

Pulsatile Insulin Release From Islets Isolated From Three Subjects With Type 2 Diabetes

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Plasma insulin in healthy subjects shows regular oscillations, which are important for the hypoglycemic action of the hormone. In individuals with type 2 diabetes, these regular variations are altered, which has been implicated in the development of insulin resistance and hyperglycemia. The origin of the change is unknown, but derangement of the islet secretory pattern has been suggested as a contributing cause. In the present study, we show the dynamics of insulin release from individually perfused islets isolated from three subjects with type 2 diabetes. Insulin release at 3 mmol/l glucose was $10.5 \pm 4.5 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{s}^{-1}$ and pulsatile ($0.26 \pm 0.05 \text{ min}^{-1}$). In islets from one subject, 11 mmol/l glucose transiently increased insulin release by augmentation of the insulin pulses without affecting the frequency. Addition of 1 mmol/l tolbutamide did not increase insulin release. In islets from the remaining subjects, insulin release was not affected by 11 mmol/l glucose. Tolbutamide transiently increased insulin release in islets from one subject. Insulin release from four normal subjects at 3 mmol/l glucose was $4.3 \pm 0.8 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{s}^{-1}$ and pulsatile ($0.23 \pm 0.03 \text{ min}^{-1}$). At 11 mmol/l glucose, insulin release increased in islets from all subjects. Tolbutamide further increased insulin release in islets from two subjects. It is concluded that islets from the three individuals with type 2 diabetes release insulin in pulses. The impaired secretory response to glucose may be related to impaired metabolism before mitochondrial degradation of the sugar. *Diabetes* 51:988–993, 2002

The discovery of plasma insulin oscillations dates back to 1977 from studies in the fasting monkey (1). Two years later, the insulin pulses were also demonstrated in humans (2). The relevance of the variations in plasma insulin was implicated when it was shown that the pattern was altered in individuals with diabetes (3). In studies in which insulin was administered either continuously or in pulses, the importance of plasma insulin oscillations was evaluated. In normal individuals, in whom the endogenous insulin production was suppressed by somatostatin, pulsatile delivery of insulin had a greater hypoglycemic effect than continuous delivery (4–6). Also, in individuals with diabetes, pulsatile delivery of

insulin was superior to continuous delivery (6–8). The greater hypoglycemic action of pulsatile delivery is probably related to the expression of insulin receptors on target tissue. When hepatocytes were perfused with either a constant or an oscillatory insulin concentration, the receptor expression was significantly higher in hepatocytes exposed to the oscillatory insulin concentration (9).

Despite numerous *in vivo* and *in vitro* studies, the origin of the generation of the plasma insulin oscillations is still unclear. Defective pulse generation in the β -cell was early suggested as a possible cause of the reported “brief, irregular oscillations” (3). This proposal was based on changes in the normally oscillatory cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$), which was thought to generate pulsatile insulin release from the β -cell (10). In the presence of β -cell toxins, the oscillatory $[\text{Ca}^{2+}]_i$ disappeared (11). Indeed, such alterations in the $[\text{Ca}^{2+}]_i$ oscillations have been suggested as an early indicator of impairment of the pancreatic β -cell (12). However, transformation of the $[\text{Ca}^{2+}]_i$ pattern does not have to be correlated with an altered, nonpulsatile release of insulin because pulsatile insulin release can occur under conditions when $[\text{Ca}^{2+}]_i$ is nonoscillatory (13–16). In one of the very few studies with islets from glucose-intolerant individuals, glucose-induced oscillations in $[\text{Ca}^{2+}]_i$ were recorded with similar frequency as in normal individuals (17). However, the kinetics of insulin release was not determined. In the present study, we analyzed the secretory pattern in response to glucose and tolbutamide of individual islets isolated from three subjects with type 2 diabetes. The results demonstrate that these islets have pulsatile insulin release.

RESEARCH DESIGN AND METHODS

Subjects. The study consisted of three male subject with type 2 diabetes and four male control subjects. All subjects were brain dead. The mean age of the subjects with type 2 diabetes was 65.7 ± 2.7 years, and they had a BMI of $27.5 \pm 0.4 \text{ kg/m}^2$, which was not significantly different from the 57.5 ± 4.2 years and $23.1 \pm 0.9 \text{ kg/m}^2$ of the control subjects (Tables 1 and 2). The type 2 diabetes group had a fasting glucose concentration of $14.3 \pm 1.5 \text{ mmol/l}$. All three subjects were on insulin treatment. The fasting glucose concentrations for the control subjects were not known. None of them had a history of diabetes. Other conditions and treatments are listed in Tables 1 and 2.

