

# Gastric Inhibitory Polypeptide and Glucagon-Like Peptide-1 in the Pathogenesis of Type 2 Diabetes

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The incretin effect denominates the phenomenon that oral glucose elicits a higher insulin response than does intravenous glucose. The two hormones responsible for the incretin effect, glucose-dependent insulintropic hormone (GIP) and glucagon-like peptide-1 (GLP-1), are secreted after oral glucose loads and augment insulin secretion in response to hyperglycemia. In patients with type 2 diabetes, the incretin effect is reduced, and there is a moderate degree of GLP-1 hyposecretion. However, the insulintropic response to GLP-1 is well maintained in type 2 diabetes. GIP is secreted normally or hypersecreted in type 2 diabetes; however, the responsiveness of the endocrine pancreas to GIP is greatly reduced. In ~50% of first-degree relatives of patients with type 2 diabetes, similarly reduced insulintropic responses toward exogenous GIP can be observed, without significantly changed secretion of GIP or GLP-1 after oral glucose. This opens the possibility that a reduced responsiveness to GIP is an early step in the pathogenesis of type 2 diabetes. On the other hand, this provides a basis to use incretin hormones, especially GLP-1 and its derivatives, to replace a deficiency in incretin-mediated insulin secretion in the treatment of type 2 diabetes. *Diabetes* 53 (Suppl. 3):S190–S196, 2004

## INCRETIN HORMONES

The study of gut hormones as stimulators of secretion of the endocrine pancreas has a long history, probably starting with attempts to therapeutically administer intestinal mucosal extracts (containing putative insulintropic hormones) in patients with diabetes (1). Due to improvements in methods to quantify plasma concentrations of potential incretins (2,3) and in the ability to estimate insulin secretion in vivo (4–6), a clear picture of the true physiological importance of the major incretin hormones, gastric inhibitory polypeptide, also interpreted as glucose-dependent insulintropic polypeptide (GIP), and glucagon-like peptide-1 (GLP-1), has emerged (7–11). From the

beginning (1), the study of intestinal incretin hormones has been driven by the hope to better understand the pathogenesis of diabetes, especially that of type 2 diabetes, or to find new treatments based on properties of gut-derived insulintropic agents. While the latter has been the object of several comprehensive reviews in recent years (9,12,13), the former will be the topic of the present article.

## QUANTIFICATION OF THE INCRETIN EFFECT

The incretin effect is a phenomenon in which oral glucose (or any other way for the administration of glucose to not bypass its absorption from the gut) elicits a much higher insulin secretory response than an intravenous infusion of glucose with similar glycemic rises (14,15) or if the same amount of glucose was given by both routes (16). In the latter case, glucose concentrations were much higher with intravenous rather than oral glucose administration (16). State of the art for exactly quantifying the incretin effect is a comparison of the insulin secretory response after oral and “isoglycemic” intravenous glucose (i.e., an infusion leading to a similar glycemic profile as after oral glucose). It has been noted that the contribution of the incretin effect to insulin responses after oral glucose is greater than that based on the measurement of C-peptide concentrations or insulin secretion rates derived thereof by deconvolution techniques (14,15). This difference points to additional changes in insulin clearance (first-pass hepatic elimination), which are greater after oral than intravenous glucose (14,17).

The quantitative impact of the incretin effect depends on the size of the glucose load and ranges between 20 and 60% of the total insulin secretory response in healthy subjects (14–16,18).

In patients with type 2 diabetes, a reduced or absent incretin effect has been described (14). This finding prompted examinations into the secretion and insulintropic action of incretin hormones in patients with type 2 diabetes, in their first-degree relatives (a high-risk group for developing diabetes later in life) (19), and in subjects with impaired oral glucose tolerance (who are also at high risk) (20,21). In first-degree relatives, no quantitative difference in the incretin effect was found ( $62 \pm 5\%$  vs.  $64 \pm 6\%$  contribution to C-peptide responses after 75 g oral glucose) (22). The incretin effect in subjects with impaired glucose tolerance has not been reported but appears to be reduced to a much lesser degree (if at all) than in patients with type 2 diabetes. These observations, however, were based on a small number of subjects (M.A.N., W. Creutzfeldt, unpublished observations).

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DPP4, dipeptidyl peptidase 4; GIP, glucose-dependent insulintropic hormone; GLP-1, glucagon-like peptide-1.

