose on arginine-stimulated insulin release. *Metabolism* 39(12):1292–1299.
- [57] Natali, A., Gastaldelli, A., Galvan, A.Q., Sironi, A.M., Ciociaro, D., Sanna, G., et al., 1998. Effects of acute alpha 2-blockade on insulin action and secretion in humans. *American Journal of Physiology* 274:E57–E64.
- [58] Caumo, A., Luzi, L., 2004. First-phase insulin secretion: does it exist in real life? Considerations on shape and function. *American Journal of Physiology. Endocrinology and Metabolism* 287:E371–E385.
- [59] Porte Jr., D., 1991. Beta-cells in type II diabetes mellitus. *Diabetes* 40(2):166–180.
- [60] Fritsche, A., Madaus, A., Stefan, N., Tschritter, O., Maerker, E., Häring, H., et al., 2002. Relationships among age, proinsulin conversion, and beta-cell function in nondiabetic humans. *Diabetes* 51(Suppl 1):S234–S239.
- [61] Kashyap, S., Belfort, R., Gastaldelli, A., Pratipanawatr, T., Berria, R., Pratipanawatr, W., et al., 2003. A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type-2 diabetes. *Diabetes* 52(10):2461–2474.
- [62] Polonsky, K.S., Given, B.D., Hirsch, L., Shapiro, E.T., Tillil, H., Beebe, C., et al., 1988. Quantitative study of insulin secretion and clearance in normal and obese subjects. *Journal of Clinical Investigation* 81(2):435–441.
- [63] Michaliszyn, S.F., Mari, A., Lee, S., Bacha, F., Tfayli, H., Farchoukh, L., et al., 2014. β -cell function, incretin effect, and incretin hormones in obese youth along the span of glucose tolerance from normal to prediabetes to type 2 diabetes. *Diabetes* 63(11):3846–3855.
- [64] Pratley, R.E., Weyer, C., 2001. The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus. *Diabetologia* 44(8):929–945.
- [65] Gerich, J.E., 2002. Is reduced first-phase insulin release the earliest detectable abnormality in individuals destined to develop type 2 diabetes? *Diabetes* 51(Suppl 1):S117–S121.
- [66] Ricordi, C., Lacy, P.E., Finke, E.H., Olack, B.J., Scharp, D.W., 1988. Automated method for isolation of human pancreatic islets. *Diabetes* 37:413–420.
- [67] Brunicaudi, F.C., Sun, Y.S., Druck, P., Goulet, R.J., Elahi, D., Andersen, D.K., 1987. Splanchnic neural regulation of insulin and glucagon secretion in the isolated perfused human pancreas. *American Journal of Surgery* 153(1):34–40.
- [68] Schwede, F., Chepurny, O.G., Kaufholz, M., Bertinetti, D., Leech, C.A., Cabrera, O., et al., 2016. Rp-cAMPS prodrugs reveal the cAMP dependence of first-phase glucose-stimulated insulin secretion. *Molecular Endocrinology* 29(7):988–1005.
- [69] Alcazar, O., Buchwald, P., 2019. Concentration-dependency and time profile of insulin secretion: dynamic perfusion studies with human and murine islets. *Frontiers in Endocrinology* 10:680.
- [70] Davalli, A.M., Ricordi, C., Socci, C., Braghi, S., Bertuzzi, F., Fattor, B., et al., 1991. Abnormal sensitivity to glucose of human islets cultured in a high glucose medium: partial reversibility after an additional culture in a normal glucose medium. *Journal of Clinical Endocrinology & Metabolism* 72(1):202–208.
- [71] Bertuzzi, F., Saccomanno, K., Socci, C., Davalli, A.M., Taglietti, M.V., Berra, C., et al., 1998. Long-term in vitro exposure to high glucose increases proinsulin-like-molecules release by isolated human islets. *Journal of Endocrinology* 158(2):205–211.
- [72] Dolai, S., Xie, L., Zhu, D., Liang, T., Qin, T., Xie, H., et al., 2016. Synaptotagmin-7 functions to replenish insulin granules for exocytosis in human islet β -cells. *Diabetes* 65(7):1962–1976.
- [73] Kelly, A.C., Smith, K.E., Purvis, W.G., Min, C.G., Weber, C.S., Cooksey, A.M., et al., 2019. Oxygen perfusion (Persufflation) of human pancreata enhances insulin secretion and attenuates islet proinflammatory signaling. *Transplantation* 103(1):160–167.
- [74] Yu, J., Shi, Y., Zhao, K., Yang, G., Yu, L., Li, Y., et al., 2020. Enhanced expression of β cell CaV3.1 channels impairs insulin release and glucose homeostasis. *Proceedings of the National Academy of Sciences of the United States of America* 117(1):448–453.
- [75] Squires, P.E., Harris, T.E., Persaud, S.J., Curtis, S.B., Buchan, A.M., Jones, P.M., 2000. The extracellular calcium-sensing receptor on human beta-cells negatively modulates insulin secretion. *Diabetes* 49(3):409–417.
- [76] Lehmann, R., Zuellig, R.A., Kugelmeier, P., Baenninger, P.B., Moritz, W., Perren, A., et al., 2007. Superiority of small islets in human islet transplantation. *Diabetes* 56(3):594–603.
- [77] Zhao, M., Muiesan, P., Amiel, S.A., Srinivasan, P., Asare-Anane, H., Fairbanks, L., et al., 2007. Human islets derived from donors after cardiac death are fully biofunctional. *American Journal of Transplantation* 7(10):2318–2325.
