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Review

First-phase insulin secretion: can its evaluation direct therapeutic approaches?

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Our work is aimed at unraveling the role of the first-phase insulin secretion in the natural history of type 2 diabetes mellitus (T2DM) and its interrelationship with insulin resistance and with β cell function and mass. Starting from pathophysiology, we investigate the impact of impaired secretion on glucose homeostasis and explore postmeal hyperglycemia as the main clinical feature, underlining its relevance in the management of the disease. We also review dietary and pharmacological approaches aimed at improving early secretory defects and restoring residual β cell function. Furthermore, we discuss possible approaches to detect early secretory defects in clinical practice. By providing a journey through human and animal data, we attempt a unification of the recent evidence in an effort to offer a new outlook on β cell secretion.

Highlights

β Cells can adapt to pathological metabolic conditions by implementing plasticity mechanisms to increase their mass and enhance insulin secretion, thus maintaining normal glucose levels at least in the early stages of type 2 diabetes mellitus (T2DM) natural history.

In T2DM the first phase of insulin secretion is impaired. This defect directly affects the clinical manifestations of the disease, primarily causing postmeal hyperglycemia, and has an impact on long-term disease-related complications.

Many studies have demonstrated the effectiveness of dietary and pharmacological strategies in improving postmeal hyperglycemia via enhancing β cell function and first-phase insulin secretion parameters in particular.

Because only stimulation tests can accurately estimate early β cell secretory impairments, detecting them in daily clinical practice remains challenging.

Pathophysiology of the secretory machinery

T2DM is a heterogeneous metabolic disorder characterized by reduced sensitivity of tissues to insulin and relative insulin deficiency [1]. However, insulin resistance [2] and reduced β cell mass alone are not sufficient to explain the metabolic decline which occurs during diabetes onset. In fact, it has been shown that manifest hyperglycemia is also associated with the decline of β cell function [3–5] as well as with the breakdown of the mechanisms that regulate insulin action and secretion [6]. In particular, impairments of first-phase insulin secretion have been demonstrated to be pivotal in the natural history of T2DM and its clinical manifestations [7].

In the complex pathophysiological scenario leading to T2DM onset, the timing and relationship between insulin resistance, insulin secretion, changes in plasma glucose, and β cell function and mass are still under investigation. The debate remains open as to whether defects in insulin secretion leading to hyperglycemia are the result of impaired β cell function, β cell mass reduction, and/or a combination the two [8–10]. The purpose of this review is to offer an updated report on the close interrelationship between β cell function, secretory machinery, and the insular environment, focusing on the pathophysiological importance of first-phase insulin secretion and underlining its clinical relevance in the diagnostic/therapeutic management of the disease.

Insulin secretion: physiological and dysfunctional mechanisms

There are several million islets of Langerhans in a human pancreas and each contains ~2000 hormone-secreting endocrine cells. Islet-derived hormones play an important role in the regulation of metabolic homeostasis: in particular, the complementary action of insulin and glucagon strictly regulates glucose homeostasis in mammals. Insulin is an anabolic hormone responsible for the storage of 'metabolic fuel', as opposed to the catabolic function of glucagon which promotes the mobilization and oxidation of stored metabolites [11]. Insulin secretion is strongly stimulated by increased plasma glucose concentrations physiologically occurring in the postprandial state

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(glucose-stimulated insulin secretion, GSIS) (Box 1 and Figure 1). Glucose enters the β cell cytoplasmic environment through the GLUT2 transporter and it is primarily catabolized by glucokinase, an enzyme which plays the major role in glucose sensing in humans. Thus, glucokinase is itself an important regulator of insulin secretion. The role of GLUT2 as a sensor of glucose levels has been demonstrated in mice, but further studies in humans are needed [12]. In addition, insulin secretion is enhanced by the action of peptides, hormones, and free fatty acids (FFAs) from the gastrointestinal tract and insular environment. α cells respond to FFAs and amino acids produced during fasting by secreting glucagon, whose production is instead inhibited by the increase in glucose and insulin concentrations. Somatostatin, produced by δ cells, suppresses both insulin and glucagon secretion [13,14]. Disruption of metabolic homeostasis and impairment of the interaction between insulin and glucagon are the fundamental defects in the genesis of T2DM, which is characterized by the inability of β cells to respond to increased insulin demand and by increased plasma glucagon levels [11].

An in-depth examination of the pathophysiological mechanisms of insulin secretion and their correlation with β cell function is essential to understand the clinical relation to the genesis and natural history of T2DM.

Insulin secretion and islet plasticity: from insulin resistance to β cell failure

Insulin resistance and β cell inadequacy represent key features in the pathogenesis of T2DM, and both are essential for the full manifestation of the disease [15]. Insulin resistance typically begins several years before hyperglycemia and is detectable throughout the entire natural history of

Box 1. Physiology of secretion

β Cells are responsible for insulin secretion. Initially synthesized as preproinsulin, during maturation preproinsulin is converted to proinsulin following conformational modifications by endoplasmic reticulum (ER) peptides. Thereafter, proinsulin is transported to the Golgi apparatus (GA) where it is cleaved into insulin and C-peptide. Once mature, insulin is stored in granules. Whenever circulating glucose levels rise, insulin secretion is stimulated via a complex pathway [140] (Figure 1), known as the triggering pathway, that is largely responsible for the so-called 'first phase' of insulin secretion. The next pathway, the amplification pathway, is also activated in the presence of maximal intracellular Ca^{2+} concentrations and is largely connected to mechanisms that are independent of K^{+}_{ATP} channels [141]. This corresponds to the 'second phase' of insulin secretion. The dynamics of the insulin response to intravenous glucose stimulation in normal and diabetic people are shown in Figure 2. This pathway is complex and finely regulated: recently, a study of the biochemical mechanisms identified numerous amplifiers of glucose-stimulated insulin secretion (GSIS), including anaplerotic metabolism of pyruvate and the consequent increase in the NADPH/$NADP^{+}$ cytosolic ratio, products of the pentose phosphate pathway, mitochondrial GTP, and free fatty acids (FFAs) [11]. Moreover, it has been demonstrated that the incretin axis plays a role as an insulin secretion amplifier via glutamate signaling [142].

