Increased β -Cell Apoptosis and Impaired Insulin Signaling Pathway Contributes to the Onset of Diabetes in OLETF Rats

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Key Words

Glucose • Insulin • Islet • β -cell mass • Insulin resistance • Glucose tolerance • Diabetes onset

Abstract

Inappropriate adaptation of β -cell mass is a primary cause of the development of diabetic hyperglycemia. However, the mechanisms underlying regulation of the β -cell mass in response to insulin resistance or in the development of type 2 diabetes remain unclear. We determined the insulin signaling in the β -cells and the adaptation of the β -cell mass in response to the progression of insulin resistance in OLETF rats. By 25 weeks of age, at the onset of diabetes, compared to control LETO rats, OLETF rats developed obesity (Body weight: LETO vs OLETF = 474.0 ± 9.5 vs 581.3 ± 21.8 g, $P < 0.001$, $n=6$), hyperlipidemia (Cholesterol: LETO vs OLETF = 1.67 ± 0.07 vs 2.19 ± 0.20 mM, $P < 0.05$, $n=6$; triglyceride: LETO vs OLETF = 0.36 ± 0.05 vs 1.36 ± 0.12 mM, $P < 0.001$, $n=6$), and impaired glucose tolerance (AUC: LETO vs OLETF = 10.3 ± 3.4 vs

29.6 ± 7.8 mM, $P < 0.001$, $n=6$). Insulin sensitivities as assessed by the insulin sensitivity index (ISI) and the homeostasis model assessment (HOMA) indicated that OLETF rats developed severe insulin resistance. The measurement of plasma insulin levels by ELISA demonstrated, at the onset of diabetes, that fasting insulin levels were increased by 1.2-fold, and 2 hr postprandial insulin levels were increased by 3-fold ($P < 0.05$, $n=6$) in OLETF rats compared to age-matched LETO mates which is suggestive of hyperinsulinemia. Immunostaining detected a significant reduction in the insulin receptor substrate 1 (IRS1) (by 54%, $P < 0.001$) and IRS2 (by 55%, $P < 0.001$) in the β -cells of the OLETF rats. Interestingly, while the β -cell mass was found to be increased (by 2.2-fold; $P < 0.001$), the β -cell insulin content as determined by immunostaining was significantly reduced by 32% ($P < 0.001$) in the OLETF rats when compared to the controls. Our findings suggest that despite increasing β -cell mass the impaired β -cell insulin signaling and reduced β -cell insulin content may contribute to the onset of overt diabetes in OLETF rats.

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Introduction

Type 2 diabetes (T2D) is a disease complex that interacts with genetic and environmental factors in which the etiology remains obscure [1]. Insulin resistance and β -cell dysfunction are the two predominant, interrelated defects in T2D. Defects of insulin action in the periphery usually precede the development of glucose intolerance [2], whereas, as long as the pancreatic β -cells are capable of compensating peripheral insulin resistance, plasma glucose levels can remain within a normal range [3, 4]. This may explain why in some subjects, despite insulin resistance and obesity, glucose tolerance remains normal [4]. Therefore, an appropriate mass of β -cells is pivotal to the maintenance of glucose homeostasis.

In non-obese diabetic (NOD) mice, which spontaneously develop type 1 diabetes (T1D) closely resembling the disease in humans [5], autoimmune-destruction of the islet β -cells via apoptosis is the major cause of the progression of the disease [6]. Despite a β -cell compensatory proliferative response, β -cell mass progressively declines because of a persistent auto-immunogenicity that leads to the onset of diabetes [7]. The onset of diabetes appears even in the presence of 30% residual β -cells. After the onset of diabetes, insulin secretion is decreased to a greater extent likely as a result of the "glucotoxicity" on β -cell function [7].