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### GLP-1 SECRETION

Plasma GLP-1 concentrations rise after meals or oral glucose, sucrose, or fat loads (2,23–25). Typical basal (fasting) concentrations are  $\sim 5$  pmol/l, and peak concentrations of  $\sim 15$ – $40$  pmol/l are observed 1 h after nutrient ingestion. These numbers refer to the total amount of GLP-1 that has been secreted, including intact (biologically active) GLP-1 (7-36 amide or 7-37) and the dipeptidyl peptidase 4 (DPP4)-degraded form (9-36 amide or 9-37), which is devoid of insulinotropic activity (26–29).

In type 2 diabetic patients, contradicting information has been published regarding the secretion of GLP-1 in response to oral glucose. Both elevated and reduced postchallenge responses have been described in earlier studies (30,31). In recent years, larger cohorts have been studied with more elaborate methods. Uniformly, a slight reduction in GLP-1 response has been found in comparison to nondiabetic subjects, especially during the second hour after the nutrient stimulus (32,33). Vilsbøll et al. (32) also examined the plasma concentrations of intact, biologically active GLP-1 and confirmed a reduced response in patients with type 2 diabetes. The percent contribution of intact GLP-1 relative to total GLP-1 was similar in patients with type 2 diabetes and healthy control subjects. In line with this observation, DPP4 activity in plasma has not been found to be different between patients with type 2 diabetes and healthy control subjects.

Toft-Nielsen et al. (33) also described a slightly reduced GLP-1 secretion in obese subjects with impaired glucose tolerance. Although ATP-dependent  $K^+$  channels have been described in GLP-1-producing L-cells (34), there is no clear influence of sulfonylurea pretreatment on GLP-1 secretion. It is not known whether improving metabolic control would normalize GLP-1 responses to nutrient ingestion (e.g., whether the GLP-1 secretion abnormality is a primary or secondary phenomenon).

In first-degree relatives of patients with type 2 diabetes, GLP-1 secretion after oral glucose (22) and meals (35) was unchanged relative to healthy control subjects.

The question arises whether small differences (by  $\sim 5$  pmol/l) (32,33) in the late phase of nutrient-induced GLP-1 secretion are of sufficient importance to have consequences for glucose metabolism. The answer probably is not because of multiple reasons. First, even in healthy subjects, antagonizing the action of exogenous GLP-1 ( $0.3$  pmol  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ , leading to increments in plasma GLP-1 concentrations of  $\sim 12$  pmol/l) by using exendin (9-39), a GLP-1 receptor antagonist (36,37), had only minor effects on insulin levels. Second, using the same antagonist to block GLP-1 actions after a liquid mixed meal enhanced rather than reduced insulin responses (38). The GLP-1 increment induced by the meal was  $\sim 30$  pmol/l in this study. The paradoxical rise in insulin is most likely explained by an acceleration in gastric emptying prompted by the GLP-1 antagonist (9). Third, the effect of infusing GLP-1 at a dose of  $0.5$  pmol  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ , leading to increments in plasma GLP-1 concentrations of  $\sim 25$ – $40$  pmol/l, on both glucose profiles and insulin secretion were minor in patients with type 2 diabetes (39).

Based on these studies, a minor reduction in GLP-1 secretion as demonstrated in patients with type 2 diabetes

cannot be expected to have a significant impact on insulin secretion and glucose metabolism.

### INSULINOTROPIC (AND OTHER) GLP-1 ACTIONS

Soon after the discovery of GLP-1, it became clear that GLP-1 is active in patients with type 2 diabetes, in contrast to the insulinotropic action of GIP, which is clearly reduced or even lost (31), and that the activity of GLP-1 infused at pharmacological concentrations would be sufficient to fully normalize fasting (40–45) and postprandial (42,46) glucose concentrations. Notably, in patients with type 2 diabetes in good metabolic control on treatment with diet/exercise with or without oral antidiabetic drugs, there was no significant difference in the insulin secretory response to glucose clamped at  $8.5$  mmol/l in comparison to age- and weight-matched healthy subjects (31). However, in other cohorts, the insulinotropic response to GLP-1 was somewhat impaired in patients with type 2 diabetes (39), and with higher fasting glucose concentrations, the responsiveness to GLP-1 is clearly reduced (33).