Materials. Reagents of analytical grade and deionized water were used. Collagenase P, HEPES, and BSA (fraction V) were obtained from Boehringer Mannheim (Mannheim, Germany). The anti-insulin serum for the enzyme-linked immunosorbent assay measurements was obtained from guinea pigs in our laboratory. The anti-insulin serum for the immunohistochemical staining was from Dako (Glostrup, Denmark). Rodamine-isothiocyanate-labeled anti-guinea pig IgG, tetramethylbenzidine, and insulin-peroxidase came from Sigma (St. Louis, MO). The rat insulin standard was from Novo Nordisk (Bagsvaerd, Denmark). IgG-certified microtiter plates were purchased from Nunc (Roskilde, Denmark).

Preparation and culture of islets. The pancreatic glands from the type 2 diabetes and control organ donors were obtained as samples from a human

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KIC, α -ketoisocaproate.

TABLE 1
Characteristics of subjects with type 2 diabetes

Subject	Age (years)	Sex	BMI (kg/m ²)	Fasting blood glucose (mmol/l)	Duration of diabetes (years)	Treatment of diabetes	Other conditions	Other treatments
NIDDM:1	62	Male	28.0	12.4	3	Insulin, 3 times daily	Hypertension (mild), hypercholesterolemia, ischemic cardiopathy	Furosemide, digoxin, acenocoumarol
NIDDM:2	64	Male	27.7	13.2	5	Insulin, 3 times daily	Ventricular and duodenal ulcers, mitral stenosis	Omeprazole, captopril, furosemide, digitalis, acenocoumarol
NIDDM:3	71	Male	26.8	17.3	4	Insulin, 3 times daily	None	None
Mean ± SE	65.7 ± 2.7		27.5 ± 0.4	14.3 ± 1.5				

islet transplantation protocol approved by the local hospital ethical committee (Hospital Clinic, Barcelona University, Barcelona, Spain) and transported to the Endocrinology and Diabetes Unit (School of Medicine, Barcelona University, Barcelona, Spain). In all cases, informed consent of family members was obtained. Parts of the pancreata were removed and frozen for later determination of islet β -cell content. The islets were isolated after collagenase digestion of the pancreas and BSA discontinuous gradient centrifugation as previously described (18). After isolation, viability of the islets ranged between 75 and 98% in all preparations as assessed by ethidium bromide/acridine orange staining. Adhering to previously described protocol (19), the islets were cultured overnight in RPMI 1640 containing 5.5 mmol/l glucose and 10% newborn calf serum. Subsequently, the islets were sent by air from Barcelona to Uppsala in fresh medium and stored for 1–3 days in RPMI 1640 medium containing 5.5 mmol/l glucose supplemented with 10% FCS.

Perfusion of islets. Individual islets were placed in a thermostated (37°C) 10- μ l chamber and perfused at a constant flow rate with the aid of a peristaltic pump placed before the islet. The perfusion medium contained 1 mg/ml BSA and (in mmol/l) 125 NaCl, 5.9 KCl, 1.2 MgCl₂, 1.28 CaCl₂, and 25 HEPES, titrated to pH 7.4 with NaOH. After 60 min of introductory perfusion in the presence or absence of 3 mmol/l glucose, collection of perfusate in 20-s fractions began. The samples were directly cooled on ice. For islets perfused in the presence of 3 mmol/l glucose, the sugar concentration was raised to 11 mmol/l, and subsequently, 1 mmol/l tolbutamide was added to the perfusion medium. For islets perfused in the absence of glucose, 11 mmol/l α -ketoisocaproate (KIC) was added to the perfusion medium. After perfusion, the islets were freeze-dried overnight and weighed on a quartz fiber balance.

Measurements of insulin release. Insulin in the perfusates was measured by a competitive enzyme-linked immunosorbent assay with the insulin-capturing antibody immobilized directly onto the solid phase (20). Amounts of insulin down to 100 amol were obtained from linear standard curves in semilogarithmic plots. The rate of insulin release was normalized to islet dry weight. The dry weights of the islets from the type 2 diabetes and control donors ranged from 0.6 to 4.0 and 1.4 to 4.2 μ g, respectively.

