Goals of Treatment for Type 2 Diabetes

β-Cell preservation for glycemic control

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Type 2 diabetes is the most common form of diabetes in humans and results from a combination of genetic and acquired factors that impair β-cell function and tissue insulin sensitivity (1,2). However, there is growing evidence that β-cell dysfunction is crucial for the development and progression of this form of diabetes (3,4). Reduced β-cell functional mass in diabetes, and other categories of glucose intolerance, has been described by several authors, and decreased islet and/or β-cell volume in the pancreas of type 2 diabetic patients has been consistently reported. In addition, studies conducted in patients, and isolated islets, have shown both quantitative and qualitative defects of glucose-stimulated insulin secretion. Predictably, therefore, much interest is focused on the possibility of preserving the β-cell to prevent the onset of diabetes, or impede the progressive deterioration of glycemic control, observed after diagnosis and developing over the years. In this brief overview, several major features of β-cell dysfunction in different conditions (from normal glucose tolerance [NGT] to overt diabetes) will be described; thereafter, the possibility/feasibility of maintaining or restoring β-cell functional mass in type 2 diabetes, to prevent deterioration of glucose control, will be discussed.

β-CELL DYSFUNCTION AND TYPE 2 DIABETES: IN VIVO
STUDIES — Several cross-sectional and prospective studies showed that β-cell dysfunction plays a major role in determining the onset and progression of

type 2 diabetes. When insulin response to an oral glucose tolerance test and insulin sensitivity during euglycemic insulin clamp were measured in 388 individuals (138 with NGT; 49 with impaired glucose tolerance [IGT], and 201 with type 2 diabetes), a progressive decline of β-cell function (with insulin release corrected for glycemic stimulus and degree of insulin resistance) was observed; this decline commenced in normal glucose tolerant subjects (5). Furthermore, in 188 subjects, spanning the range from NGT to IGT or overt diabetes, it was found that the sensitivity of β-cells to glucose decreased already within the range of NGT in association with rising 2-h glucose levels during an oral glucose tolerance test (6). Moreover, dynamic parameters of β-cell function were independent determinants of prevailing plasma glucose concentrations throughout the glucose tolerance interval (6).

More stringent results have derived from longitudinal studies. Insulin action and insulin secretion were measured in 17 Pima Indians, in whom glucose tolerance deteriorated from NGT to IGT, or diabetes, over 5.1 ± 1.4 years (7). Transition from NGT to IGT was associated with a decline in insulin-stimulated glucose disposal and a more marked decrease of the acute insulin secretory response to intravenous glucose. Progression from IGT to diabetes was accompanied by further reductions in insulin sensitivity and acute insulin response. Nevertheless, 31 subjects who retained NGT over a similar period also showed reduced insulin-stimulated glucose disposal, but their

acute insulin response increased sufficiently to maintain normoglycemia (7). Changes in β-cell function and insulin sensitivity were evaluated in Caucasian and African American individuals with NGT, IGT, or type 2 diabetes, over 5.2 years of follow-up (8). At baseline, decreasing levels of both β-cell function (acute insulin response) and insulin sensitivity (obtained from a frequently sampled intravenous glucose tolerance test) mirrored deteriorating glucose tolerance condition at baseline and at follow-up. Over time, insulin sensitivity declined in each glucose tolerance category. However, subjects who maintained NGT exhibited a compensatory increase in insulin secretion, whereas failure to augment insulin release led to IGT, or overt diabetes (8).

Secondary failure of plasma glucose control after initial successful response to diet therapy in 432 newly diagnosed type 2 diabetic patients was evaluated in the Belfast Diet Study (9). Secondary failure to diet therapy occurred in 41 patients in years 2–4, in 67 patients in years 5–7, and in 51 patients in years 8–10; 173 patients remained on diet alone until death or the end of the study (10 years). Loss of efficacy of diet alone was associated with greater β-cell failure, and the ongoing decline in β-cell function (assessed by homeostasis model assessment [HOMA]-β) closely mirrored the steady rise in fasting plasma glucose. It is of interest that there was no change in mean insulin sensitivity in any of the groups (9).