In contrast to GLP-1 actions at pharmacological plasma concentrations (such as seen during the infusion of  $1.2$  pmol  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ ) (31,40), significant insulinotropic responses at doses mimicking a physiological “replacement” of postprandial GLP-1 increments have failed to significantly stimulate insulin secretion in patients with type 2 diabetes in some (31) but not in all (47) studies. This adds further evidence against the small differences in GLP-1 secretion typical for patients with type 2 diabetes being of pathophysiological importance.

GLP-1 is fully effective in subjects with impaired glucose tolerance (45,48). GLP-1 effects in first-degree relatives of patients with type 2 diabetes have not been studied.

### GIP SECRETION

The secretion of GIP in response to oral glucose or mixed meals has been examined in numerous studies. Using different radioimmunological methods, most studies agree that GIP responses, on average, are higher in patients with type 2 diabetes than in healthy control subjects (49–53). Creutzfeldt et al. (52) suggested a bimodal distribution with GIP hypo- as well as hyper-secretors among 141 type 2 diabetic patients studied. The overall differences between diabetic and healthy subjects, however, were minor on average, and other studies have not detected significant differences between healthy controls and type 2 diabetic patients (32,33). In subjects with impaired oral glucose tolerance, GIP responses appear to be normal (33). In first-degree relatives of patients with type 2 diabetes studied during a 24-h period with regular meals, the GIP response after breakfast and lunch was significantly greater than in control subjects without diabetic relatives. The 24-h integrated incremental GIP response was also significantly greater (35). In our own study of first-degree relatives, no significant difference (but a similar trend) was detected (22).

### INSULINOTROPIC GIP ACTIONS

Earlier studies used GIP of the porcine sequence and glycemic conditions that were less controlled than during a hyperglycemic clamp. The amino acid sequence difference between porcine and human GIP caused differential

TABLE 1  
Insulinotropic effects of GIP in patients with type 2 diabetes

Study: author, year (ref. no.)	Conditions (glycemia)	GIP amino acid sequence	Dose (pmol · kg <sup>-1</sup> · min <sup>-1</sup> )	Duration (min)	Stimulation of insulin secretion (% of normal)	Comments
Amland et al., 1985 (56)	Fasting hyperglycemia (~11 mmol/l)	Porcine	5.0	20	~7 to ~13 mU/l	No effect in normoglycemic control subjects* (glucose dependence of insulinotropic action of GIP)
Jorde and Burhol, et al., 1987 (57)	Glucose infusion hyperglycemia (~17 mmol/l)	Porcine	3.4	20	~18 to ~42 mU/l	Minor insulinotropic effects, no healthy control group
Jones et al., 1987 (58)	Glucose infusion (25 g/30 min)	Porcine	0.75	30	~20%	Type 2 diabetic patients studied at higher glucose concentrations
Krarup et al., 1987 (59)	Hyperglycemic clamp (~8 mmol/l)	Porcine	2.0	30	~15%	First comparison at equivalent glycemic level
Nauck et al., 1993 (31)	Hyperglycemic clamp (~8.5 mmol/l)	Human	0.8 and 2.4	60 (each)	~43%	Insulinotropic GLP-1 effects well preserved (~74% of normal) in the same type 2 diabetic patients
Meier et al., 2001 (60)	Hyperglycemic clamp (~8 mmol/l)	Human	2.0	60	~32%	Reduced insulinotropic effect of GIP also in first-degree relatives (~65% of normal)
Vilsbøll et al., 2002 (61)	Hyperglycemic clamp (~15 mmol/l)	Human	4 and 16	240	<10%	Only "late" response diminished, insulin secretory response to GIP bolus injection only reduced like that to GLP-1 (by ~45%)

\*Glucose dependence of insulinotropic actions of GIP (7) prevents effects in normoglycemic healthy control subjects.

affinity to GIP antisera, and thus it was difficult to exactly compare plasma concentrations of endogenous (human) GIP and infused (porcine) GIP (54,55). Nevertheless, all studies found only minor insulinotropic effects of exogenous GIP in patients with type 2 diabetes (31,56–61) (Table 1). If a healthy control group was compared under similar glycemic conditions, insulin secretory responses to exogenous GIP were shown to be greatly reduced in patients with type 2 diabetes (31,59,60), even when extremely high doses of GIP leading to far supraphysiological plasma concentrations were used (61). GIP at a dose leading to a "physiological replacement" of postprandial plasma concentrations (0.8 pmol · kg<sup>-1</sup> · min<sup>-1</sup>) was without significant effect on parameters of insulin secretion (31).