The incretin hormones GIP (glucose-dependent insulinotropic peptide), secreted by K cells located in the duodenal and upper jejunal mucosa, and GLP-1 (glucagon-like peptide 1), produced by L cells mainly located in the ileum, are the main actors in the 'incretin effect' that is responsible for a higher insulin secretion rate in response to an oral glucose load compared to intravenous glucose administration. The two hormones can be considered to be gut-derived endocrine signals to the islets that modulate insulin and glucagon secretion in a glucose-dependent manner [143]. β cells express, at the membrane level, receptors for GIP (GIPRs) and for GLP-1 (GLP-1Rs): ligand binding to these receptors activates adenylate cyclase, causing an increase in cAMP concentrations which activates protein kinase A (PKA) [144]. This pathway cannot by itself cause insulin release because only hyperglycemia can activate K^{+}_{ATP} channels and the subsequent cascade needed for the fusion of granules to the cell membrane: for these reasons, the insulinotropic action of incretins necessarily requires hyperglycemia [145].

In addition to the multiple metabolic signaling mechanisms described above, insulin secretion is strictly regulated by crosstalk of β cells with other islet endocrine cells – α cells in particular – via the secretion of proglucagon-derived peptides [146–148]. Other islet cells affect β cell function through paracrine signals by activating transduction pathways that typically converge on the increase of cAMP levels in β cells and the amplification of GSIS [11].

In most individuals with impaired glucose tolerance (IGT) or overt diabetes we observe a reduction in the early insulin response to glucose (first phase), whereas there is an enhancement of the second phase [149,150]. These data suggest that β cell dysfunction is already present in the early stages of the disease and that worsening of the secretory machinery can strongly influence the onset and natural history of T2DM.

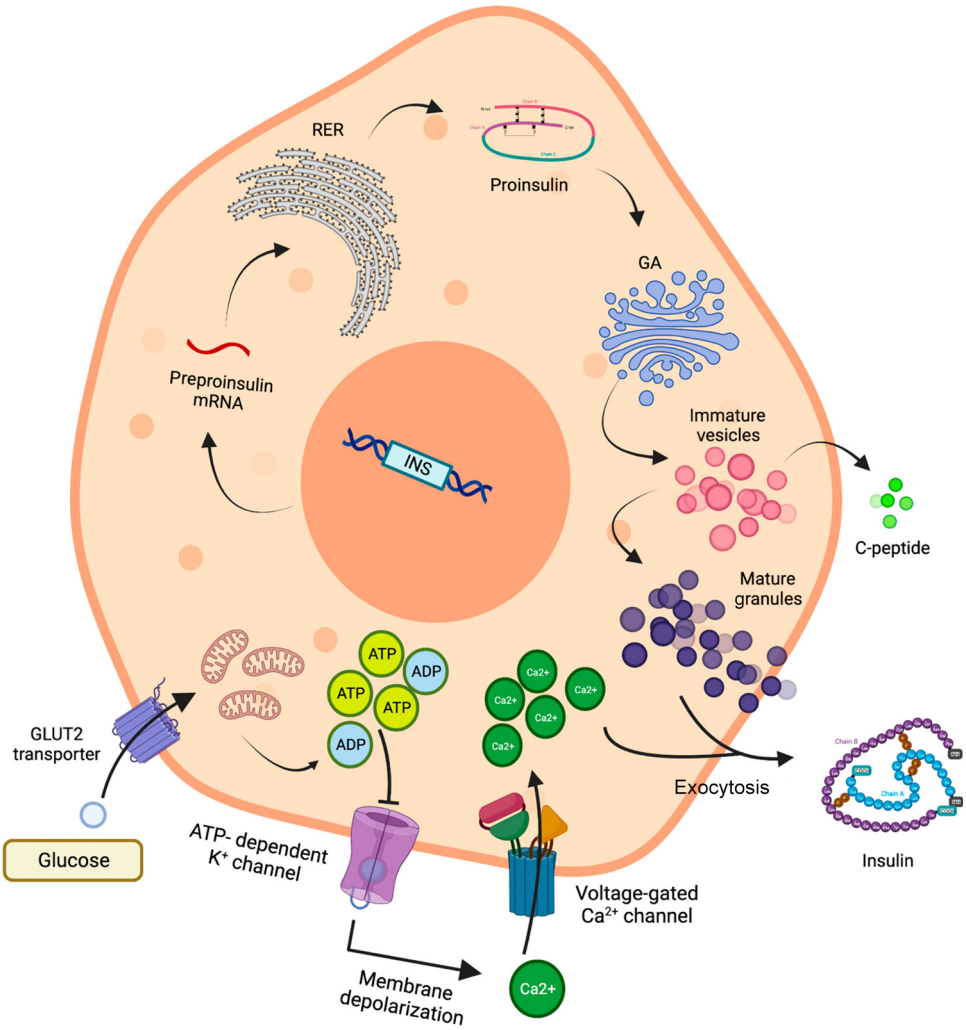

Figure 1. Main steps involved in insulin synthesis and glucose-stimulated insulin secretion (GSIS). Insulin is encoded by the *INS* gene and is originally synthesized as preproinsulin, which is directly translated in the rough endoplasmic reticulum (RER). Preproinsulin is cleaved here into proinsulin and then folded. Folded proinsulin then enters the Golgi apparatus (GA) and is packaged into immature vesicles where it is cleaved into C-peptide and active insulin. Insulin is finally stored in mature granules in a form that is available for secretion. Following an elevation of plasma glucose levels, glucose enters the β cell cytoplasmic environment via the GLUT2 transporter, and glucose metabolism results in increased ATP:ADP ratio which causes closure of ATP-dependent K^+ channels; this leads to (i) membrane depolarization, (ii) opening of voltage-gated Ca^{2+} channels (iii), an increase in intracellular Ca^{2+} levels, (iv) fusion of preformed secretory granules with the cytoplasmic membrane, and (v) insulin exocytosis.

the disease; on the other hand, β cell secretory function has a compensatory phase during which insulin secretion rises in response to insulin resistance [16].