Measurements of islet β -cell content. Pieces of pancreas were obtained from each donor. The pieces were frozen, serial-sectioned, and immunostained for insulin using immunofluorescence technique. The total islet surface area was defined after toluidine staining, which made it possible to determine the border between the endocrine and the exocrine pancreas, using an optical picture image analyzer (Model MOP-01; Olympus, Tokyo, Japan) on a projected image of the pancreatic sections. The β -cell content was calculated by relating the immunostained area of the islet to the total islet area. The islet β -cell percentage of the subjects with type 2 diabetes was not different from that of the control subjects.

Data analysis. The data points in the figures represent three-point moving averages. Frequency determination of oscillations in insulin release was done

by Fourier transformation using the Igor software (Wave Metrics, Lake Oswego, OR). Determination of significant insulin pulses was based on the signal-to-noise ratio as described previously (21).

Statistical analysis. Results are presented as means \pm SE. Differences in secretory rates and frequencies were evaluated with Student's two-tailed *t* test for paired and unpaired observations.

RESULTS

Insulin release in the presence of 3 mmol/l glucose from individual islets ($n = 16$) isolated from three subjects with type 2 diabetes was pulsatile (Fig. 1, Table 3). In one subject (NIDDM:1) elevation of the glucose concentration to 11 mmol/l caused a transient secretory response (Fig. 1A, Table 3). In the remaining two patients, 11 mmol/l glucose did not change the secretory rate or frequency of the insulin pulses. Subsequent addition of 1 mmol/l tolbutamide increased the secretory rate only in the subject NIDDM:2 (Fig. 1B, Table 3). The addition of tolbutamide did not affect the frequency of the insulin oscillations.

Insulin release was also examined from 13 islets isolated from four control subjects (Fig. 2, Table 4). In the presence of 3 mmol/l glucose, secretion showed regular variations with a frequency similar to that of type 2 diabetes islets. When the glucose concentration was increased to 11 mmol/l, pulsatile insulin release rose two- to threefold in 10 of 13 islets. Addition of tolbutamide only increased insulin release in Control:1 (Fig. 2A, Table 4). In Control:2, there was an initial response to the sulfonylurea, but secretion was not maintained. The remaining two subjects did not respond to the addition of tolbutamide (Fig. 2B–D, Table 4). Elevation of glucose or addition of tolbutamide did not affect the frequency of the insulin pulses.

The limited supply of islets allowed us to investigate only the secretory response to KIC in four islets from one of the patients (NIDDM:2). Insulin secretion after \sim 60 min perfusion in the absence of glucose was 5.0 pmol \cdot g⁻¹ \cdot

TABLE 2
Characteristics of control subjects

Subject	Age (years)	Sex	BMI (kg/m ²)	Conditions	Treatment
Control:1	47	Male	20.7	None	None
Control:2	55	Male	23.0	Uremia	Allopurinol, acetyl salicylic acid
Control:3	62	Male	24.5	Arthropathy	Nonsteroidal anti-inflammatory drug
Control:4	66	Male	24.2	Hypertension (mild)	Antihypertensive agents
Mean \pm SE	57.5 \pm 4.2		23.1 \pm 0.9		