Taken together, these studies suggest that a major abnormality of the entero-insular axis in patients with type 2 diabetes is reduced insulinotropic activity of GIP. Given the importance of GIP as a physiological incretin hormone, this reduced responsiveness may well explain the reduced incretin effect typical for type 2 diabetes (14) and could contribute to the abnormalities of insulin secretion associated with this condition (6,62). Based on the observation that also in a significant proportion (~50%) of

nondiabetic first-degree relatives of patients with type 2 diabetes a reduced GIP effect can be observed (60), it has been postulated that reduced expression of  $\beta$ -cell GIP receptors is an early, potentially genetically determined step in the pathogenesis of type 2 diabetes (63). Indeed, in an animal model of type 2 diabetes, reduced number and activity of GIP receptors have been described (64). It is, however, not known at what stage in the pathogenesis of type 2 diabetes such a deficient signaling via GIP receptors occurs, and if it can be quantitatively modified by glycemic control, nutrition, or other environmental factors. It remains a challenge to explain why insulinotropic actions of GLP-1 (31,39,41) or its derivatives (65) are relatively well preserved in patients with type 2 diabetes, while GIP effects are greatly abnormal. This is the more surprising since GIP and GLP-1 share many common features in their mode of action, involving the majority of mechanisms of postreceptor signaling (11,66–68), primarily via cAMP and protein kinase A. Recent studies by Vilsbøll et al. (61) make it unlikely that the GIP receptors of pancreatic  $\beta$ -cells would be defective in patients with type 2 diabetes. They reported a relatively well-preserved insulinotropic effect of GIP injected intravenously as a bolus. The insulin response to this bolus was somewhat reduced in compar-