Several studies in rodents [17–19] and humans [20–22] have demonstrated the ability of islets to compensate for increased peripheral insulin demand by increasing β cell mass and insulin secretion [23]. In this scenario, β cells appear to be capable of adapting to new physiological and pathological metabolic conditions by implementing plasticity mechanisms to increase their mass and maintain normal glucose levels [24–26]. However, insulin resistance is not invariably associated with high

glucose levels because several conditions including obesity [27,28], pregnancy [29,30], and polycystic ovary syndrome [31] are characterized by insulin resistance, increased insulin secretion, and increased β cell mass without hyperglycemia. Thus, β cells seem to be able to compensate for the augmented insulin demand occurring in many different metabolic states. In particular, we found that insulin-resistant subjects exhibited increased islet size, which was strongly inversely correlated with insulin sensitivity calculated during euglycemic hyperinsulinemic clamp (EHC), demonstrating that insulin resistance directly impacts on islet biology in nondiabetic humans by inducing an increase in β cell area to compensate for the increased insulin demand [32]. Alongside this prevailing view of the natural history of T2DM, in which the early decline of insulin sensitivity precedes and causes a progressive increase in insulin secretion, another pathophysiological hypothesis proposes that hyperinsulinemia is the primary trigger for insulin resistance [33]. This new theory comes from the evidence that, in some mildly glucose intolerant, obese, non-diabetic human subjects, hyperinsulinemia occurs during both the fasting and the postprandial state, without a detectable increase in blood glucose levels that is theoretically necessary to stimulate β cells to increase insulin secretion [33]. Possible mechanisms may include downregulation of insulin signaling; enhanced skeletal muscle conversion of glucose to lactate, a substrate for gluconeogenesis and hepatic glucose output; and insulin-enhanced inflammatory and cytokine pathways which can impair tissue responsiveness to insulin [33]. Studies on animals [34–40] and humans [41–46] further support this hypothesis, demonstrating that an inappropriate increase of insulin secretion, independent of insulin resistance, could be the first trigger for T2DM. However, further studies will be necessary to identify molecular mechanisms leading to primary insulin hypersecretion and to develop potential therapeutic approaches.

These data confirm the complexity of the regulation of insular responses to insulin resistance and the plasticity of islets as a mechanism for increased insulin secretion in the first phases of T2DM. However, this phenomenon seems to be unable to cope with β cell loss in the last stages of the disease, as observed in insulin-dependent T2DM. In fact, breakdown of the mechanisms regulating β cell secretory machinery and the inability of islets to compensate for the progressive worsening of insulin sensitivity leads to β cell failure and overt hyperglycemia [47].

Although the decline in islet function has traditionally been associated with β cell death and reduction of β cell mass [48], the most recent evidence suggests that this process may be due to disruption of the complex network of interactions between the environment and different molecular patterns, probably driven by increased metabolic demand and β cell workload [49]. Furthermore, *in vitro* studies on murine β cell lines and human pancreatic islets have identified endoplasmic reticulum (ER) stress as a mechanism leading to β cell failure and decreased insulin production in T2DM [50].

In this regard, we previously highlighted the crucial role of insulin resistance in the impairment of insulin synthesis and/or processing, and confirmed this in a model of increased β cell workload induced by acute surgical removal of 50% of β cell mass against a background of insulin resistance [51]. Furthermore, proinsulin biosynthesis has been demonstrated to increase up to 50-fold in response to insulin resistance [52]. An increased rate of proinsulin synthesis, together with alterations in the ER environment, can cause an overload of misfolded proinsulin – a possible key factor that could contribute to ER stress and subsequently to β cell impairment [53]. Indeed, increased circulating proinsulin levels and biosynthesis [54–57] represent the primary driver of ER protein overload in β cells, causing accumulation of misfolded proinsulin [58–60]. This leads to activation of the adaptive, proapoptotic, unfolded protein response (UPR) pathway and, if unresolved, to terminal UPR and ER stress [60–63]. In a recent study, we found that chronic increase in β cell workload induced by increased insulin demand and insulin resistance produces

ER stress and impairment of the β cell secretory machinery, as shown by an altered *in situ* proinsulin/insulin ratio, increased expression of ER stress marker genes, and a progressive decrease in markers associated with β cell identity [64], leading to dedifferentiation. Previous reports [65,66] proposed that glucotoxicity is another main factor possibly leading to β cell failure by inducing dedifferentiation. Further, increased noradrenergic innervation in T2DM islets may also play an important role in β cell dedifferentiation [67,68].

In addition, metabolic stress resulting from lipo- and glucotoxicity is able to activate the UPR pathway through numerous mechanisms, including inhibition of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump, activation of the inositol trisphosphate receptor (IP3), and direct toxicity on ER homeostasis, leading to the accumulation of misfolded insulin, islet amyloid polypeptide (IAPP), and reactive oxygen species (ROS) [69]. These events alter the physiological mobilization of Ca^{2+} from the ER, favoring proapoptotic signals and the degradation of proinsulin mRNAs, thus inducing the release of interleukin (IL)-1β, which recalls macrophages and increases insular inflammation [49].