- [78] Johnson, J.D., Ao, Z., Ao, P., Li, H., Dai, L.J., He, Z., et al., 2009. Different effects of FK506, rapamycin, and mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets. *Cell Transplantation* 18:833–845.
- [79] Butcher, M.J., Hallinger, D., Garcia, E., Machida, Y., Chakrabarti, S., Nadler, J., et al., 2014. Association of proinflammatory cytokines and islet resident leucocytes with islet dysfunction in type 2 diabetes. *Diabetologia* 57(3):491–501.
- [80] Krogvold, L., Skog, O., Sundström, G., Edwin, B., Buanes, T., Hanssen, K.F., et al., 2015. Function of isolated pancreatic islets from patients at onset of Type 1 Diabetes: insulin secretion can be restored after some days in a nondiabetogenic environment in vitro: results from the DiViD Study. *Diabetes* 64(7):2506–2512.
- [81] Roomp, K., Kristinsson, H., Schwartz, D., Ubhayasekera, K., Sargsyan, E., Manukyan, L., et al., 2017. Combined lipidomic and proteomic analysis of isolated human islets exposed to palmitate reveals time-dependent changes in insulin secretion and lipid metabolism. *PLoS One* 12(4):e0176391.
- [82] Zuellig, R.A., Cavallari, G., Gerber, P., Tschopp, O., Spinaz, G.A., Moritz, W., et al., 2017. Improved physiological properties of gravity-enforced

- reassembled rat and human pancreatic pseudo-islets. *Journal of Tissue Engineering and Regenerative Medicine* 11(1):109–120.
- [83] Nagao, M., Esguerra, J.L.S., Asai, A., Ofori, J.K., Edlund, A., Wendt, A., et al., 2020. Potential protection against Type 2 Diabetes in obesity through lower CD36 expression and improved exocytosis in β -cells. *Diabetes* 69(6):1193–1205.
- [84] Ferner, R.E., Ashworth, L., Tronier, B., Alberti, K.G., 1986. Effects of short-term hyperglycemia on insulin secretion in normal humans. *American Journal of Physiology* 250:E655–E661.
- [85] Toschi, E., Camastra, S., Sironi, A.M., Masoni, A., Gastaldelli, A., Mari, A., et al., 2002. Effect of acute hyperglycemia on insulin secretion in humans. *Diabetes* 51(Suppl 1):S130–S133.
- [86] Lim, E.L., Hollingsworth, K.G., Aribisala, B.S., Chen, M.J., Mathers, J.C., Taylor, R., 2011. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia* 54(10):2506–2514.
- [87] Lang, D.A., Matthews, D.R., Burnett, M., Ward, G.M., Turner, R.C., 1982. Pulsatile, synchronous basal insulin and glucagon secretion in man. *Diabetes* 31(1):22–26.
- [88] Song, S.H., McIntyre, S.S., Shah, H., Veldhuis, J.D., Hayes, P.C., Butler, P.C., 2000. Direct measurement of pulsatile insulin secretion from the portal vein in human subjects. *Journal of Clinical Endocrinology & Metabolism* 85(12):4491–4499.
- [89] Porksen, N., Grøfte, T., Greisen, J., Mengel, A., Juhl, C., Veldhuis, J.D., et al., 2002. Human insulin release processes measured by intraportal sampling. *American Journal of Physiology. Endocrinology and Metabolism* 282(3):E695–E702.
- [90] Porksen, N., 2002. The in vivo regulation of pulsatile insulin secretion. *Diabetologia* 45(1):3–20.
- [91] Satin, L.S., Butler, P.C., Ha, J., Sherman, A.S., 2015. Pulsatile insulin secretion, impaired glucose tolerance and type 2 diabetes. *Molecular Aspects of Medicine* 42:61–77.
- [92] Lin, J.M., Fabregat, M.E., Gomis, R., Bergsten, P., 2002. Pulsatile insulin release from islets isolated from three subjects with type 2 diabetes. *Diabetes* 51(4):988–993.
- [93] Song, S.H., Kjems, L., Ritzel, R., McIntyre, S.M., Johnson, M.L., Veldhuis, J.D., et al., 2002. Pulsatile insulin secretion by human pancreatic islets. *Journal of Clinical Endocrinology & Metabolism* 87(1):213–221.
- [94] Marchetti, P., Scharp, D.W., Mclear, M., Gingerich, R., Finke, E., Olack, B., et al., 1994. Pulsatile insulin secretion from isolated human pancreatic islets. *Diabetes* 43(6):827–830.
- [95] Hellman, B., Salehi, A., Gylfe, E., Dansk, H., Grapengiesser, E., 2009. Glucose generates coincident insulin and somatostatin pulses and antisynchronous glucagon pulses from human pancreatic islets. *Endocrinology* 150:5334–5340.
- [96] Simpson, N., Maffei, A., Freeby, M., Burroughs, S., Freyberg, Z., Javitch, J., et al., 2012. Dopamine-mediated autocrine inhibitory circuit regulating human insulin secretion in vitro. *Molecular Endocrinology* 26(10):1757–1772.
- [97] Ritzel, R.A., Veldhuis, J.D., Butler, P.C., 2003. Glucose stimulates pulsatile insulin secretion from human pancreatic islets by increasing secretory burst mass: dose-response relationships. *Journal of Clinical Endocrinology & Metabolism* 88(2):742–747.
- [98] Hellman, B., 2009. Pulsatility of insulin release: a clinically important phenomenon. *Uppsala Journal of Medical Sciences* 114:193–205.