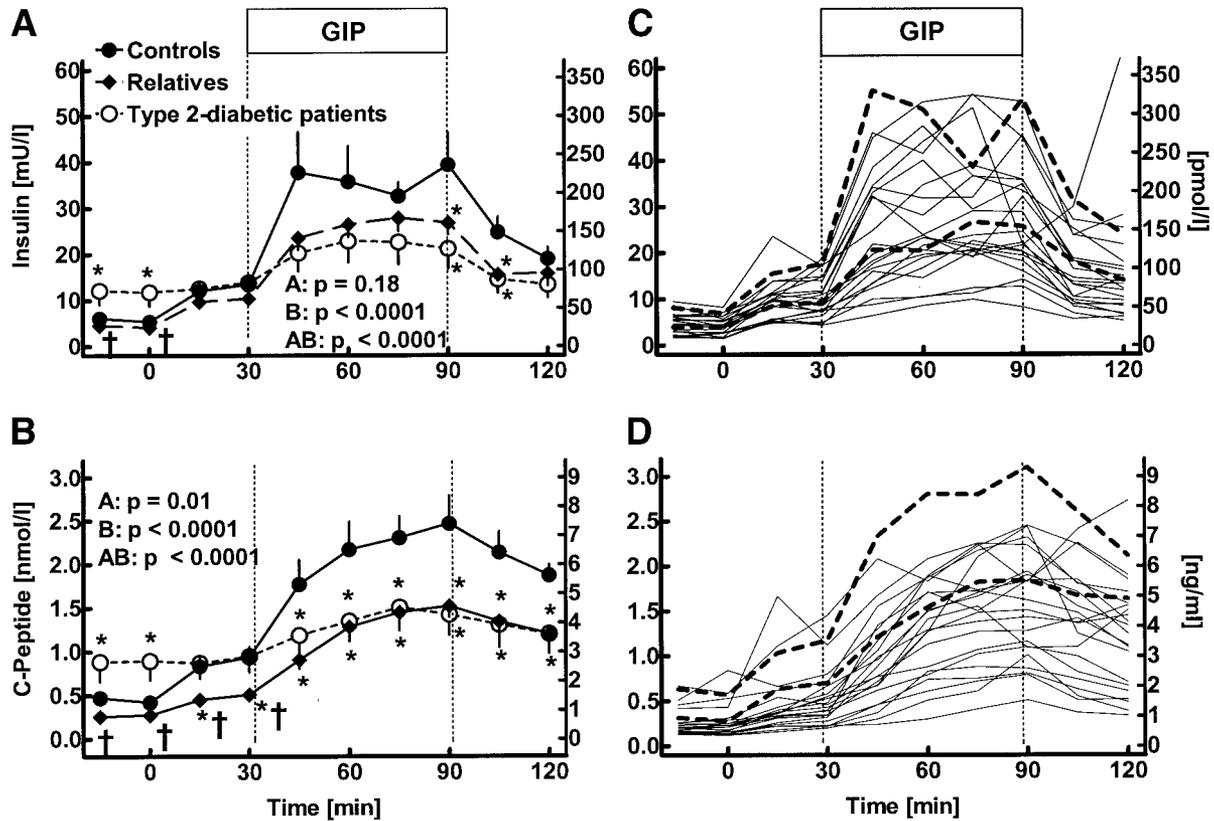


FIG. 1. Effect of an intravenous infusion of GIP during hyperglycemic clamp experiments ( $\sim 145$  mg/dl; 8 mmol/l) in healthy control subjects without diabetic relatives ( $\bullet$ ), in type 2 diabetic patients ( $\circ$ ), and in first-degree relatives of type 2 diabetic patients ( $\blacklozenge$ ) on insulin (A and C) and C-peptide (B and D) concentrations. *P* values were the result of repeated-measures ANOVA (A: by group; B: with time; A and B: interaction of group assignment and time). \*Significant differences at specific time points ( $P \leq 0.05$ , Duncan's post hoc test). In the right panels, individual insulin (C) and C-peptide (D) responses in first-degree relatives of patients with type 2 diabetes are shown. Dashed lines are 95% confidence intervals for responses in healthy control subjects. Approximately half of the relatives have subnormal insulin and C-peptide responses. Modified from Meier et al. (60).

ison to that in control subjects, but only to the same degree as for a similar bolus administration of GLP-1. Only when GIP was infused over longer periods (up to 240 min) during hyperglycemic clamps (glucose concentration  $\sim 15$  mmol/l) could a clear reduction in insulinotropic effectiveness, even at vastly supraphysiological GIP doses, be demonstrated (61). This finding suggests a rapid desensitization of the signaling via GIP receptors in type 2 diabetic patients, the molecular nature of which needs to be studied in greater detail. It might be related to GIP hypersecretion (vide supra), chronic hyperglycemia (69), other metabolic abnormalities, or genetic traits. Along the same line, in first-degree relatives of type 2 diabetic patients, GIP bolus injection (rather than continuous infusion) (60) did not reveal differences in insulinotropic responses (62).

If the impaired insulinotropic response to GIP in normal glucose tolerant first-degree relatives of patients with type 2 diabetes is of pathophysiological importance, the subgroup with a subnormal stimulation of insulin secretion by GIP (Fig. 1) should be at especially high risk to progress to diabetes. We are currently following up our cohort (60) to assess their glucose tolerance status. After 4 years, only in a few subjects was a deterioration in glucose tolerance noted, and there was no clear difference related to their previous insulinotropic response with GIP infused during a hyperglycemic clamp (M.A.N., B.B., J.J.M., unpublished observations).

#### GIP/GLP-1 RECEPTOR KNOCKOUT STUDIES

The quantitative impact of incretin hormones can be derived from studies of animals with a targeted disruption of the GIP (71) and GLP-1 (72,73) receptor genes. In both cases, oral glucose tolerance is diminished. The disruption of signaling through a single incretin receptor may not fully disclose the importance of that particular pathway, because compensatory mechanisms may be active. For example, GLP-1 receptor knockout mice display enhanced secretion of and sensitivity to GIP (74). Nevertheless, double incretin receptor knockout mice, which lack the possibility for compensation through any known important incretin hormone, do not present with an overtly diabetic phenotype (75,76). Therefore, it is unlikely that impairments in incretin secretion and insulinotropic action alone explain the phenotype of type 2 diabetes.