First-degree relatives of patients with type 2 diabetes are at increased risk of developing hyperglycemia. When 531 first-degree relatives with no known history of diabetes were studied (10), it was found that in all ethnic groups (Caucasian, African American, Asian-American, and Hispanic-American), impaired β-cell function was more important than insulin resistance in determining alterations of glucose metabolism. Accordingly, in a group of 33 nondiabetic first-degree relatives followed-up for 7 years, decline in glucose tolerance over time was strongly associated with loss of β-cell function (11). All this is in agreement with the data from the U.K. Prospective Diabetes Study,

showing that at the time of diagnosis of diabetes, there is an ∼50% loss of β-cell function (as calculated by HOMA-β), which is followed by a further progressive decline over time, whatever the treatment (12).

Whereas all this work provides valuable information, it has to be kept in mind that each test used in the aforementioned studies for the assessment of β-cell function in vivo has of course merits but also caveats (rev. in 13,14). The HOMA-β index is useful when studying large populations, but it provides an estimate of how the β-cell is performing under fasting conditions only. The oral glucose tolerance test is relatively easy to administer, but it provides limited information on early phase insulin release, and the intravenous glucose tolerance test does not allow the assessment of the incretin effect. On the other hand, measurement of β-cell mass in vivo in humans remains elusive (13,14). Work done with human donors who underwent hemipancreatectomy (15) and recipients of islet auto- or allograft (16,17) has shown that acute insulin response to glucose or arginine and glucose potentiation of arginine-induced insulin secretion appear to best correlate with β-cell mass.

β-CELL DYSFUNCTION AND TYPE 2 DIABETES: HISTOLOGICAL AND EX VIVO STUDIES

The role of reduced β-cell mass in human type 2 diabetes, the primary importance of β-cell apoptosis, and the insufficiency of replication/neogenesis have been studied by several authors using histological pancreatic samples, or isolated islets. The number of islets in the pancreas of diabetic subjects has been generally found to be reduced (up to 40%) compared with nondiabetic individuals (18–21). Moreover, β-cell mass and/or volume have been consistently found to be ∼20–60% lower in type 2 diabetic pancreata (21–24), with reduction already occurring in the presence of impaired fasting glycemia (IFG) (23). In this latter study, the authors found that obesity in nondiabetic individuals was accompanied by a 50% increase in relative β-cell volume compared with lean nondiabetic individuals. However, obese subjects with IFG and type 2 diabetes had a 40 and 63% deficit, respectively, in relative β-cell volume, and lean subjects with type 2 diabetes had a 41% deficit in relative β-cell volume than lean nondiabetic subjects. These differences
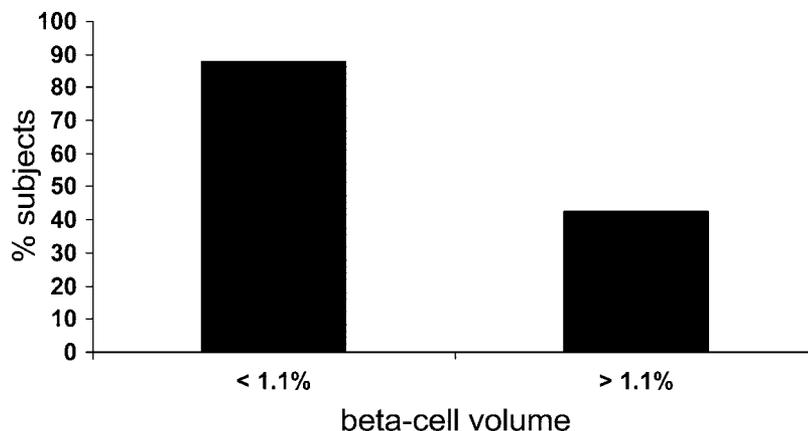
Figure 1—*Percentage of subjects with fasting plasma glucose higher than 100 mg/dl in relation to β-cell volume (lower or greater than 1.1%). Case subjects were obese and nondiabetic either with IFG or with diabetes. A higher percentage of individuals with increased glucose values had reduced β-cell volume. Adapted from Ritzel RA, Butler AE, Rizza RA, Veldhuis JD, Butler PC: Relationship between β-cell mass and fasting blood glucose concentration in humans. Diabetes Care 2006;29:717–718.*