These defects in the synthesis of insulin precursors and insulin itself, together with alteration of the secretory mechanisms, may be pivotal in leading to the β cell dysfunction and inadequate insulin secretion that characterize T2DM.

Impaired insulin secretion and T2DM natural history: a matter of 'phase'?

Cerasi *et al.* were the first to demonstrate the existence of a biphasic insulin secretion pattern in response to square-wave glucose infusion [70]. Within 10 min of a rapid elevation in plasma glucose levels, β cells start first-phase insulin secretion followed by a sustained second phase (Figure 2). Insulin plays a fundamental role in glucose homeostasis, and the magnitude of its effects depends on insulin concentration at the liver and in the interstitial fluids. In particular, insulin is secreted directly into the portal system, and this ensures a greater hepatic than peripheral insulinization (porto-systemic gradient). For these reasons, the liver is the most obvious target of first-phase insulin secretion [71]. Many studies on animals and humans [72–74] have shown that an increased insulin concentration at the portal level rapidly inhibits hepatic glucose production, even independently of an increase in peripheral plasma insulin concentration [75]. Thus, by acutely elevating liver sinusoidal insulin levels, first-phase insulin secretion brings about an inhibitory effect on hepatic glucose production. In addition, the first-phase insulin response can act on the liver through the interaction with glucagon: when the first-phase insulin response is lacking, the insulin-to-glucagon ratio is rapidly altered in favor of glucagon, leading to increased hepatic glucose production and hyperglycemia [76]. It should be noted that a clear-cut first phase occurs only when β cells are exposed to a rapidly changing glucose stimulus such as that one induced by intravenous glucose administration. By contrast, peripheral insulin concentration following glucose ingestion and early insulin response in particular do not show any clear sign of a biphasic shape. Compared to intravenous stimulation, oral glucose load elicits other factors, in addition to rising glucose levels, that contribute to stimulating β cell secretion – such as the neurally activated cephalic phase and incretin hormones secreted by the gastrointestinal tract in response to glucose and other nutrients. The cephalic phase corresponds to a rapid (≤2 min) and small (~30 pmol/l) insulin secretion from β cells evoked by the sight, taste, and smell of food, and is activated before a meal by pancreatic innervation [71]. Even though modest, this phenomenon still plays a significant physiological role because there seems to be an inverse relationship between the cephalic phase and the initial increase in plasma glucose concentration [77]. Furthermore, the incretin effect is responsible by itself for nearly 50% of insulin release after glucose ingestion, and hence plays an important role even in the early insulin response. There is experimental evidence supporting the conjecture that the first-phase insulin response to a rapid intravenous glucose challenge and the early insulin response to

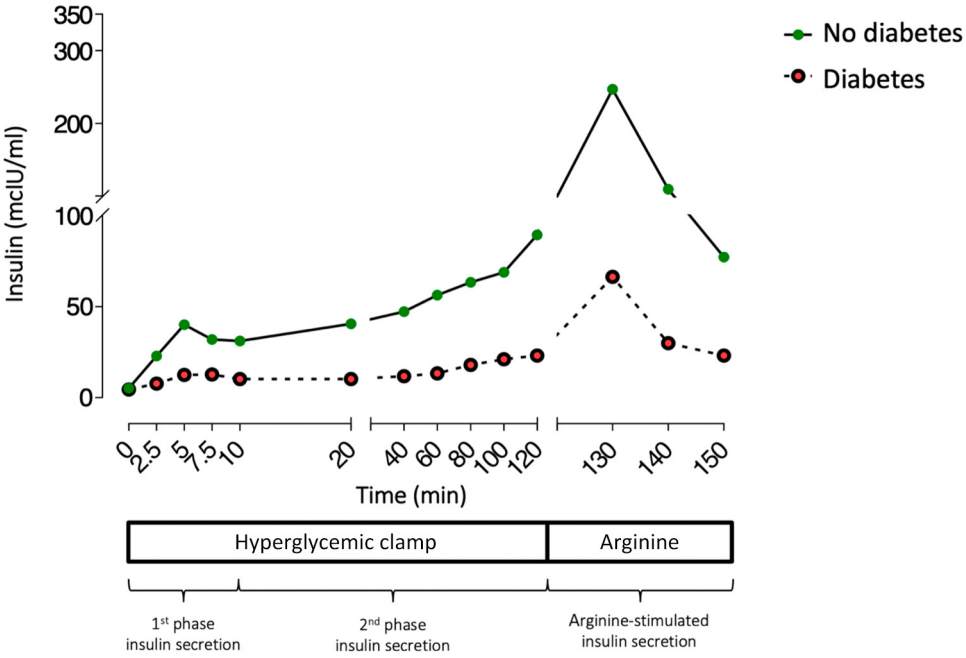

Figure 2. Insulin levels over time during hyperglycemic clamp and arginine administration in diabetic (dashed line) and non-diabetic (continuous line) subjects. The first phase of insulin secretion is characterized by a switch from a rapid increase to a rapid decrease in the insulin secretion rate during the first 10–20 min following a hyperglycemic stimulus. The second phase, which lasts several hours, is characterized by a lower but relatively constant insulin secretion rate compared to the first phase: it is responsible for 60–70% of total insulin release in response to a sustained hyperglycemic stimulus overtime. Note that both first/second-phase and arginine-stimulated insulin secretion are reduced in diabetic subjects. Abbreviation: mcIU/ml, micro-international units per ml.

oral glucose are emanations of common underlying β cell mechanisms. It is plausible that the β cell dynamic properties that can generate biphasic secretion in response to a rapid intravenous glucose infusion are the same as those operating with gradual entry of glucose from the gut. Thus, even though insulin secretion tends to lose its biphasic shape under physiological conditions, the early insulin response may be aimed at rapidly shifting glucose metabolism from the fasting to the prandial state by primarily acting on the liver [71,78–83].