- [99] Jones, C.N., Abbasi, F., Carantoni, M., Polonsky, K.S., Reaven, G.M., 2000. Roles of insulin resistance and obesity in regulation of plasma insulin concentrations. *American Journal of Physiology. Endocrinology and Metabolism* 278(3):E501–E508.
- [100] Brandt, A., Katschinski, M., Arnold, R., Polonsky, K.S., Göke, B., Byrne, M.M., 2001. GLP-1-induced alterations in the glucose-stimulated insulin secretory dose-response curve. *American Journal of Physiology. Endocrinology and Metabolism* 281(2):E242–E247.
- [101] Wang, S., Oestriker, L.Z., Wallendorf, M.J., Sterl, K., Dunai, J., Kilpatrick, C.R., et al., 2018. Cholinergic signaling mediates the effects of xenin-25 on secretion of pancreatic polypeptide but not insulin or glucagon in humans with impaired glucose tolerance. *PLoS One* 13:e0192441.
- [102] Fritsche, A., Madaus, A., Renn, W., Tschirner, O., Teigeler, A., Weisser, M., et al., 2001. The prevalent Gly1057Asp polymorphism in the insulin receptor substrate-2 gene is not associated with impaired insulin secretion. *Journal of Clinical Endocrinology & Metabolism* 86:4822–4825.
- [103] Stefan, N., Fritsche, A., Häring, H., Stumvoll, M., 2001. Effect of experimental elevation of free fatty acids on insulin secretion and insulin sensitivity in healthy carriers of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 gene. *Diabetes* 50:1143–1148.
- [104] Cerasi, E., Luft, R., Efendic, S., 1972. Decreased sensitivity of the pancreatic beta cells to glucose in prediabetic and diabetic subjects. A glucose dose-response study. *Diabetes* 21(4):224–234.
- [105] Rasouli, N., Kern, P.A., Reece, E.A., Elbein, S.C., 2007. Effects of pioglitazone and metformin on beta-cell function in nondiabetic subjects at high risk for type 2 diabetes. *American Journal of Physiology. Endocrinology and Metabolism* 292(1):E359–E365.
- [106] Raju, B., Cryer, P.E., 2005. Mechanism, temporal patterns, and magnitudes of the metabolic responses to the KATP channel agonist diazoxide. *American Journal of Physiology. Endocrinology and Metabolism* 288(1):E80–E85.
- [107] Kraegen, E.W., Lazarus, L., Campbell, L.V., 1983. Failure of insulin infusion during euglycemia to influence endogenous basal insulin secretion. *Metabolism* 32(6):622–627.
- [108] Banarer, S., McGregor, V.P., Cryer, P.E., 2002. Intra-islet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic response. *Diabetes* 51(4):958–965.
- [109] Walker, J.N., Ramracheya, R., Zhang, Q., Johnson, P.R., Braun, M., Rorsman, P., 2011. Regulation of glucagon secretion by glucose: paracrine, intrinsic or both? *Diabetes, Obesity and Metabolism* 13(Suppl 1):95–105.
- [110] Henquin, J.C., Dufrane, D., Nenquin, M., 2006. Nutrient control of insulin secretion in isolated normal human islets. *Diabetes* 55:3470–3477.
- [111] Ling, Z., Pipeleers, D.G., 1996. Prolonged exposure of human beta cells to elevated glucose levels results in sustained cellular activation leading to a loss of glucose regulation. *Journal of Clinical Investigation* 98(12):2805–2812.
- [112] Henquin, J.C., Sempoux, C., Marchandise, J., Godecharles, S., Guiot, Y., Nenquin, M., et al., 2013. Congenital hyperinsulinism caused by hexokinase I expression or glucokinase-activating mutation in a subset of β -cells. *Diabetes* 62(5):1689–1696.
- [113] Breda, E., Cobelli, C., 2001. Insulin secretion rate during glucose stimuli: alternative analyses of C-peptide data. *Annals of Biomedical Engineering* 29(8):692–700.
- [114] Kjems, L.L., Vølund, A., Madsbad, S., 2001. Quantification of beta-cell function during IVGTT in Type II and non-diabetic subjects: assessment of insulin secretion by mathematical methods. *Diabetologia* 44(10):1339–1348.
- [115] Axelsson, A.S., Mahdi, T., Nenonen, H.A., Singh, T., Hänzelmann, S., Wendt, A., et al., 2017. Sox5 regulates beta-cell phenotype and is reduced in type 2 diabetes. *Nature Communications* 8:15652.
- [116] Dai, C., Walker, J.T., Shostak, A., Padgett, A., Spears, E., Wisniewski, S., et al., 2020. Tacrolimus- and sirolimus-induced human β cell dysfunction is reversible and preventable. *JCI Insight* 5 pii:130770.
- [117] Seino, S., Shibasaki, T., Minami, K., 2011. Dynamics of insulin secretion and the clinical implications for obesity and diabetes. *Journal of Clinical Investigation* 121:2118–2125.
- [118] Pedersen, M.G., Tagliavini, A., Henquin, J.C., 2019. Calcium signaling and secretory granule pool dynamics underlie biphasic insulin secretion and its

- amplification by glucose: experiments and modeling. *American Journal of Physiology. Endocrinology and Metabolism* 316:E475–E486.
- [119] Dominguez-Gutierrez, G., Xin, Y., Gromada, J., 2019. Heterogeneity of human pancreatic β -cells. *Molecular Metabolism* 27:S7–S14.