With T2D, the time at which β -cell mass begins to fall before the onset of diabetes is controversial [8-10]. The discrepancies of pancreatic pathology are partially due to the scarcity of human data since pancreatic tissues from humans become available usually at autopsy. In animal studies, the time for completion of the pancreas isolation is usually rapid (i.e. within five min) [11], whereas extensive autolysis of the human pancreas may occur before the pancreas is isolated from the cadavers [10]. Autolysis contributes to the incongruity. Nevertheless, the reduction of the β -cells reported in many T2D subjects is much more moderate than in T1D subjects [12].

Given that the pancreas obtained from type 2 diabetic cadaveric donors are usually at a post-diabetic stage, it is difficult to precisely determine the β -cell mass at the time of diabetes onset. Interestingly, in the T2D mouse model, db/db mice, the β -cell mass is increased at the onset of diabetes when compared to their lean age-matched background strain; however, when compared to age-matched obese mates who received glucagon-like peptide-1 (GLP-1) treatment and displayed normal glycemia, the β -cell mass of these untreated diabetic db/db mice is significantly reduced [11]. This suggests that an

inadequate β -cell mass and loss of the β -cells compensatory capacity may lead to the onset of diabetes in subjects who have an increased demand for insulin.

Maintenance of the β -cell mass is a dynamic process which involves increasing (replication/neogenesis) and decreasing (apoptosis) numbers of β -cells to maintain glucose levels within a normal range [13]. The mass of β -cells is regulated by a variety of different hormones such as β -cell growth factors, including GLP-1, growth hormone, prolactin, the insulin-like growth factors [14-16] and insulin [17].

In an autocrine manner, insulin stimulates insulin secretion from the β -cells [18] and promotes insulin biosynthesis by enhancing insulin gene transcription [19]. The insulin signal-transduction pathway is important in regulating β -cell growth and prevents the death of β -cells [17, 20]. Studies using knockout techniques [21, 22] demonstrated that mice lacking β -cell-specific insulin receptors developed severe diabetes associated with glucose intolerance and hyperinsulinemia. Furthermore, interference with insulin signaling in the β -cells could obstruct upregulation of β -cell mass and function, and result in the failure of β -cells to compensate for peripheral insulin resistance [17]. These findings indicate that the positive feedback mechanism of insulin on the β -cell is critical in the control of glucose homeostasis.

The present study therefore determined β -cell mass and β -cell secretory function during the development and progression of diabetes in the Otsuka Long-Evans Tokushima Fatty (OLETF) rats. The OLETF rat is a model of type 2 diabetes, and has a congenital defect of the cholecystokinin-A receptor gene, resulting in hyperphagia and obesity [23]. Our results showed that at the onset of diabetes, while the β -cell mass was increased despite increased β -cell apoptosis, the insulin signaling, as determined by IRS1 and IRS2 expression, and the insulin content were significantly reduced. These findings indicate that the β -cells mass is important in maintaining blood glucose homeostasis. Our results also suggest that defects in the insulin signaling pathway may contribute to the loss of the β -cell compensatory capacity leading to the onset of diabetes.

Materials and Methods

Animals

Male Otsuka Long-Evans Tokushima Fatty (OLETF) rats were purchased from Otsuka Research Institute (Tokushima, Japan) at four weeks of age. The OLETF rats are an established genetic model that resembles human type 2 diabetes [24, 25].

The characteristic features of OLETF rats are late onset of hyperglycemia which usually occurs at 25 weeks of age and pancreatic islets that undergo histological changes during the feeding course [24]. The rats were allowed free access to food and water in a specific pathogen-free environment. All procedures were conducted in accordance with the guidelines on the regulation of animal care approved by the Fudan University Animal Care Committee.

Oral glucose tolerance test (OGTT) and glucose-stimulated insulin secretion in vivo

Rats had fasted for 15 h before glucose was applied orally by gavage (2.0 g/kg); blood samples were collected from the tail vein at 0, 30, 60 and 120 min and glucose concentrations were measured using a Freestyle glucose meter (TheraSense, Alameda, USA). In parallel experiments, the blood samples were taken at 0 and 120 min after glucose gavage; the plasma insulin concentrations were determined using an ACTIVE Insulin ELISA kit (Diagnostic Systems Laboratories, Webster, USA).