In summary, insulin release is not intrinsically biphasic, but it can have a biphasic secretion pattern depending on the type and magnitude of glucose stimulation. In particular, biphasic secretion is elicited only when β cells are exposed to rapidly changing glucose concentrations (i.e., intravenous administration), whereas after an oral stimulus the first and second phases are not clearly distinguishable. Moreover, the dynamics allowing β cell machinery to determine a biphasic secretion pattern under certain conditions are the same as those ensuring prompt insulin release after a meal. As we will discuss below, loss of these dynamics is a common defect in T2DM even in its early stages, and can be a factor contributing to the development and progression of the disease.

The importance of first-phase insulin secretion in clinical practice

Although many factors (e.g., insulin resistance, inadequacy of glucagon suppression, and elevated FFAs levels after meals) may lead to increased glucose levels after a meal, impairment of the early insulin response has been identified as one of the major factors contributing to postprandial hyperglycemia in people with impaired glucose tolerance (IGT) or in the early stages of T2DM [7]. In this regard, a strong inverse correlation has been found between the efficacy of first-phase insulin

secretion and the early rise in plasma glucose during intravenous glucose administration [77]. Another inverse correlation has been observed between insulin levels detected at 30 min after an oral glucose tolerance test (OGTT) and 2 h blood glucose levels [84], showing that an effective early insulin response impacts positively on glucose tolerance to ensure more physiological glucose levels in the postprandial state. Another study showed that the rise in 2 h plasma glucose levels after OGTT was associated with decreased β cell glucose sensitivity (βCGS), a β cell functional parameter, in a group of normal glucose-tolerant (NGT) patients [85]. Furthermore, we have recently demonstrated that reduced βCGS and rate sensitivity, a functional parameter expressing the effectiveness of first-phase insulin secretion, can together predict diabetes appearance in a cohort of non-diabetic patients eligible for partial pancreatectomy after surgery determining ~50% acute removal of total pancreatic β cell mass [86]. This finding shows that β cell mass reduction *per se* is not sufficient to cause hyperglycemia, at least when β cell mass is reduced acutely, whereas the true determinant of diabetes onset is the already present prediabetic functional milieu (i.e., impaired first phase) [87]. These data together confirm the relevance of an ineffective first-phase insulin secretion in the history of diabetes onset even in the earliest stages.

The consequences of impaired first-phase/early insulin response and its clinical manifestation, namely postmeal hyperglycemia, have been analyzed in several epidemiological studies showing a strong association between postchallenge and postmeal hyperglycemia and cardiovascular mortality, regardless of fasting glucose values [88–92]. Furthermore, there is strong evidence for a causal relationship between postmeal hyperglycemia, oxidative stress and inflammation [93], carotid intima media thickness [94], and nitric oxide-mediated endothelial dysfunction [95,96], all of which are known markers of cardiovascular disease. Postmeal hyperglycemia is also linked to retinopathy [97] and cognitive dysfunction in elderly people [98]. In this regard, many mechanisms such as increased glomerular rate and renal flow, increased retinal blood flow, reduced conduction of motor and sensory nerves, and non-enzymatic glycation of proteins are well known to be pivotal for the development of the above-mentioned micro- and macrovascular complications [7].

In conclusion, based on current evidence, the primary mechanism driving impairments of insulin secretion is still unclear. As previously reported, our studies support the classical pathophysiological view according to which insulin resistance could be the *primum movens* that drives β cell failure. By contrast, other studies postulate that hypersecretion itself could be the primary trigger leading to reduced insulin secretion in some subjects, although further investigations will be necessary to clarify the possible role of primary insulin hypersecretion in non-diabetic and T2DM subjects, as well as its link to the insular environment and molecular setting. Whatever the *primum movens*, there is much evidence to show that early secretory defects are mainly characterized by an impairment of first-phase insulin secretion which has a direct impact on T2DM clinical manifestations and complications. In this scenario, we have shown that reduced rate sensitivity is a good marker of early first secretion impairment, even if its use in clinical practice is still limited [87].

Because postprandial hyperglycemia is an early feature of T2DM, it follows that normalization of postmeal glucose levels should be considered to be an important therapeutic goal of a personalized therapy already in the first stages of the disease.

Diet

Physical exercise and medical nutrition therapy are fundamental strategies in T2DM management, and their effectiveness in lowering glycated hemoglobin (HbA1c) levels has been amply demonstrated [99]. Among macronutrients, dietary carbohydrates are the main factors that influence

postmeal glucose levels (on the basis of their specific biological composition) a well as other widely used measures such as glycemic index and glycemic load [100]. Many studies have been conducted to test specific nutritional strategies for the control of postmeal hyperglycemia. In this regard, dietary carbohydrate restriction is re-emerging as an effective approach to glycemic control [101,102]. Given that the rise in blood glucose concentration after a meal is largely dependent on carbohydrate composition [103], reducing exogenous carbohydrate intake at each meal is a logical strategy to lower postprandial glucose [104–106]. Further, nutritional strategies favoring foods with low glycemic index and glycemic load, but high in soluble dietary fibers, have proved to be effective in improving the postmeal glycemic response in diabetic patients, in line with the recommendation from the International Diabetes Federation [107–109]. The effectiveness of these nutritional regimens could be explained by a lower rate of gastric emptying, leading to a lower rate of carbohydrate absorption in the gastrointestinal tract, thereby compensating for the impaired early insulin response and exerting a positive impact on the postmeal glycemic response [100].