- [120] Horwitz, D.L., Starr, J.I., Mako, M.E., Blackard, W.G., Rubenstein, A.H., 1975. Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. *Journal of Clinical Investigation* 55(6):1278–1283.
- [121] Ward, W.K., LaCava, E.C., Paquette, T.L., Beard, J.C., Wallum, B.J., Porte Jr, D., 1987. Disproportionate elevation of immunoreactive proinsulin in type 2 (non-insulin-dependent) diabetes mellitus and in experimental insulin resistance. *Diabetologia* 30(9):698–702.
- [122] Kahn, S.E., Halban, P.A., 1997. Release of incompletely processed proinsulin is the cause of the disproportionate proinsulinemia of NIDDM. *Diabetes* 46(11):1725–1732.
- [123] Hou, X., Ling, Z., Zambre, Y., Foriers, A., Houssa, P., Deberg, M., et al., 1997. Proinsulin and its conversion intermediates in human pancreas and isolated islet tissue: kinetics and steady-state analysis. *Pancreas* 15(2):113–121.
- [124] Hostens, K., Ling, Z., Van Schravendijk, C., Pipeleers, D., 1999. Prolonged exposure of human beta-cells to high glucose increases their release of proinsulin during acute stimulation with glucose or arginine. *Journal of Clinical Endocrinology & Metabolism* 84(4):1386–1390.
- [125] Björklund, A., Grill, V., 1999. Enhancing effects of long-term elevated glucose and palmitate on stored and secreted proinsulin-to-insulin ratios in human pancreatic islets. *Diabetes* 48(7):1409–1414.
- [126] Marshak, S., Leibowitz, G., Bertuzzi, F., Soggi, C., Kaiser, N., Gross, D.J., et al., 1999. Impaired beta-cell functions induced by chronic exposure of cultured human pancreatic islets to high glucose. *Diabetes* 48(6):1230–1236.
- [127] Varlamov, O., Bethea, C.L., Roberts Jr., C.T., 2015. Sex-specific differences in lipid and glucose metabolism. *Frontiers in Endocrinology* 5:241.
- [128] Gannon, M., Kulkarni, R.N., Tse, H.M., Mauvais-Jarvis, F., 2018. Sex differences underlying pancreatic islet biology and its dysfunction. *Molecular Metabolism* 15:82–91.
- [129] Tramunt, B., Smati, S., Grandgeorge, N., Lenfant, F., Arnal, J.F., Montagner, A., et al., 2020. Sex differences in metabolic regulation and diabetes susceptibility. *Diabetologia* 63(3):453–461.
- [130] Basu, R., Dalla Man, C., Campioni, M., Basu, A., Klee, G., Toffolo, G., et al., 2006. Effects of age and sex on postprandial glucose metabolism: differences in glucose turnover, insulin secretion, insulin action, and hepatic insulin extraction. *Diabetes* 55(7):2001–2014.
- [131] Faerch, K., Borch-Johnsen, K., Vaag, A., Jørgensen, T., Witte, D.R., 2010. Sex differences in glucose levels: a consequence of physiology or methodological convenience? The Inter99 study. *Diabetologia* 53(5):858–865.
- [132] Anderwald, C., Gastaldelli, A., Tura, A., Krebs, M., Promintzer-Schifferl, M., Kautzky-Willer, A., et al., 2011. Mechanism and effects of glucose absorption during an oral glucose tolerance test among females and males. *Journal of Clinical Endocrinology & Metabolism* 96(2):515–524.
- [133] Hall, E., Volkov, P., Dayeh, T., Esguerra, J.L., Salö, S., Eliasson, L., et al., 2014. Sex differences in the genome-wide DNA methylation pattern and impact on gene expression, microRNA levels and insulin secretion in human pancreatic islets. *Genome Biology* 15(12):522.
- [134] Kong, Y., Sharma, R.B., Ly, S., Stamateris, R.E., Jesdale, W.M., Alonso, L.C., 2018. CDKN2A/B T2D GWAS risk-SNPs impact locus gene expression and proliferation in human islets. *Diabetes* 67(5):872–884.
- [135] Chang, A.M., Halter, J.B., 2003. Aging and insulin secretion. *American Journal of Physiology. Endocrinology and Metabolism* 284:E7–E12.
- [136] Scheen, A.J., 2005. Diabetes mellitus in the elderly: insulin resistance and/or impaired insulin secretion? *Diabetes & Metabolism* 31:5S27–5S34.
- [137] Gumbiner, B., Polonsky, K.S., Beltz, W.F., Wallace, P., Brechtel, G., Fink, R.I., 1989. Effects of aging on insulin secretion. *Diabetes* 38(12):1549–1556.
- [138] Ahrén, B., Pacini, G., 1998. Age-related reduction in glucose elimination is accompanied by reduced glucose effectiveness and increased hepatic insulin extraction in man. *Journal of Clinical Endocrinology & Metabolism* 83:3350–3356.
- [139] 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. *Journal of Clinical Endocrinology & Metabolism* 84(3):863–868.
- [140] Basu, R., Breda, E., Oberg, A.L., Powell, C.C., Dalla Man, C., Basu, A., et al., 2003. Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearance. *Diabetes* 52:1738–1748.
- [141] 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. *Scientific Reports* 7:11015.
- [142] Gregg, T., Poudel, C., Schmidt, B.A., Dhillon, R.S., Sdao, S.M., Truchan, N.A., et al., 2016. Pancreatic β -cells from mice offset age-associated mitochondrial deficiency with reduced K_{ATP} channel activity. *Diabetes* 65(9):2700–2710.