Total cholesterol and triglyceride levels

Rats were fasted for 15 h before blood sampling from the tail vein. Plasma total cholesterol and triglyceride levels were measured enzymatically with a biochemical autoanalyzer (Hitachi Auto Analyzer 7170; Hitachi, Tokyo, Japan).

Assessment of insulin resistance

Insulin sensitivity was evaluated by the insulin sensitivity index (ISI) which was calculated as $1/(\text{fasting plasma glucose (FPG)} \times \text{fasting plasma insulin (FINS)})$ and the homeostasis model assessment of insulin resistance (HOMA-IR) which was calculated as $(\text{FPG} \times \text{FINS})/22.5$ [26, 27].

Pancreatic tissue processing and immunohistochemistry

Pancreas was isolated and fixed in 4% paraformaldehyde for 60 min at 4°C, rinsed with phosphate-buffered saline (PBS), immersed in 30% sucrose in PBS at 4°C overnight, cut into 12–14 segments and then embedded in optimal cutting temperature (OCT) compound (Mile Laboratories, Elkhart, USA). The embedded tissues were sectioned at 7 µm in a cryostat. Cryosections from every segment were chosen, and incubated overnight at 4°C with primary antibodies (mouse anti-insulin IgG (1:1000), and rabbit anti-IRS1 IgG (1:500), or rabbit anti-IRS2 IgG (1:500), or rabbit anti phosphor-Akt IgG (1:500), all from Santa Cruz, California, USA), and followed by incubation with the corresponding secondary antibodies conjugated Cy-3 or Cy-2 (1:500) (Jackson Immunoresearch Laboratories, West Grove, PA, USA) for 1 h at room temperature.

All multiple staining procedures were performed sequentially, and the images acquired by sequentially scanning using a confocal microscope (Leica, TCS-NT, Microsystems Ltd, Germany). Quantification of the staining intensity was made by the mean immunofluorescence intensity (MFI) using Image/J software (National Institute of Health, USA) and the total numbers of pixels were then calculated per individual cell. To minimize variations, the tissues were processed simultaneously with identical batches of all solutions from the beginning of tissue harvesting to the end of staining. The background of

the image was subtracted from the total number of pixels in the threshold. Typically, >30 islets from each animal in each group were analyzed.

β-cell mass analysis

β-cell mass from the insulin antibody-stained sections was measured using Image/J software, and the cross-sectional area occupied by all of the β-cells and the cross-sectional area of all pancreatic tissue was quantified. Total β-cell area and total pancreas mass for each animal was calculated as the sum of the determinations from each whole pancreatic section. A total of ~3000 β-cells were counted for each pancreas. Total β-cell mass for each pancreas was determined as the product of the total cross-sectional β-cell area divided by the total tissue area and the weight of the pancreas before fixation as described previously [11].

Detection of β-cell apoptosis

Apoptosis of pancreatic islet β-cells was evaluated by 4'-6-diamidino-2-phenylindole (DAPI) nuclear staining, and using the quantitative terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling (TUNEL) using the In Situ Cell Death Detection Kit, TMR Red (Roche Diagnostics, Mannheim, Germany) in paraffin-embedded pancreatic sections. For the DAPI analysis, the pancreatic sections pre-stained with mouse anti-insulin IgG (1:1000) and Cy3-conjugated anti-mouse IgG (1:500) were incubated with DAPI (1 µg/ml in PBS, 5 min, Sigma, Missouri, USA). The apoptotic β-cells were identified by the typical morphologic criteria of chromatin condensation and fragmentation [28]. For the TUNEL assay, the pancreatic sections pre-stained with guinea-pig anti-insulin IgG (1:1,000, DakoCytomation, Mississauga, ON, Canada) and FITC-conjugated secondary antibodies were incubated with the tunel reaction mixture (60 min, RT, in dark) according to the manufacturer's instructions. The pancreatic sections were incubated with DAPI prior to visualisation using confocal microscopy (Zeiss LSM 510).