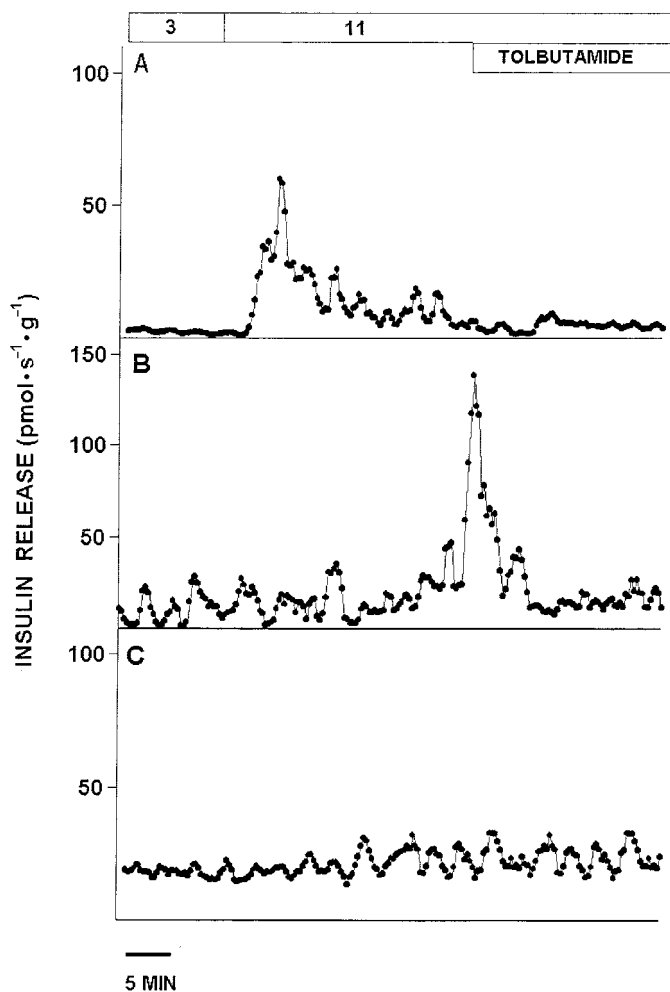


FIG. 1. Insulin release from individual islets isolated from human subjects NIDDM:1 (A), NIDDM:2 (B), and NIDDM:3 (C) perfused in the presence of 3 and 11 mmol/l glucose and 1 mmol/l tolbutamide. Representative experiments of 3 (A), 5 (B), and 8 (C).

s⁻¹ and pulsatile with a frequency of 0.23 ± 0.03 min⁻¹ (Fig. 3A). When the leucine deamination product was added to the perfusion medium, insulin transiently increased more than twofold. Subsequently, insulin release returned to basal levels, showing oscillations with similar frequency as before introduction of KIC. Similar results were obtained from four islets isolated from Control:4 (Fig. 3B).

TABLE 3
Type 2 diabetes islet secretory characteristics

Subject	Insulin release						n
	3 mmol/l glucose		11 mmol/l glucose		11 mmol/l glucose + 1 mmol/l tolbutamide		
	(pmol · g ⁻¹ · s ⁻¹)	(osc · min ⁻¹)	(pmol · g ⁻¹ · s ⁻¹)	(osc · min ⁻¹)	(pmol · g ⁻¹ · s ⁻¹)	(osc · min ⁻¹)	
NIDDM: 1	2.8 ± 0.7	0.30 ± 0.01	11.1 ± 1.8	0.28 ± 0.01	3.4 ± 0.9	0.27 ± 0.01	3
NIDDM: 2	10.9 ± 2.2	0.21 ± 0.04	15.2 ± 2.6	0.18 ± 0.02	68.3 ± 36.1	0.25 ± 0.02	5
NIDDM: 3	18.5 ± 2.8	0.28 ± 0.01	19.9 ± 2.9	0.26 ± 0.01	22.0 ± 2.9	0.27 ± 0.01	8

Data are means ± SE for the number (n) of islets perfused from each subject. Islets isolated from subjects with type 2 diabetes were perfused in the presence of 3 and 11 mmol/l glucose and 11 mmol/l glucose and 1 mmol/l tolbutamide. The perfusate was sampled every 20 s and analyzed for insulin content and oscillatory activity.