- [143] Westacott, M.J., Farnsworth, N.L., St Clair, J.R., Poffenberger, G., Heintz, A., Ludin, N.W., et al., 2017. Age-dependent decline in the coordinated $[Ca^{2+}]$ and insulin secretory dynamics in human pancreatic islets. *Diabetes* 66(9):2436–2445.
- [144] Ihm, S.H., Matsumoto, I., Sawada, T., Nakano, M., Zhang, H.J., Ansite, J.D., et al., 2006. Effect of donor age on function of isolated human islets. *Diabetes* 55(5):1361–1368.
- [145] Niclauss, N., Bosco, D., Morel, P., Demuylder-Mischler, S., Brault, C., Milliat-Guittard, L., et al., 2011. Influence of donor age on islet isolation and transplantation outcome. *Transplantation* 91(3):360–366.
- [146] Lakey, J.R., Warnock, G.L., Rajotte, R.V., Suarez-Alamazor, M.E., Ao, Z., Shapiro, A.M., et al., 1996. Variables in organ donors that affect the recovery of human islets of Langerhans. *Transplantation* 61(7):1047–1053.
- [147] Almaça, J., Molina, J., Arrojo e Drigo, R., Abdulreda, M.H., Jeon, W.B., Berggren, P.O., et al., 2014. Young capillary vessels rejuvenate aged pancreatic islets. *Proceedings of the National Academy of Sciences of the United States of America* 111(49):17612–17617.
- [148] Camastra, S., Manco, M., Mari, A., Baldi, S., Gastaldelli, A., Greco, A.V., et al., 2005. β -cell function in morbidly obese subjects during free living: long-term effects of weight loss. *Diabetes* 54(8):2382–2389.
- [149] Shankar, S.S., Shankar, R.R., Mixson, L.A., Miller, D.L., Chung, C., Ciliissen, C., et al., 2016. Linearity of β -cell response across the metabolic spectrum and to pharmacology: insights from a graded glucose infusion-based investigation series. *American Journal of Physiology. Endocrinology and Metabolism* 310(11):E865–E873.
- [150] Rahier, J., Guiot, Y., Goebbels, R.M., Sempoux, C., Henquin, J.C., 2008. Pancreatic beta-cell mass in European subjects with type 2 diabetes. *Diabetes, Obesity and Metabolism* 10(Suppl 4):32–42.
- [151] Kou, K., Saisho, Y., Satoh, S., Yamada, T., Itoh, H., 2013. Change in β -cell mass in Japanese nondiabetic obese individuals. *Journal of Clinical Endocrinology & Metabolism* 98(9):3724–3730.
- [152] 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(1):111–117.
- [153] Matsumoto, I., Sawada, T., Nakano, M., Sakai, T., Liu, B., Ansite, J.D., et al., 2004. Improvement in islet yield from obese donors for human islet transplants. *Transplantation* 78(6):880–885.
- [154] Reitano, G., Grasso, S., Distefano, G., Messina, A., 1971. The serum insulin and growth hormone response to arginine and to arginine with glucose in the premature infant. *Journal of Clinical Endocrinology & Metabolism* 33:924–928.

- [155] Grasso, S., Messina, A., Distefano, G., Vigo, R., Reitano, G., 1973. Insulin secretion in the premature infant. Response to glucose and amino acids. *Diabetes* 22(5):349–353.
- [156] Isles, T.E., Dickson, M., Farquhar, J.W., 1968. Glucose tolerance and plasma insulin in newborn infants of normal and diabetic mothers. *Pediatric Research* 2(3):198–208.
- [157] Falorni, A., Fracassini, F., Massi-Benedetti, F., Amici, A., 1972. Glucose metabolism, plasma insulin, and growth hormone secretion in newborn infants with erythroblastosis fetalis compared with normal newborns and those born to diabetic mothers. *Pediatrics* 49(5):682–693.
- [158] Otonkoski, T., Andersson, S., Knip, M., Simell, O., 1988. Maturation of insulin response to glucose during human fetal and neonatal development. Studies with perfusion of pancreatic islet-like cell clusters. *Diabetes* 37(3):286–291.
- [159] Henquin, J.C., Nenquin, M., 2018. Immaturity of insulin secretion by pancreatic islets isolated from one human neonate. *Journal of Diabetes Investigation* 9:270–273.
- [160] Mericq, V., Ong, K.K., Bazaes, R., Peña, V., Avila, A., Salazar, T., et al., 2005. Longitudinal changes in insulin sensitivity and secretion from birth to age three years in small- and appropriate-for-gestational-age children. *Diabetologia* 48(12):2609–2614.
- [161] Robert, J.J., Deschamps, I., Chevenne, D., Roger, M., Mogenet, A., Boitard, C., 1991. Relationship between first-phase insulin secretion and age, HLA, islet cell antibody status, and development of type 1 diabetes in 220 juvenile first-degree relatives of diabetic patients. *Diabetes Care* 14(8):718–723.
- [162] Carel, J.C., Boitard, C., Bougnères, P.F., 1993. Decreased insulin response to glucose in islet cell antibody-negative siblings of type 1 diabetic children. *Journal of Clinical Investigation* 92(1):509–513.
- [163] Caprio, S., Bronson, M., Sherwin, R.S., Rife, F., Tamborlane, W.V., 1996. Coexistence of severe insulin resistance and hyperinsulinaemia in pre-adolescent obese children. *Diabetologia* 39(12):1489–1497.