Immunoblotting

Pancreas tissues were lysed in buffer containing 1% Triton X-100 and a mixture of protease and phosphatase inhibitors. The protein content was measured by Bradford assay (Bio-Rad Laboratories, Hercules, Calif., USA) and equal amounts of protein (50 µg) were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and electrotransferred on to polyvinylidene difluoride filters (Bio-Rad). After probing with rabbit primary antibodies that recognise isoforms of phospho-Ser473-Akt (1:500; EMD Biosciences, Darmstadt, Germany), the immunoreactive bands were visualised with HRP-conjugated sheep anti-rabbit IgG using an electrochemiluminescent detection technique as described previously [11]. The blots were stripped and re-probed for β-actin using actin antibody (Santa Cruz, California, USA).

Statistical analysis

An unpaired Student's T-test was used for statistical analysis. A *P* value of <0.05 was considered significant. All data are expressed as mean ± SE.

Fig. 1. Glucose tolerance is reduced in OLETF rats. A, OGTT were performed in OLETF and LETO rats at 25 weeks of age. After 15 h fasting, rats were gavaged with glucose (2.0 g/kg) and blood samples were collected at 0, 30, 60, and 120 min and glucose levels were measured B, The area under curve (AUC) of the OGTT. Data are expressed as mean $\pm$ SE. * $P < 0.001$, $n=6$.

Group	n	Body weight (g)	Body length (cm)	FINS (mIU/L)	2h insulin (mIU/L)	ISI	HOMA-IR	Total cholesterol (mmol/L)	Triglyceride (mmol/L)
LETO	6	474.0 $\pm$ 9.5	23.7 $\pm$ 0.67	2.34 $\pm$ 0.28	1.75 $\pm$ 0.24	0.1169 $\pm$ 0.0003	0.38 $\pm$ 0.17	1.67 $\pm$ 0.07	0.36 $\pm$ 0.05
OLETF	6	581.3 $\pm$ 21.8**	25.0 $\pm$ 0.58	2.66 $\pm$ 0.44	5.45 $\pm$ 1.06*	0.0336 $\pm$ 0.002*	1.32 $\pm$ 1.2*	2.19 $\pm$ 0.20*	1.36 $\pm$ 0.12***

Table 1. Metabolic Parameters of LETO and OLETF rats. Data are expressed as mean $\pm$ SE (OLETF rats, $n=6$; LETO rats, $n=6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. OLETF vs. LETO rats. FINS: fasting insulin. HOMA: homeostasis model assessment. ISI: insulin sensitivity index.

Results

Metabolic studies

At 25 weeks of age, OLETF rats showed elevated fasting blood glucose and impaired glucose tolerance during the OGTT time course (Fig 1A). The area under the curve (AUC) showed that the differences between the two groups were statistically significant (OLETF vs control LETO rats = 14.8 ± 3.9 vs 5.2 ± 1.7 mM*120min, $P < 0.001$, $n=6$) (Fig 1B).

As shown in Table 1, the body weight of OLETF rats at 25 weeks of age was ~ 1.25 -fold higher than control LETO rats ($P < 0.01$), whereas there was no significant difference in the body length between the two groups. Although the fasting insulin levels remained unchanged (OLETF vs LETO = 2.66 ± 0.44 vs 2.34 ± 0.28 mIU/L,

$P > 0.05$, $n=6$), the insulin levels of OLETF rats at two hours after oral glucose challenge were threefold higher than those of control rats (OLETF vs LETO = 5.45 ± 1.06 vs 1.75 ± 0.24 mIU/L, $P < 0.05$, $n=6$) which is indicative of glucose intolerance and insulin resistance. Consistently, while the ISI was significantly lower in the OLETF rats than the LETO rats (OLETF vs LETO = 0.0336 ± 0.0021 vs 0.1169 ± 0.0003 , $P < 0.05$, $n=6$), the HOMA-IR was significantly higher in OLETF when compared to those of LETO control rats (OLETF vs LETO = 1.32 ± 1.21 vs 0.38 ± 0.17 , $P < 0.05$, $n=6$).