#### CONCLUSIONS AND HYPOTHESIS

Although clearly and uniformly a reduced insulinotropic response to exogenous GIP has been described in patients with type 2 diabetes (Table 1), and despite the fact that such a secretory abnormality can be found in  $\sim 50\%$  of first-degree relatives (60), it must now be considered unlikely that type 2 diabetes is simply accompanied (or even preceded) by an underexpression of endocrine pancreatic GIP receptors as previously postulated by us (63). The reasons are 1) a relatively well-preserved insulin

## Consequences of impaired GIP action in the pathogenesis of type 2 diabetes

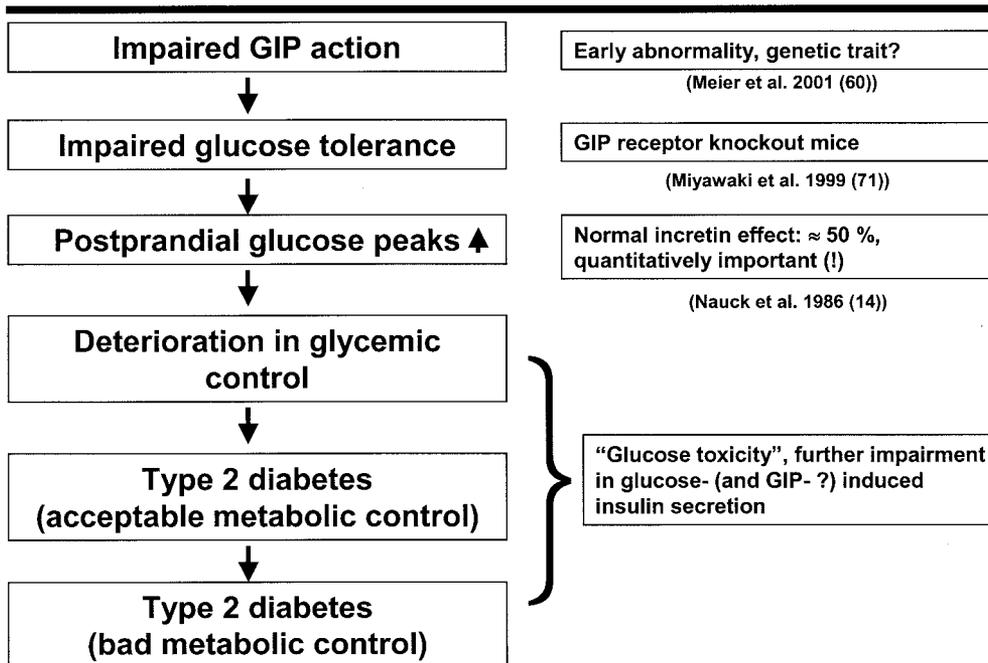


FIG. 2. Hypothetical sequence of events concerning the insulinotropic action of GIP in the pathogenesis of type 2 diabetes. GIP may play a role in the initiation of impaired  $\beta$ -cell function in first-degree relatives of type 2 diabetic patients. However, GIP action may be reversibly impaired at later stages in the development of type 2 diabetes.

secretory response to acute stimulation with a GIP bolus injection (61,70); 2) an unimpaired incretin effect in subjects characterized by a reduced insulinotropic responsiveness toward GIP during hyperglycemic clamps (22); and 3) the failure of a reduced insulinotropic response to exogenous GIP to predict deteriorations in oral glucose tolerance (M.A.N., B.B., J.J.M., unpublished observations.)

Nevertheless, abnormalities in the response to GIP may theoretically initiate the process leading from normal to impaired glucose tolerance (Fig. 2), as suggested by studies with incretin receptor knockout mice (71). Given the quantitative impact of a normal incretin effect to the overall insulin secretory response to oral glucose (14–16,18,77), and the quantitatively significant reduction of the incretin effect in patients with type 2 diabetes (14) along with the reduced insulinotropic effectiveness of GIP (Table 1), it is very likely that this contributes to the phenotypic abnormalities in  $\beta$ -cell function typical for type 2 diabetic patients (6,62). Such processes may be the initial trigger to the deterioration of glucose tolerance, or mediate, via “glucose toxicity” or other mechanisms, the loss of glucose control also at later stages of type 2 diabetes (Fig. 2). These aspects require more detailed studies into the secretion and action of incretin hormones in patients with type 2 diabetes, their first-degree relatives, and other high-risk groups. Such knowledge will certainly provide the pathophysiological background required for tailoring incretin-based therapeutics to the needs of patients or subjects at risk to develop type 2 diabetes.

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