- [164] Arslanian, S., Suprasongsin, C., Janosky, J.E., 1997. Insulin secretion and sensitivity in black versus white prepubertal healthy children. *Journal of Clinical Endocrinology & Metabolism* 82(6):1923–1927.
- [165] Manning Fox, J.E., Seeberger, K., Dai, X.Q., Lyon, J., Spigelman, A.F., Kolic, J., et al., 2013. Functional plasticity of the human infant β -cell exocytotic phenotype. *Endocrinology* 154(4):1392–1399.
- [166] Henquin, J.C., Nenquin, M., Sempoux, C., Guiot, Y., Bellanné-Chantelot, C., Otonkoski, T., et al., 2011. In vitro insulin secretion by pancreatic tissue from infants with diazoxide-resistant congenital hyperinsulinism deviates from model predictions. *Journal of Clinical Investigation* 121(10):3932–3942.
- [167] Henquin, J.C., Nenquin, M., 2016. Dynamics and regulation of insulin secretion in pancreatic islets from normal young children. *PLoS One* 11(11):e0165961.
- [168] Eizirik, D.L., Pasquali, L., Cnop, M., 2020. Pancreatic β -cells in type 1 and type 2 diabetes mellitus: different pathways to failure. *Nature Reviews Endocrinology* 16:349–362.
- [169] Marchetti, P., Suleiman, M., De Luca, C., Baronti, W., Bosi, E., Tesi, M., et al., 2020. A direct look at the dysfunction and pathology of the β cells in human type 2 diabetes. *Seminars in Cell & Developmental Biology* 103:83–93.
- [170] Marrano, N., Biondi, G., Cignarelli, A., Perrini, S., Laviola, L., Giorgino, F., et al., 2020. Functional loss of pancreatic islets in type 2 diabetes: how can we halt it? *Metabolism* 110:154304.
- [171] Mattis, K.K., Gloyn, A.L., 2020. From genetic association to molecular mechanisms for islet-cell dysfunction in type 2 diabetes. *Journal of Molecular Biology* 432(5):1551–1578.
- [172] Kahn, S.E., Zraika, S., Utschneider, K.M., Hull, R.L., 2009. The beta cell lesion in type 2 diabetes: there has to be a primary functional abnormality. *Diabetologia* 52(6):1003–1012.
- [173] Ferrannini, E., Mari, A., 2014. β -Cell function in type 2 diabetes. *Metabolism* 63(10):1217–1227.
- [174] Zhyzhneuskaya, S.V., Al-Mrabeh, A., Peters, C., Barnes, A., Aribisala, B., Hollingsworth, K.G., et al., 2020. Time course of normalization of functional β -cell capacity in the diabetes remission clinical trial after weight loss in type 2 diabetes. *Diabetes Care* 43(4):813–820.
- [175] Brunzell, J.D., Robertson, R.P., Lerner, R.L., Hazzard, W.R., Ensink, J.W., Bierman, E.L., et al., 1976. Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. *Journal of Clinical Endocrinology & Metabolism* 42(2):222–229.
- [176] Godsland, I.F., Jeffs, J.A.R., Johnston, D.G., 2004. Loss of beta cell function as fasting glucose increases in the non-diabetic range. *Diabetologia* 47(7):1157–1166.
- [177] Kanat, M., Mari, A., Norton, L., Winnier, D., DeFronzo, R.A., Jenkinson, C., et al., 2012. Distinct β -cell defects in impaired fasting glucose and impaired glucose tolerance. *Diabetes* 61(2):447–453.
- [178] Hosker, J.P., Rudenski, A.S., Burnett, M.A., Matthews, D.R., Turner, R.C., 1989. Similar reduction of first- and second-phase B-cell responses at three different glucose levels in type II diabetes and the effect of gliclazide therapy. *Metabolism* 38(8):767–772.
- [179] van Haefen, T.W., Van Maarschalkerweerd, W.W., Gerich, J.E., Van der Veen, E.A., 1991. Decreased insulin secretory capacity and normal pancreatic B-cell glucose sensitivity in non-obese patients with NIDDM. *European Journal of Clinical Investigation* 21(2):168–174.
- [180] Karam, J.H., Sanz, N., Salamon, E., Nolte, M.S., 1986. Selective unresponsiveness of pancreatic beta-cells to acute sulfonylurea stimulation during sulfonylurea therapy in NIDDM. *Diabetes* 35(12):1314–1320.
- [181] Lohmann, D., Jahr, H., Verlohren, H.J., Schmidt, S., Heilmann, W., Zühlke, H., et al., 1980. Insulin secretion in maturity-onset diabetes. Function of isolated islets. *Hormone and Metabolic Research* 12(8):349–353.
- [182] Lundberg, M., Stenwall, A., Tegehall, A., Korsgren, O., Skog, O., 2018. Expression profiles of stress-related genes in islets from donors with progressively impaired glucose metabolism. *Islets* 10(2):69–79.
- [183] Campbell, S.A., Golec, D.P., Hubert, M., Johnson, J., Salamon, N., Barr, A., et al., 2020. Human islets contain a subpopulation of glucagon-like peptide-1 secreting α -cells that is increased in type 2 diabetes. *Molecular Metabolism* 39:101014.
- [184] Liang, T., Qin, T., Kang, F., Kang, Y., Xie, L., Zhu, D., et al., 2020. SNAP23 depletion enables more SNAP25/calcium channel excitosome formation to increase insulin exocytosis in type 2 diabetes. *JCI Insight* 5(3):e129694.