We found that the total cholesterol levels in OLETF rats were significantly higher than those in the control rats (OLETF vs LETO = 2.19 ± 0.20 vs 1.67 ± 0.07 mM, $P < 0.05$, $n=6$); the triglyceride levels in the OLETF rats were found to be 3.8-fold higher than those in the LETO

Fig. 2. Reduction in the β -cell insulin content, IRS-1 and IRS-2 in OLETF rats. A, A pancreatic section from a LETO rat was stained for insulin (red), IRS1 (green) or IRS2 (green). B, Quantification of the staining intensity was made by the mean immunofluorescence intensity (MFI) using Image/J software. Data are expressed as mean $\pm$ SE. * $P < 0.001$.

Fig. 3. Decreased β -cell phospho-Akt in OLETF rats. A-B: A pancreatic section from a LETO rat was stained for insulin (red) and phospho-Akt(green), C-D: A pancreatic section from a OLETF rat was stained for insulin(red) and phospho-Akt(green), E: Phospho-Akt expression was quantified by the staining intensity as the mean immunofluorescence intensity (MFI) using Image/J software. Data are expressed as mean $\pm$ SE. * $P < 0.05$, n=6.

control rats (OLETF vs LETO = 1.36 ± 0.12 vs 0.36 ± 0.05 mM, $P < 0.001$, n=6) (Table 1).

These findings indicate that OLETF rats at 25 weeks of age have developed frank diabetic hyperglycemia, glucose intolerance and insulin resistance, associated with dyslipidemia.

Quantification of β -cell insulin, IRS1, IRS2 and phosphor-Akt

Immunostaining showed that the β -cell insulin content as determined by the total pixels per individual β -cell was found decreased by 32% ($P < 0.001$) in OLETF rats (Fig 2). The expression levels of IRS1 and IRS2 proteins

Fig. 4. Western blot detects reduction of pancreatic phosphor-Akt in OLEFT rats. A: Immunoblotting was performed using rabbit anti-phospho-Akt IgG (upper) or actin antibody (lower) and detected using ECL techniques. Quantitative analysis of the immunoblotting (B, pospho-Akt; C, actin content). Data are mean±SEM (n=6, *p<0.05).

Group	n	Pancreas mass (g)	Pancreas area (mm ²)	islet size (μm ²)	β-cell size (μm ²)	β-cell mass (mg)
LETO	6	0.94 ± 0.05	537.26 ±46.85	14988 ±1931	101.22 ± 5.82	7.4 ± 0.8
OLEFT	6	0.83 ±0.03	481.73 ±34.15	34246 ±3564*	114.18 ± 9.46	14.9 ± 1.2*

Table 2. The Characteristics of islets in LETO and OLEFT rats. Data are mean ± SE(OLEFT rats, n=6; LETO rats, n=6). *P<0.001. OLEFT vs. LETO rats.

in the β-cells of OLEFT were decreased by 54% ($P<0.001$) and 55% ($P<0.001$), respectively (Fig 2); phospho-Akt expression in the β-cells of OLEFT was decreased by 39% ($P<0.05$), when compared to those of control LETO rats (Fig 3). Consistent with immunostaining data, the semi-quantitative Western blot analysis, demonstrated that while the actin content was not significantly altered, a significant reduction of pancreatic phosphorylated Akt levels was found in the OLEFT rats compared to those of control group (LETO vs OLEFT = 100 vs 51.6±6.9%, $P<0.05$, n=6)(Fig 4). These results suggest that, at the diabetes onset, the insulin storage in the OLEFT rats was significantly reduced and associated with decreased insulin signaling.