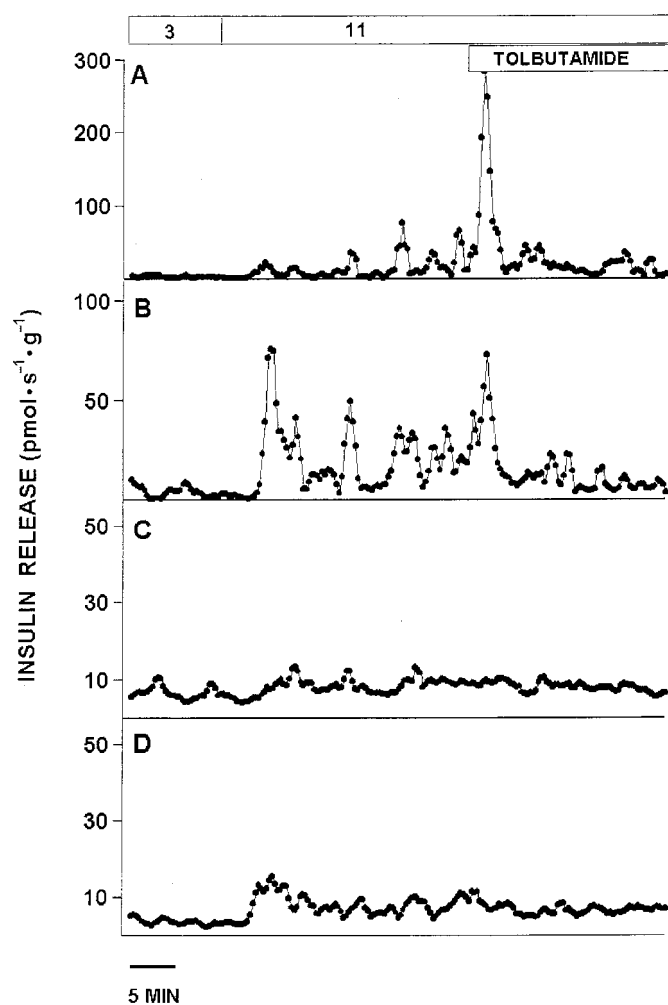


FIG. 2. Insulin release from individual islets isolated from human subjects Control:1 (A), Control:2 (B), Control:3 (C), and Control:4 (D) perfused in the presence of 3 and 11 mmol/l glucose and 1 mmol/l tolbutamide. Representative experiments of 2 (A), 2 (B), 6 (C), and 3 (D).

DISCUSSION

The main finding of the present study is that all 20 individual islets isolated from three subjects with type 2 diabetes released insulin in a pulsatile manner. The finding is in agreement with previous reports of pulsatile insulin release from individual islets isolated from the NOD mouse (22), an animal model of type 1 diabetes (23,24), and the GK rat and congenics thereof (25), an animal

TABLE 4
Control islet secretory characteristics

Subject	Insulin release						<i>n</i>
	3 mmol/l glucose		11 mmol/l glucose		11 mmol/l glucose + 1 mmol/l tolbutamide		
	(pmol · g ⁻¹ · s ⁻¹)	(osc · min ⁻¹)	(pmol · g ⁻¹ · s ⁻¹)	(osc · min ⁻¹)	(pmol · g ⁻¹ · s ⁻¹)	(osc · min ⁻¹)	
Control: 1	5.7 ± 3.0	0.24 ± 0.03	10.7 ± 3.3	0.22 ± 0.02	19.9 ± 12.8	0.21 ± 0.09	2
Control: 2	8.2 ± 4.2	0.19 ± 0.06	14.5 ± 5.4	0.16 ± 0.03	10.9 ± 2.8	0.24 ± 0.04	2
Control: 3	10.4 ± 2.0	0.20 ± 0.03	9.6 ± 0.7	0.15 ± 0.02	11.5 ± 1.4	0.17 ± 0.03	6
Control: 4	3.6 ± 0.5	0.30 ± 0.01	29.5 ± 21.7	0.26 ± 0.02	19.6 ± 13.3	0.19 ± 0.04	3

Data are means ± SE for the number (*n*) of islets perfused from each subject. Islets isolated from normal human subjects were perfused in the presence of 3 and 11 mmol/l glucose and 11 mmol/l glucose and 1 mmol/l tolbutamide. The perfusate was sampled every 20 s and analyzed for insulin content and oscillatory activity.

model of type 2 diabetes (26). From this perspective, it is difficult to see how the deranged plasma insulin pattern could be explained by loss of pulsatile insulin release from the pancreatic β -cell (12). How then are regular plasma insulin oscillations converted into "brief irregular oscillations" in patients with type 2 diabetes (3)?

The appearance of regular plasma insulin oscillations in the portal vein requires not only coordination of the β -cells in the islet but also coordination between the islets of the pancreas. It has been suggested that this coordinating function, for which the intrapancreatic ganglia may be responsible (27), may be impaired in individuals with type 2 diabetes (15). Supporting such a view, the regularity of plasma insulin pulses from the perfused pancreas was

decreased when the neurotoxin tetrodotoxin was included in the perfusate (28).