- [185] Oh, E., Stull, N.D., Mirmira, R.G., Thurmond, D.C., 2014. Syntaxin 4 up-regulation increases efficiency of insulin release in pancreatic islets from humans with and without type 2 diabetes mellitus. *Journal of Clinical Endocrinology & Metabolism* 99:E866–E870.
- [186] Del Guerra, S., Lupi, R., Marselli, L., Masini, M., Bugliani, M., Sbrana, S., et al., 2005. Functional and molecular defects of pancreatic islets in human type 2 diabetes. *Diabetes* 54(3):727–735.
- [187] Anello, M., Lupi, R., Spampinato, D., Piro, S., Masini, M., Boggi, U., et al., 2005. Functional and morphological alterations of mitochondria in pancreatic beta cells from type 2 diabetic patients. *Diabetologia* 48(2):282–289.
- [188] Ostenson, C.G., Gaisano, H., Sheu, L., Tibell, A., Bartfai, T., 2006. Impaired gene and protein expression of exocytotic soluble N-ethylmaleimide attachment protein receptor complex proteins in pancreatic islets of type 2 diabetic patients. *Diabetes* 55(2):435–440.
- [189] Ehehalt, F., Knoch, K., Erdmann, K., Krautz, C., Jäger, M., Steffen, A., et al., 2010. Impaired insulin turnover in islets from type 2 diabetic patients. *Islets* 2(1):30–36.
- [190] Rosengren, A.H., Braun, M., Mahdi, T., Andersson, S.A., Travers, M.E., Shigeto, M., et al., 2012. Reduced insulin exocytosis in human pancreatic β -cells with gene variants linked to type 2 diabetes. *Diabetes* 61(7):1726–1733.
- [191] Locke, J.M., da Silva Xavier, G., Dawe, H.R., Rutter, G.A., Harries, L.W., 2014. Increased expression of miR-187 in human islets from individuals with type 2

- diabetes is associated with reduced glucose-stimulated insulin secretion. *Diabetologia* 57(1):122–128.
- [192] Daneshpajoo, M., Eliasson, L., Bacos, K., Ling, C., 2018. MC1568 improves insulin secretion in islets from type 2 diabetes patients and rescues β -cell dysfunction caused by Hdac7 upregulation. *Acta Diabetologica* 55(12):1231–1235.
- [193] Deng, S., Vatamaniuk, M., Huang, X., Doliba, N., Lian, M.M., Frank, A., et al., 2004. Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects. *Diabetes* 53(3):624–632.
- [194] Batchuluun, B., Al Rijjal, D., Prentice, K.J., Eversley, J.A., Burdett, E., Mohan, H., et al., 2018. Elevated medium-chain acylcarnitines are associated with gestational diabetes mellitus and early progression to Type 2 Diabetes and induce pancreatic β -cell dysfunction. *Diabetes* 67(5):885–897.
- [195] Solimena, M., Schulte, A.M., Marselli, L., Ehehalt, F., Richter, D., Kleeberg, M., et al., 2018. Systems biology of the IMIDIA biobank from organ donors and pancreatectomised patients defines a novel transcriptomic signature of islets from individuals with type 2 diabetes. *Diabetologia* 61(3):641–657.
- [196] Taneera, J., Dhaiban, S., Mohammed, A.K., Mukhopadhyay, D., Aljaibaji, H., Sulaiman, N., et al., 2019. GNAS gene is an important regulator of insulin secretory capacity in pancreatic β -cells. *Gene* 715:144028.
- [197] Byrne, M.M., Sturis, J., Sobel, R.J., Polonsky, K.S., 1996. Elevated plasma glucose 2 h postchallenge predicts defects in beta-cell function. *American Journal of Physiology* 270:E572–E579.
- [198] Lupi, R., Del Guerra, S., D'Aleo, V., Boggi, U., Filipponi, F., Marchetti, P., 2010. The direct effects of GLP-1 and GIP, alone or in combination, on human pancreatic islets. *Regulatory Peptides* 165:129–132.
- [199] Ferdaoussi, M., Smith, N., Lin, H., Bautista, A., Spigelman, A.F., Lyon, J., et al., 2020. Improved glucose tolerance with DPPIV inhibition requires β -cell SENP1 amplification of glucose-stimulated insulin secretion. *Physiological Reports* 8:e14420.
- [200] Nauck, M.A., Heimesaat, M.M., Orskov, C., Holst, J.J., Ebert, R., Creutzfeldt, W., 1993. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *Journal of Clinical Investigation* 91(1):301–307.
- [201] Müller, T.D., Finan, B., Bloom, S.R., D'Alessio, D., Drucker, D.J., Flatt, P.R., et al., 2019. Glucagon-like peptide 1 (GLP-1). *Molecular Metabolism* 30:72–130.
- [202] Keenan, H.A., Sun, J.K., Levine, J., Doria, A., Aiello, L.P., Eisenbarth, G., et al., 2010. Residual insulin production and pancreatic β -cell turnover after 50 years of diabetes: Joslin Medalist Study. *Diabetes* 59(11):2846–2853.
- [203] Campbell-Thompson, M., Fu, A., Kaddis, J.S., Wasserfall, C., Schatz, D.A., Pugliese, A., et al., 2016. Insulinitis and β -cell mass in the natural history of type 1 diabetes. *Diabetes* 65(3):719–731.
- [204] Lam, C.J., Jacobson, D.R., Rankin, M.M., Cox, A.R., Kushner, J.A., 2017. β Cells persist in T1D pancreata without evidence of ongoing β -cell turnover or neogenesis. *Journal of Clinical Endocrinology & Metabolism* 102(8):2647–2659.