were due to a reduced number of β-cells, rather than a smaller volume of individual cells. Neogenesis, while increased in obesity, was comparable in all groups. Furthermore, β-cell replication was found to be not significantly decreased in patients with diabetes or IFG. However, a significantly increased frequency of apoptotic events was detected in type 2 diabetic subjects versus nondiabetic subjects. In a detailed study published more recently (25), the authors analyzed autopsy samples from 57 type 2 diabetic and 52 nondiabetic European subjects and confirmed that β-cell mass was lower in the former. However, there was marked inter-subject variability and overlap between the two groups. β-Cell mass reduction was more pronounced with longer duration of diabetes (25). Using electron microscopy, it was observed that diabetic β-cells have a decreased number of insulin granules (24). Interestingly, the percentage of pancreas volume occupied by β-cells (as assessed in autopsy pancreas samples from obese humans with NGT, IFG, or diabetes) has been found to be significantly correlated with fasting plasma glucose (26) (Fig. 1). Furthermore, research with isolated human islets has consistently shown that β-cells from type 2 diabetic subjects display several defects that include reduced insulin content, diminished insulin mRNA expression, and decreased, or absent, first-phase insulin secretion in response to glucose (3,27–29).

Obviously, these studies are cross-sectional. Therefore, prospective information on β-cell mass changes to be correlated with diabetes progression is not available. In addition, at the present time, it is not possible to exclude that subjects who develop diabetes start with a lower β-cell mass due to genetic reasons. As a matter of fact, several genes associated with type 2 diabetes may affect β-cell development (30,31).

β-CELL PRESERVATION BY CURRENT PHARMACOLOGICAL THERAPIES: IN VIVO STUDIES

As aforementioned, research performed in different categories of subjects by cross-sectional and longitudinal studies, together with histological analysis and ex vivo islet investigations, strongly suggest that β-cell failure is crucial for the onset of diabetes and progressive deterioration of glycemic control. Consequently, much interest is being focused on the potential of preserving the β-cell during the different natural history stages of type 2 diabetes.

Prevention of diabetes has been achieved in variable percentages of high-risk individuals by lifestyle changes, or pharmacological intervention, but only in a few cases has β-cell function been assessed. In the U.S. Diabetes Prevention Program study, subjects with IGT were assigned to either placebo, a lifestyle modification program (with a goal of 150 min of physical activity per week and 7% weight loss), or metformin (32). During an average follow-up of 2.8 years, the incidence of diabetes was significantly

lower in the metformin-treated individuals (4.8%) than in those receiving placebo (7.8%). When the role of insulin sensitivity and insulin secretion was evaluated (33), it was found that diabetes prevention was associated with a significant improvement of insulin secretion relative to insulin sensitivity. Thiazolidinediones have also been shown to be effective in preventing diabetes. In the Troglitazone Prevention of Diabetes (TRIPOD) study, a significant reduction in the incidence of type 2 diabetes (from 45 to 20%) was observed in the group of individuals treated with troglitazone, which was associated with preservation of β-cell compensation for insulin resistance (34). In addition, preliminary results of the ACT NOW study showed that pioglitazone was able to significantly reduce the incidence of type 2 diabetes in patients with IGT, which was associated with better insulin secretory function (DeFronzo et al., late-breaking abstract, American Diabetes Association 68th Scientific Sessions, San Francisco, 6–10 June 2008).