The type 2 diabetes-related changes in the plasma insulin pattern could also be explained by reduced amplitude of the blood insulin pulses. When the pulse amplitude is reduced, the pulse is less likely to be detected. Indeed, in a study when sampling both portal and peripheral blood from the same animal, the peripheral pulses were much less accentuated as a result of preferential extraction of the pulsatile insulin component by the liver (29). With improved sensitivity of both insulin measurements and analysis, it has become increasingly clear that plasma insulin oscillations from subjects with type 2 diabetes are regular (30,31). Reduced amplitude of the insulin pulses in subjects with type 2 diabetes, without any dysfunction in the frequency generation of the pulses, could therefore explain the observed changes in the type 2 diabetes plasma insulin pattern. Such a view is supported not only by the present report, which shows no increase in insulin pulse amplitude of individual islets in response to a high glucose concentration from two of three subjects, but also by previous reports of small or absent increases of amplitude of insulin pulses in response to the sugar of islets isolated from animal models of diabetes (22,25). This impaired secretory response to glucose in type 2 diabetes may reside in early steps of glucose handling because the mitochondrial substrate KIC elicited a secretory response comparable to that observed in the normal subject.

The secretory response to tolbutamide in the type 2 diabetes islets was absent in two of three subjects. This does not necessarily mean that the sulfonylurea was unable to depolarize β -cells of these islets but could reflect an inadequate energy supply to support secretion in these cells (14). Although mutations of the sulfonylurea receptor have been found in subjects with type 2 diabetes, the receptor seems to be functional in most subjects (32). Supporting such a view, tolbutamide caused depolarization in glucose-intolerant subjects (17). Similarly, GK islets, which show poor secretory response to tolbutamide (25), have functional sulfonylurea receptors (33). The poor secretory response of the GK islets and the gradual reduction of the amplitude of the insulin pulses observed in the type 2 diabetes islet responding to the sulfonylurea in the present study support the idea that type 2 diabetes is associated with an inadequate supply of energy in the β -cell to maintain secretion. From this perspective, the treatment of individuals with type 2 diabetes with sulfonyl-

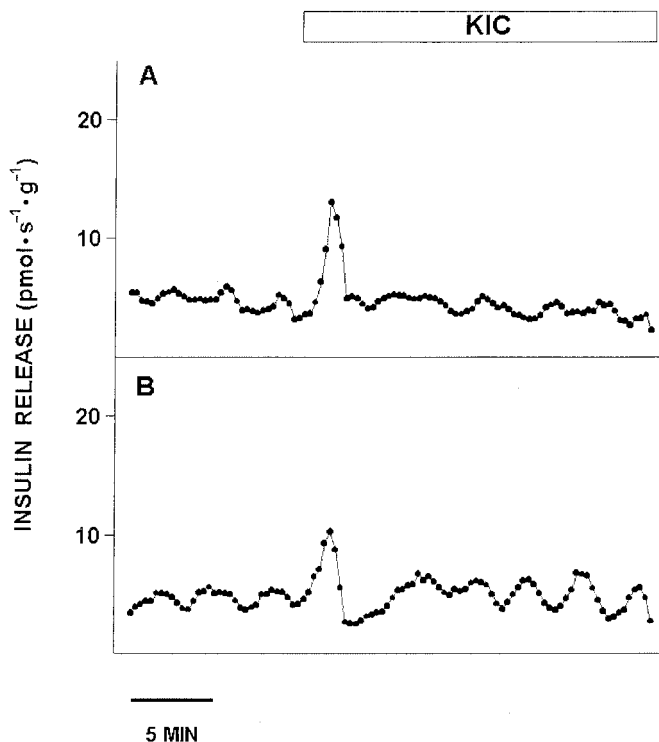


FIG. 3. Insulin release from individual islets isolated from human subjects with type 2 diabetes NIDDM:2 (A) and Control:4 (B) perfused in the presence of 11 mmol/l KIC. Representative experiments of 4 (A) and 4 (B).

ureas has its obvious limitations in not alleviating the problem with impaired metabolism.

Age-related impairment of β -cell function has been suggested to play a role in the worsening of glucose tolerance in type 2 diabetes (34,35). Although many conditions influence the secretory function, an age-related deterioration of the islet insulin secretory response was observed in both the type 2 diabetes and control subjects. The present and previous (36) findings of comparable percentages of β -cells in type 2 diabetes and control islets promote the notion that age-related development of glucose intolerance may be associated with impaired β -cell function rather than with loss of β -cell mass (37,38). Future studies addressing causes of impaired metabolism of β -cells in type 2 diabetes are therefore crucial for the understanding of the pathophysiology of impaired pulsatile insulin release in type 2 diabetes.

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