β-cell mass, islet morphology and β-cell apoptosis

At the diabetes onset, while the pancreatic mass was not significantly changed, the β-cell mass was found to be increased by 2.2-fold in the OLEFT rats when compared to the controls ($P<0.001$, n=6, Table 2). Consistently, the islet size of OLEFT rats was significantly increased over that of the controls (2.4-fold, $P<0.001$, n=6) (Table 2). In a contrary, the β-cell size of OLEFT rats was only increased by 11% which was not statistically significant when compared to the control group. Therefore, it was thus unlikely that the β-cell hypertrophy significantly contributed to the increased β-cell mass in the OLEFT rats.

Fig. 5. β -cell apoptosis is increased at diabetes onset in the OLETF rats. Representative images of pancreatic sections stained for insulin (red, A,D) and DAPI (blue, A,B,D,E). The enlarged insets of B,E are shown in C and F, respectively. Arrows indicate the apoptotic β -cells. G: Data are expressed as mean $\pm$ SE. * $P < 0.05$, $n=6$.

Fig. 6. Quantification of β -cell apoptosis at diabetes onset in the OLETF rats. Representative images of pancreatic sections of LETO (A,B) and OLETF (C,D) rats stained for insulin (green, A,B,C,D) TUNEL (red, A,B,C,D) and DAPI (blue, A,B,C,D), respectively. The quantitative data are shown in E (mean $\pm$ SE. ** $P < 0.001$, $n=4$).

The rate of β -cell apoptosis, as evaluated by dual staining with DAPI and insulin antibody, was found to be increased in the OLETF rats when compared to the rats in the control group (OLETF vs LETO = $0.34 \pm 0.06\%$ vs $0.13 \pm 0.04\%$, $P < 0.05$, $n=6$) (Fig 5). Consistent with data from the DAPI staining assay, the quantitative TUNEL TMR Red assay, which sensitively measures the earlier stages of apoptosis, also showed increased β -cell apoptosis in the pancreas of OLETF rats when compared to those of control group (OLETF vs LETO = $25.9 \pm 3.9\%$ vs $5.0 \pm 2.5\%$, $P < 0.001$, $n=4$) (Fig 6).

Interestingly, an extended prolonged feeding study showed that at the age of 56 wks, when the mice developed severe diabetes, the β -cell mass of OLETF rats was decreased by 79.4% ($P < 0.001$, $n=6$), when compared to the age-matched controls.

Discussion

The adaptation of β -cells to hyperglycemia is to increase insulin production [2, 29]. Long-term adaptation

of pancreatic islets to an increased demand for insulin is achieved primarily by increasing β -cell mass [30, 31]. The maintenance of β -cell mass is a dynamic process that results from a balance of β -cell renewal and death. While an absolute decrease in the β -cell mass is known to be a major cause that leads to the onset of type 1 diabetes, in type 2 diabetes the time at which β -cell mass begins to fall before the onset of diabetes is controversial [8-10].

In the present study we found that with OLETF rats at the age of 25 wks the β -cell mass was increased, although the β -cell apoptosis was increased at the time of onset of frank diabetes. The increased β -cell mass might be a compensatory mechanism of the body in response to insulin resistance. It is conceivable, at this stage, although the β -cells are functioning at maximum capacity, they are not able to make enough insulin to fully compensate peripheral insulin resistance resulting in the onset of frank diabetes. These results are in good agreement with the concept that the type 2 diabetes only occurs when the β -cell loses its compensatory capacity [4]. In subjects with severe insulin resistance, hyperglycemia occurs despite hyperinsulinemia, with the latter being an unsuccessful compensatory response of the islet β -cells. Previous studies demonstrated that the onset of diabetes could be prevented or delayed if the β -cell mass was adequately expanded. For example, in type 2 diabetic db/db mice and Goto-Kakizaki rat models, appropriate expansion of the β -cell mass during the prediabetic period with GLP-1 prevented or delayed the onset of diabetes in these animals with severe insulin resistance [11, 32].