After the diagnosis of type 2 diabetes, the use of intensive treatment with insulin, metformin, glibenclamide, or chlorpropamide was effective in improving glycemic control compared with conventional therapy in the U.K. Prospective Diabetes Study (35). However, the various drug treatments were similarly unable to prevent progressive deterioration of glucose control and reduction of β-cell function, as assessed by measuring the HOMA-β index. Successive studies have shown that treatment with some medications is associated with improved β-cell function. When 53 type 2 diabetic patients were randomized to receive 4 months of treatment with placebo, 45 mg/day pioglitazone, or 8 mg/day rosiglitazone, improved glycemic control was achieved in the thiazolidinedione groups (36). In this study, glucose values were significantly associated with β-cell function (36). Postprandial β-cell performance was assessed by a mathematical model in a group of subjects with type 2 diabetes treated with metformin or a sulfonylurea, who were administered exenatide as add-on for 4 weeks (37). The authors showed that the glucagon-like peptide 1 (GLP-1) mimetic improved β-cell function significantly at the end of the treatment period. In a recent study (38), 69 metformin-treated patients with type 2 diabetes were randomized to exenatide or insulin glargine, and β-cell function was measured during an argin-

Table 1—Measures of β-cell function during hyperglycemic clamp and ratio to pretreatment in the groups of type 2 diabetic patients treated with exenatide or insulin glargine

	Pretreatment	End of treatment	Off-drug	End of treatment ratio to pretreatment (geometric mean)	End of treatment ratio to pretreatment (between group difference)	End of treatment ratio to pretreatment (P)	Off-drug ratio to pretreatment (geometric mean)	Off-drug ratio to pretreatment (between group difference)	Off-drug ratio to pretreatment (P)
First-phase C-peptide response to glucose									
Glargine	5.4 ± 0.6	6.1 ± 0.5	6.1 ± 0.6	1.17 ± 0.06			1.13 ± 0.05		
Exenatide	5.4 ± 0.6	9.4 ± 1.0	5.0 ± 0.6	1.78 ± 0.11	1.53 ± 0.11	<0.0001	1.00 ± 0.05	0.90 ± 0.06	0.11
Second-phase C-peptide response to glucose									
Glargine	77.4 ± 8.8	80.7 ± 6.9	86.2 ± 9.1	1.08 ± 0.05			1.10 ± 0.05		
Exenatide	78.5 ± 8.3	235.6 ± 23.0	79.5 ± 9.1	3.05 ± 0.22	2.85 ± 0.22	<0.0001	1.01 ± 0.04	0.92 ± 0.06	0.19
C-peptide response to arginine at 15 mM glucose									
Glargine	20.0 ± 2.5	24.8 ± 2.2	21.4 ± 2.5	1.31 ± 0.07			1.03 ± 0.08		
Exenatide	19.7 ± 2.1	62.2 ± 7.0	22.0 ± 2.6	3.19 ± 0.24	2.46 ± 0.20	<0.0001	1.12 ± 0.06	1.08 ± 0.10	0.40