Other coingested macronutrients, proteins in particular, have been shown to influence the magnitude of the postmeal glycemic excursion as a result of the so-called mixed meal effect. By being digested more slowly that carbohydrates, they are able to delay the peak glucose response and sustain late postmeal hyperglycemia [110]. In particular, whey proteins have been demonstrated to exert an insulinotropic effect on β cells, thus attenuating the postmeal glucose rise [111]. The bioactive peptides or amino acids generated during the digestion of whey protein may inhibit the enzyme dipeptidyl-peptidase 4 (DPP-4) and enhance the incretin effect, leading to increased incretin-induced insulin stimulation and improved postmeal glycemic control. In addition, whey protein is a rapid-acting protein and contains higher amounts of branched chain amino acids which also support the insulinotropic effect to reduce postmeal hyperglycemia [112].

Meal timing – breakfast in particular – can also affect glycemic fluctuations not only during the immediate postprandial period but even following the second meal of the day, probably because of the 'second meal phenomenon' (or Staub–Traugott effect), that describes the ability of a prior meal to improve glucose tolerance after a subsequent meal. In particular, a study found an increase in insulin secretion rate and early insulin response during lunch in a group of T2DM patients who had previously had breakfast compared to those who had not [113]. It is important to underline that all the other interventions mentioned above do not demonstrate specific improvement in first-phase insulin secretion and early insulin response because they seem to act mostly on reducing calorie intake and glucose load. By contrast, manipulating meal sequence can play a role in postmeal glycemic control by delaying gastric emptying rate and enhancing insulin secretion by increasing the incretin effect. Altering meal sequence can also include an increase in dietary fiber or obtaining a satiating effect through proteins and fats, leading to an overall reduction in total calorie intake and improvement in postmeal glycemic control, as in a study on a group of Japanese diabetic patients [114].

In summary, modifying and improving nutritional aspects (i.e., selecting an appropriate amount and type of carbohydrates, using specific dietary proteins, and manipulating mealtimes) has been shown to exert immediate beneficial effects on postmeal glucose tolerance. Further investigations are required into the efficacy, compliance, durability, and metabolomic implication of different nutritional strategies and their associations with pharmacological agents.

Pharmacotherapy

Among the pharmacological agents available for treatment of T2DM, sulfonylureas, glinides, DPP4 inhibitors, the newest GLP-1 receptor agonists (GLP-1 RAs), and a dual gastric inhibitory

polypeptide GIP/GLP-1 receptor agonist (tirzepatide) are known to exert positive effects in the management of T2DM via enhancement of insulin secretion, albeit with different mechanisms. Sulfonylureas, widely prescribed over the past 30 years, have been progressively abandoned in clinical practice owing to their side effects (hypoglycemia, weight gain) and poor cardiovascular safety. However, their stimulatory effect on insulin secretion (in particular, benzoic acid derivatives – glinides) has been shown to reduce postprandial glycemic excursions through a secretory stimulus that 'simulates' the first phase of secretion in patients with T2DM [7]. Further, over recent decades many studies have been conducted to test the effectiveness of non-secretagogue pharmacologic agents in improving specific insulin secretion parameters (Table 1).

DPP4 inhibitors, which represent a possible therapeutic approach for the management of T2DM, have demonstrated beneficial effects on the first phase of insulin secretion: in particular, vildagliptin has been shown to significantly improve the first-phase insulin response both in diabetic patients [115] after 3 months of treatment and in patients with impaired glucose tolerance after 6 weeks

Table 1. Clinical studies evaluating the effects of different non-secretagogue pharmacologic agents on specific β cell secretory parameters[a]

Year	Population	Intervention	Outcomes	Refs
2014	10 Diabetic subjects already under treatment with one antidiabetic drug (met, Sus, TZDs)	Vildagliptin 100 mg daily versus baseline (3 months)	↑ $AUC_{C\text{-}peptide}$ during the first 20 min of IVGTT ↑ CS1 during the first 5 min of IVGTT	[115]
2007	22 IFG subjects	Vildagliptin 100 mg daily versus placebo (6 weeks)	↑ AIR_g and ACR_g during IVGTT (+27% and +24% respectively)	[116]
1992	4 Non-diabetic and 11 diabetic subjects	GLP-1 (7–37) infusion versus placebo (30 min)	↑ Insulin secretion: fasting (during GLP-1 infusion) postmeal (after GLP-1 infusion) ↓ Postmeal glucose levels	[119]
2004	13 Diabetic subjects	Liraglutide (NN2211) 6 μg/kg daily versus placebo (1 week)	↑ AIR_g and ↑ ISR_{ARG} during HC ↓ P:I ratio during HC	[125]
2008	39 Diabetic subjects	Liraglutide 0.65 mg, 1.25 mg, or 1.9 mg daily versus placebo (14 weeks)	↑ $AUC_{insulin\ 0–10\ min}$ during FSIGTT (+34% in the 0.65 group; +118% in the 1.25 group; +103% in the 1.9 group) ↑ AIR_g during FSIGTT (similar to previous) ↑ ISR_{ARG} during FSIGTT (+32% in the 0.65 group; +114% in the 1.25 group; +94% in the 1.9 group)	[123]
2011	69 Diabetic subjects in treatment with metformin	Exenatide 20 μg t.i.d. (after titration from 5 μg b.i.d. to 10 μg b.i.d.) versus insulin glargine (starting from 10 IU daily and increasing the dose to reach fasting glucose levels <5.6 mmol/l) (52 weeks)	↑ AIR_{ARG} during HC in the EXE group compared to the GLAR group ↑ First-phase C-peptide secretion during HC in the EXE group compared to the GLAR group	[122]
2017	75 Diabetic subjects in treatment with diet and exercising and/or metformin	Semaglutide 1.0 mg weekly (after titration from 0.25 to 0.5 mg) versus placebo (12 weeks)	↑ $AUC_{insulin\ 0–10\ min}$ during IVGTT compared to placebo ↑ $ISR_{0–10\ min}$ during IVGTT compared to baseline in the semaglutide group ↑ ISR_{ARG} during IVGTT compared to placebo	[127]
2022	117 Diabetic subjects in treatment with lifestyle advice plus met with/without another antidiabetic drug	Tirzepatide 15 mg weekly versus semaglutide 1 mg weekly versus placebo (28 weeks)	↑ Incremental $ISR_{0–8\ min}$ during HC compared to baseline; compared to placebo; compared to semaglutide	[130]