- [205] Oram, R.A., Jones, A.G., Besser, R.E., Knight, B.A., Shields, B.M., Brown, R.J., et al., 2014. The majority of patients with long-duration type 1 diabetes are insulin microsecretors and have functioning beta cells. *Diabetologia* 57(1):187–191.
- [206] Davis, A.K., DuBose, S.N., Haller, M.J., Miller, K.M., DiMeglio, L.A., Bethin, K.E., et al., 2015. Prevalence of detectable C-Peptide according to age at diagnosis and duration of type 1 diabetes. *Diabetes Care* 38(3):476–481.
- [207] Rickels, M.R., Evans-Molina, C., Bahnson, H.T., Yescupidez, A., Nadeau, K.J., Hao, W., et al., 2020. High residual C-peptide likely contributes to glycemic control in type 1 diabetes. *Journal of Clinical Investigation* 130(4):1850–1862.
- [208] Conget, I., Fernández-Alvarez, J., Ferrer, J., Sarri, Y., Novials, A., Somoza, N., et al., 1993. Human pancreatic islet function at the onset of type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 36(4):358–360.
- [209] Marchetti, P., Dotta, F., Ling, Z., Lupi, R., Del Guerra, S., Santangelo, C., et al., 2000. Function of pancreatic islets isolated from a type 1 diabetic patient. *Diabetes Care* 23(5):701–703.
- [210] Lupi, R., Marselli, L., Dionisi, S., Del Guerra, S., Boggi, U., Del Chiaro, M., et al., 2004. Improved insulin secretory function and reduced chemotactic properties after tissue culture of islets from type 1 diabetic patients. *Diabetes/Metabolism Research and Reviews* 20:246–251.
- [211] Walker, J.N., Johnson, P.R., Shigeto, M., Hughes, S.J., Clark, A., Rorsman, P., 2011. Glucose-responsive beta cells in islets isolated from a patient with long-standing type 1 diabetes mellitus. *Diabetologia* 54:200–202.
- [212] Brissova, M., Haliyur, R., Saunders, D., Shrestha, S., Dai, C., Blodgett, D.M., et al., 2018. α -Cell function and gene expression are compromised in Type 1 Diabetes. *Cell Reports* 22(10):2667–2676.
- [213] Panzer, J.K., Hiller, H., Cohrs, C.M., Almaça, J., Enos, S.J., Beery, M., et al., 2020. Pancreas tissue slices from organ donors enable in situ analysis of type 1 diabetes pathogenesis. *JCI Insight* 5(8):e134525.
- [214] Sims, E.K., DiMeglio, L.A., 2019. Cause or effect? A review of clinical data demonstrating beta cell dysfunction prior to the clinical onset of type 1 diabetes. *Molecular Metabolism* 27(Suppl):S129–S138.
- [215] Arya, V.B., Mohammed, Z., Blankenstein, O., De Lonlay, P., Hussain, K., 2014. Hyperinsulinaemic hypoglycaemia. *Hormone and Metabolic Research* 46(3):157–170.
- [216] Stanley, C.A., 2016. Perspective on the genetics and diagnosis of congenital hyperinsulinism disorders. *Journal of Clinical Endocrinology & Metabolism* 101(3):815–826.
- [217] Adzick, N.S., De Leon, D.D., States, L.J., Lord, K., Bhatti, T.R., Becker, S.A., et al., 2019. Surgical treatment of congenital hyperinsulinism: results from 500 pancreatectomies in neonates and children. *Journal of Pediatric Surgery* 54:27–32.
- [218] de Lonlay, P., Fournet, J.C., Rahier, J., Gross-Morand, M.S., Poggi-Travert, F., Foussier, V., et al., 1997. Somatic deletion of the imprinted 11p15 region in sporadic persistent hyperinsulinemic hypoglycemia of infancy is specific of focal adenomatous hyperplasia and endorses partial pancreatectomy. *Journal of Clinical Investigation* 100:802–807.
- [219] Straub, S.G., Cosgrove, K.E., Ammälä, C., Shepherd, R.M., O'Brien, R.E., Barnes, P.D., et al., 2001. Hyperinsulinism of infancy: the regulated release of insulin by K_{ATP} channel-independent pathways. *Diabetes* 50(2):329–339.
- [220] Calabria, A.C., Li, C., Gallagher, P.R., Stanley, C.A., De León, D.D., 2012. GLP-1 receptor antagonist exendin-(9-39) elevates fasting blood glucose levels in congenital hyperinsulinism owing to inactivating mutations in the ATP-sensitive K^+ channel. *Diabetes* 61(10):2585–2591.
- [221] Sempoux, C., Capito, C., Bellanné-Chantelot, C., Verkarre, V., de Lonlay, P., Aigrain, Y., et al., 2011. Morphological mosaicism of the pancreatic islets: a novel anatomopathological form of persistent hyperinsulinemic hypoglycemia of infancy. *Journal of Clinical Endocrinology & Metabolism* 96(12):3785–3793.
- [222] Kulkarni, R.N., Stewart, A.F., 2014. Summary of the Keystone islet workshop (April 2014): the increasing demand for human islet availability in diabetes research. *Diabetes* 63(12):3979–3981.
- [223] Poitout, V., Satin, L.S., Kahn, S.E., Stoffers, D.A., Marchetti, P., Gannon, M., et al., 2019. A call for improved reporting of human islet characteristics in research articles. *Diabetologia* 62(2):209–211.