Consistently, we also observed that at the time of diabetes onset, the expression levels of β -cell IRS1, IRS2 and Akt were decreased, which was associated with reduced β -cell insulin content and increased β -cell apoptosis, suggesting that impaired insulin signaling, reduced insulin storage and increased β -cell apoptosis might have contributed to the β -cell incompetence in compensating for peripheral insulin resistance, leading to the onset of diabetes.

The autocrine insulin action has a significant role in promoting β -cell insulin synthesis and secretion [18, 19]. Recent studies also suggested that insulin signal transduction is critical in the regulation of β -cell mass and functions [33] via modulating the rate of β -cell survival and death [17, 20]. The scaffold proteins IRS1 and IRS2 mediate most of the insulin signal to its downstream molecules [34]. Studies with transgenic mice suggested that while IRS1 controls body growth and peripheral insulin

action, IRS2 regulates specific organ growth, body weight control and glucose homeostasis. In particular, the mice lacking the IRS1 gene developed a mild form of insulin resistance, but not diabetes [35, 36], likely due to β -cell compensation [37]. On the contrary, the homozygous IRS2 knockout mice displayed peripheral insulin resistance, impaired insulin secretion and diabetes [38].

Given that skeletal muscle IRS2 does not appear to be necessary for insulin- or exercise-stimulated glucose transport [39], it has been proposed that the insulin resistance observed in the IRS2 knockout mice is a secondary event occurring as a consequence of alterations in β -cell function [37]. Using rat primary islet β -cells, a recent study by Lingohr MK et al [40] demonstrated that expression levels of IRS2 are regulated by glucose and insulin which in turn control β -cell survival via modulating Akt kinase activities. Consistent with the animal studies that mice with β -cell specific knockout of insulin receptor developed diabetes [21, 22], and overexpressing IRS2 in the β -cells prevented diabetes onset [41, 42], our observation of reduced expression of both IRS1 and IRS2 proteins in OLETF rats at the onset of diabetes further supports an important concept that the insulin signaling pathway plays a pivotal role in modulating β -cell function [43] and survival [42]. It is likely that the impaired insulin signaling might have contributed to the loss of the β -cell mass and function during the progression of the disease.

It has been recently suggested that impaired insulin signaling in the α -cells, referred to as α -cell insulin resistance may be a contributor to the development of diabetic hyperglycemia [44]. Recent studies by Ueki K et al have suggested that β -cell insulin resistance might have resulted in the onset of diabetes in the mice lacking functional insulin and insulin-like growth factor 1 (IGF-1) receptors [43]. It is therefore, of great interest to investigate the interplay between islet cell insulin resistance and the precise mechanism underlying the regulation of islet cell mass and their impact on the development of diabetes in subjects under insulin resistant conditions.

It is possible, in addition to peripheral insulin resistance which brings to the β -cells an overload burden, that glucolipotoxicity is an additional factor that leads to β -cell death and dysfunction [45, 46]. At the stage when OLETF rats developed severe diabetes, their β -cell mass was found to be significantly decreased. It is conceivable, while the glucolipotoxicity might have contributed to the loss of the mass of β -cells in the OLETF rats during the progression of diabetes, that the impairment of insulin signaling might have initiated this process supporting the

proposal by Prentki and Nolan recently that β -cell failure is controlled by the factors that are under two broad categories: those with initiation and those with progression roles [46].

In summary, the present study shows that in the type 2 diabetic model, OLETF rats, despite the increased β -cell mass, the impaired β -cell insulin signaling, reduced β -cell insulin content and increased β -cell apoptosis may contribute to the β -cell incompetence in compensating peripheral insulin resistance, leading to the onset of overt diabetes in OLETF rats.

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