[a]Key and abbreviations: ↑: increased; ↓: decreased; ACR_g, acute C-peptide response to glucose; AIR_{ARG}, arginine-stimulated C-peptide secretion; AIR_g, acute insulin response to glucose; AUC, area under the curve; b.i.d., twice daily; CS1, first-phase C-peptide secretion rate; EXE, exenatide; FSIGTT, frequent sampled intravenous glucose tolerance test; HC, hyperglycemic clamp; IFG, impaired fasting glucose; GLAR, insulin glargine; $ISR_{0–8\ min}$/$ISR_{0–10\ min}$, first-phase insulin secretion rate; ISR_{ARG}, arginine-stimulated insulin secretion; IU, international units; IVGTT, intravenous glucose tolerance test; met, metformin; P:I ratio, proinsulin-to-insulin ratio; Sus, sulfonylureas; T2DM, type 2 diabetes mellitus; b.i.d., two times per day; t.i.d., three times per day; TZDs, thiazolidinediones.

of treatment [116] by increasing GIP levels and thus enhancing insulin secretion. Furthermore, the VERIFY (Vildagliptin Efficacy in Combination with Metformin for Early Treatment of Type 2 Diabetes) study has demonstrated the durability of vildagliptin, particularly in combination with metformin, in patients with newly diagnosed T2DM in ensuring long-term benefits for glycemic control compared to metformin monotherapy. These findings could be attributed to insulin sensitization by metformin and restoration of β cell function, including improved first-phase insulin secretion, by vildagliptin [117,118]. These data suggest that DPP4 inhibitors can partially attenuate β cell dysfunction by primarily acting on the first-phase secretion deficit through potentiation of the deficient incretin effect and incretin-stimulated insulin secretion.

In this regard, in 1992 Nathan *et al.* [119] demonstrated that a 30 min intravenous infusion of GLP-1 caused an increase in insulin secretion during the meal. As expected, the early increase in plasma insulin concentrations during the infusion led to a reduced postprandial hyperglycemic peak. The effect on postprandial glycemic control persisted even after interrupting the GLP-1 infusion, demonstrating that early modulation of postprandial hyperglycemia can produce an overall improvement in glucose metabolism even without a decrease in plasma insulin levels. Nowadays, GLP-1 RAs are widely used in the therapeutic management of T2DM. GLP-1 RAs have several beneficial effects on β cell function, guaranteeing sustained glycemic control and preventing glucolipotoxicity [120,121]. In particular, exenatide was shown to improve first- and second-phase insulin secretion, as well as arginine-stimulated insulin secretion, in T2DM subjects compared to baseline and compared to insulin glargine after 52 weeks of treatment, even if this effect was lost after 4 weeks of treatment cessation [122]. Similarly, the daily administration of liraglutide produced, compared to placebo, an increase in first- and second-phase insulin secretion, an increase in arginine-stimulated insulin secretion [123–125], and an increase in homeostatic model assessment (HOMA-B), C-peptide levels, and a reduction in the proinsulin:insulin ratio, demonstrating benefits to the cellular processes that regulate the secretory function of β cells [126]. According to these data, a study [127] demonstrated that weekly administration of semaglutide, compared to placebo, significantly improved glycemic control and β cell function in treated subjects, positively impacting all β cell functional parameters, including increased first- and second-phase insulin secretion and decreased fasting and postprandial glucose and glucagon levels. More recently, tirzepatide, a dual GIP/GLP-1 receptor agonist, showed robust improvements in glycemic control and body weight without increased risk of hypoglycemia in adults with T2DM [128]. There was a significant reduction of proinsulin/insulin levels compared to placebo and dulaglutide [129], probably by positively impacting on the molecular mechanisms regulating the β cell secretory machinery. Moreover, in a recent study, tirzepatide, compared to placebo and semaglutide, was found to improve first-phase insulin secretion together with other β cellular functional parameters and insulin sensitivity [130].

In summary, mechanisms contributing to the genesis of β cell dysfunction, especially the first-phase secretion deficit, strongly influence the development of T2DM even in the early stages of its natural history; among these, postprandial hyperglycemia directly correlates with the effectiveness of early insulin response. It seems clear, therefore, that pharmacological treatments aimed at improving and restoring first-phase insulin secretion can significantly modify the natural history of the disease. In this scenario, DPP4 inhibitors, GLP-1 RAs, and the double GIP/GLP-1 receptor antagonist tirzepatide have demonstrated direct beneficial effects on first-phase insulin secretion, albeit in the absence of robust data concerning durability.

