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

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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.

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...
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-μm diameter spherical structures (islet equivalent). Such a theoretical islet equivalent contains about 12–14 ng of insulin, for a total of ~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 KATP channels with diazoxide (Dz 100 μM) and this inhibition was reversed by closing the channels with tolbutamide (Tolb 100 μM). 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 ± standard error of the mean for seven islet preparations. B: Measurements of islet [Ca2+]i show that the changes in secretion produced by diazoxide and tolbutamide are secondary to changes in the triggering Ca2+ 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 KATP 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 ± standard error of the mean for five islet preparations tested in parallel. Data are taken from [37] (A) and [39] (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<sub>ATP</sub> channels are open in the plasma membrane to keep the cells hyperpolarized. The influx of Ca<sup>2+</sup> is minimal, the concentration of cytosolic Ca<sup>2+</sup> (Ca<sup>2+</sup><sub>c</sub>) 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<sub>ATP</sub> 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<sup>2+</sup><sub>c</sub> influx into the cell [32]. The ensuing increase in [Ca<sup>2+</sup><sub>c</sub>] [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<sub>ATP</sub> channels to produce the triggering Ca<sup>2+</sup> signal can be demonstrated with two drugs [37]. Through direct opening of K<sub>ATP</sub> channels, diazoxide inhibits the effects of glucose on membrane potential, Ca<sup>2+</sup> influx, [Ca<sup>2+</sup><sub>c</sub>]c, and insulin secretion. Conversely, tolbutamide, a sulfonylurea that closes K<sub>ATP</sub> 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<sub>ATP</sub> 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<sup>2+</sup> would not be fully effective without operation of an amplifying pathway that does not further raise [Ca<sup>2+</sup><sub>c</sub>], but augments its effectiveness on exocytosis. When islets are stimulated by 15 mM glucose (G15) or by 500 μM 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<sup>2+</sup><sub>c</sub>]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<sup>2+</sup><sub>c</sub>]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<sup>2+</sup> 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 effective in subjects with T2D [49]. The amplifying action of glucose is most likely implicated in this potentiation, even though changes in the triggering Ca<sup>2+</sup> 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 perifused 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 [50]. 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-G17 [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 (min) | Delta G | Sampling 1st phase | Ratio 1st/2nd | Stimulation index |
|-------|------------------------|-----------|------------------|---------|------------------|--------------|------------------|
| [77]  | Alcazar, 2019          | 12        | 20 G3-G20        | 0.5 min | 1.35             | 3.2          |
| [76]  | Davalli, 1991          | 20        | 40 G3-G17        | 1 min   | 1.45             | 3.4          |
| [75]  | Henquin, 2015          | 35        | 20 G3-G17        | 1 min   | 1.8              | 3.8          |
| [74]  | Alcazar, 2019          | 20        | 20 G3-G17        | 1 min   | 1.7               | 4            |
| [73]  | Kelly, 2019            | 20        | 40 G3-G17        | 1 min   | 1.8              | 4.3          |
| [72]  | Doli, 2016             | 20        | 40 G3-G17        | 1 min   | 1.8              | 4.7          |
| [71]  | Bertucci, 1998         | 30        | 20 G3-G17        | 1 min   | 1.5              | 4.3          |
| [70]  | Davalli, 1991          | 20        | 40 G3-G17        | 1 min   | 1.6              | 4.7          |
| [69]  | Alcazar, 2019          | 20        | 20 G3-G17        | 1 min   | 1.7               | 5             |
|       | Mean                   |           |                  |         | 1.87             | 5.49      |
|       | Median                 |           |                  |         | 2.00             | 4.70      |

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.
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 perifusion 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: stepwise increases of blood glucose to successive plateaus [85], 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 perifusions with a jump to a single glucose concentration [37,69]; single perifusions with sequential, stepwise increases in glucose concentration [29,110]; and single perifusions 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
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 [25,144–146], 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 [23,153]. 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 hyperreactivity 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 progression 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 in-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

| Refs. | First author and year | Donors ND/T2D | Method | Test | SI Glucose ND T2D | ISR T2D/ND (%) | Ins content ND T2D (%)
|-------|------------------------|---------------|--------|------|-----------------|----------------|----------------------|
| [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] | Ekehalt, 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] | Daneshpapoh, 2018      | 3/3           | Incub  | G17/G3 | 3.0              | 1.8            | 100                  | 60                   | 49                   |
| Mean  |                        |               |        |       | 138             | 73             |                      |                      |                      |
| [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             | 5.2            | 150                  | 70                   |                      |
| [83]  | Nagao, 2020            | 49/28         | Peri   | G20/G2 | 11.5             | 5.2            | 150                  | 70                   |                      |
| [188] | Ostenson, 2006         | 4/4           | Incub  | G17/G3 | 3.9              | 1.5            | 90                   | 30                   | 72                   |
| [190] | Rosenberg, 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                   |                      |
| [198] | Taneera, 2019          | 6/6           | Incub  | G16/G1 | 10               | 3.0            | 100                  | 30                   |                      |
| Mean  |                        |               |        |       | 99              | 49             | 152                  | 75                   | 64                   |

ND: non diabetic. T2D: Type 2 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 Ekehalt 2010 (2 days in G11).
Insulin secretion in isolated islets from subjects with T1D.

For the second phase but are discordant regarding the increase measured in subjects with T2D 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 perifusions [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, whereas G17 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.

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% × 0.75 × 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 perifusions [198,199] and the total response was similar to ND controls over 1 h of incubation [195]. At the very high concentration of 100 μM in G3, it showed the same effect in T2D as in ND islets during perifusions [186] but was 40% less effective during incubations [198,199]. 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

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

| Refs. | First author and year | Donors | Duration diabetes (days) | Culture | SI Glucose Basal Stim T1D/ND (%) | ISR T1D/ND (%) | Ins content T1D/ND (%) |
|-------|-----------------------|--------|--------------------------|---------|---------------------------------|----------------|------------------------|
| [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                 |
| [211] | Bissova, 2018        | 7/4    | 2–7 y                    | 2–4     | 3.8                             | 2.6            | 95 80 25               |

Results per islet

| Refs. | First author and year | Donors | Duration diabetes (days) | Culture | SI Glucose Basal Stim T1D/ND (%) | ISR T1D/ND (%) | Ins content T1D/ND (%) |
|-------|-----------------------|--------|--------------------------|---------|---------------------------------|----------------|------------------------|
| [198] | Krogvold, 2015       | 15/6   | 3–9 w                    | 3       | 16                              | 7.0            | 50 25                  |
| [206] | Congt, 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: Type 1 diabetic. ISR: Insulin secretion rate. When a pre-testing culture period was mentioned, the medium always contained G5S.
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 KATP 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 perifusions 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 KATP 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 a few adjacent lobules of the pancreas [221]. As they are not caused by mutations in KATP channels, these mosaic forms may benefit from long-term treatment with diazoxide but can be cured by resection of the affected region [217,221]. Perifusing 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.

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CONFLICT OF INTEREST

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

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