Assessment of insulin secretion in clinical practice: the role of C-peptide

The data in the previous sections demonstrate the great complexity of the mechanisms leading to impairments of the insulin secretion machinery and their relevance to the dynamics of T2DM

natural history, and show how these defects – reduced first-phase insulin secretion in particular – can be present even in the early stages of the disease, directly impacting on clinical manifestations and outcomes. Because many nutritional strategies and pharmacological agents have been reported to exert positive effects on insulin secretion, it seems clear that identifying early secretory defects in individuals at risk of or with newly diagnosed T2DM could be a valid strategy to address clinical decision-making to restore residual β cell secretory capacity. The use of formal stimulation tests is currently proposed as the most accurate approach to the clinical investigation of insulin secretion. In addition, stimulation tests also allow evaluation of the dynamics of β cell response to provocative stimuli. In particular, the HC technique is the gold standard for the assessment of insulin secretion [131] but it is rarely performed in clinical settings owing to its invasiveness, cost, and technical complexity. It is therefore necessary to identify a non-invasive, inexpensive, and practical method that best reflects the physiology of insulin secretion. Since the discovery of proinsulin, plasma or urinary C-peptide has been used as a biomarker of β cell dysfunction because it is secreted in equimolar amounts with insulin but is not processed by the liver [132]. By mathematically modeling (by deconvolution) the time-sequentially measured C-peptide and insulin concentrations under various conditions that stimulate β cells, such as HC, mixed meal test (MMT), and oral glucose tolerance test (OGTT), many β cellular parameters of secretion can be deduced, including first-phase insulin secretion rate ($ISR^{1st\ phase}$, calculated only during HC stimulation) and rate sensitivity (RS), and both specifically correlate with the effectiveness of the first phase [87]. Other dynamic tests, such as the glucagon stimulation test (GST) and the arginine stimulation test (AST), are considered to be validated tests for estimating residual secretory capacity in individuals affected by autoimmune diabetes [133], although possible confounding factors (e.g., glucotoxicity, glucose variability, hypoglycemia, differences in the incretin effect, insulin resistance, and renal function) must be considered in the interpretation of results. Furthermore, it is important to consider that the C-peptide values detected under stimulation depend on the nature of the stimulus itself (e.g., intravenous glucagon administration vs. meal). Moreover, a recent study showed better validity and repeatability of AST compared to GST for the evaluation of residual β cell function [134]. Recently, circulating 1,5-anhydroglucitol has been proposed as a biomarker of β cell mass independently of diabetes phenotype, although further studies are needed for validation and to identify a possible link with effectiveness of insulin secretion [135].

Compared to dynamic tests, fasting C-peptide is an expression of the static response of β cells to plasma glucose values, whereas the 'random' (non-fasting) C-peptide is primarily affected by the incretin effect of the previous meal [136]. Compared to stimulation tests, fasting and random C-peptide are more easily usable in clinical practice because only blood sampling is required. However, the use of C-peptide to address clinical decisions in the management of T2DM remains under debate. There is no strong evidence showing a clear role of C-peptide in terms of risk assessment and response to therapies. As discussed above, the early stage of the natural history of T2DM is characterized by hyperinsulinemia in response to insulin resistance, followed by β cell functional decline. In this phase, elevated C-peptide levels are strongly associated with a higher risk of progression to overt diabetes, with a stronger relationship compared to insulin levels alone [137,138]. It follows that the use of C-peptide for estimating β cell function in T2DM can be strongly influenced by concomitant insulin resistance, and may therefore be misleading for clinical decisions. In general, most potential clinical uses of C-peptide in T2DM remain hypothetical: to date, there is no evidence for the use of C-peptide in clinical practice except under stimulation and with appropriate mathematical modeling. Future clinical studies will be necessary to clarify the utility of C-peptide in T1DM and T2DM differential diagnosis and in clinical decisions. Furthermore, it will also be necessary to evaluate and possibly establish, through a precision medicine approach, clear C-peptide cut-offs that can find effective clinical use.

Concluding remarks

Our review of insulin secretion defects in the context of T2DM reveals several important knowledge gaps. A main goal in clinical practice is to identify new biomarkers to define insulin secretory patterns and concomitant β cell dysfunctional features so as to improve patient treatment personalization and outcome. Further, in-depth evaluation of insulin secretion in patients with prediabetes and/or early-onset T2DM will be necessary to understand whether insulin secretory defect can define specific patient clusters and how it can be reverted with current therapeutic strategies.

In particular, we show the relevance of first-phase insulin secretion to glucose homeostasis and analyze the consequences of its reduction on the clinical manifestations – in particular on postprandial hyperglycemia – and outcomes of the disease. In this context, restoring β cell function could be the game-changer in preventing and treating diabetes, as demonstrated by the Diabetes Remission Clinical Trial (DiRECT) in which diabetic subjects showed improved β cell function and partial restoration of first-phase insulin secretion after weight loss over a 2 year follow-up [139]. However, despite weight loss and diabetes 'remission', first-phase insulin secretion did not return to normal, suggesting that irreversible defects could affect β cell function. Investigating the mechanisms underlying defects in insulin secretory machinery will help to identify new potential therapeutic approaches to prevent β cell dysfunction and diabetes onset.

In conclusion, we emphasize the importance of β cell dysfunction and alteration of the secretory machinery in the pathogenesis and natural history of T2DM and their relevance to the clinical manifestation of the disease. Clinical identification of early secretory defects is an unmet need to treat patients at risk of T2DM by timely improvement of β cell function and by halting or slowing the decline towards relative and absolute insulin deficiency (see Outstanding questions).

Outstanding questions

Is insulin resistance the very first *primum movens* leading to insulin deficiency in T2D? What is the role of primary insulin hypersecretion in the decline of β cell function?

What are the intrainsular and molecular mechanisms leading to first-phase deficiency at the early stages of T2D? Are there any strategies to delay or even block them?

Should we put more effort into identifying an easy, non-invasive, and cost-effective but still accurate way to detect early secretory defects in people at risk or with overt T2D?

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Declaration of interests

The authors declare no conflicts of interest.

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