Data are means ± SD. Table adapted from Bunck MC, Diamant M, Cornér A, Eliasson B, Malloy JL, Shaginian RM, Deng W, Kendall DM, Taskinen MR, Smith U, Yki-Järvinen H, Heine RJ: One-year treatment with exenatide improves β-cell function, compared with insulin glargine, in metformin-treated type 2 diabetes patients: a randomized, controlled trial. Diabetes Care 2009;32:762–768.
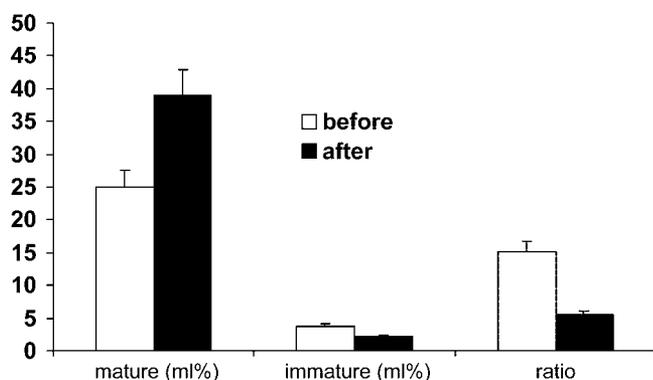
Figure 2—*Volume density of mature and immature insulin granules and its ratio in β-cells from type 2 diabetic subjects before and after ex vivo metformin exposure. Treatment caused significant increase of mature granules, decrease of immature granules, and higher ratio. Adapted from Marchetti P, Del Guerra S, Marselli L, Lupi R, Masini M, Pollera M, Bugliani M, Boggi U, Vistoli F, Mosca F, Del Prato S: Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. J Clin Endocrinol Metab 2004;89:5535–5541.*

ine-stimulated hyperglycemic clamp at week 0, at week 52, and after a 4-week off-drug period. Both drugs reduced A1C levels significantly during treatment, which was associated with a significant improvement of β-cell function parameters in the exenatide arm (Table 1). However, glycemic control and β-cell function measures returned to pretreatment values in both groups after 4 weeks off-drug (Table 1).

Therefore, a few studies have shown that certain pharmacological treatments can prevent the onset of diabetes and slow its progression in humans and that these effects are associated with ameliorated β-cell function. However, from such in vivo studies, it is impossible to understand whether the effects on the insulin-secreting cells are mediated by an improvement of the metabolic milieu or are due to an action of the drug(s) directly on β-cells.

β-CELL PRESERVATION BY CURRENT PHARMACOLOGICAL THERAPIES: EX VIVO STUDIES

— The possibility that pancreatic β-cell damage can be prevented, or even reverted, has been tested in isolated human nondiabetic islets exposed to different metabolic perturbations and, more importantly, with islets from type 2 diabetic donors. In early work, it was assessed whether metformin could affect the phenomenon of glucotoxicity (39). Human islets were incubated for 24 h in culture medium containing either 5.5 or 22.2 mmol/l glucose, with or without a

therapeutic concentration of metformin. After incubation in the absence of metformin, the islets pre-exposed to 22.2 mmol/l glucose showed no significant increase in insulin release when challenged acutely with 16.7 mmol/l glucose (confirming that hyperglycemia desensitizes pancreatic β-cells). In the presence of metformin, the islets maintained the ability to significantly increase their insulin release in response to 16.7 mmol/l glucose, even when previously cultured with high glucose. Metformin could also protect human islets from lipotoxicity. When islets were incubated for 48 h in the presence of 2.0 mmol/l free fatty acid (oleate to palmitate, 2 to 1), acute insulin secretion in response to 16.7 mmol/l glucose was significantly reduced (40). Impairment of insulin secretion after exposure to free fatty acids was mainly accounted for by defective early-phase release. Addition of metformin to high–free fatty acid media prevented the impairment of glucose-mediated insulin release and the decline of first-phase insulin secretion (40). Other drugs used to reduce insulin resistance in peripheral tissues (i.e., peroxisome proliferator–activated receptor [PPAR]-γ) seem to protect islet cells from metabolic insults. Exposure of isolated islets to free fatty acids decreased glucose-stimulated insulin release and islet insulin content, and these alterations were prevented by the PPAR-γ agonist rosiglitazone (41). Interestingly, it has been reported that β-cell rest induced by an ATP-dependent potassium channel (K_{ATP}) opener protected human islets from the functional damage induced by

prolonged pre-exposure to relatively (11 mmol/l) high glucose concentrations (42).

Of greater importance, however, is to assess whether the functional and molecular alterations of human type 2 diabetic β-cells are reversible. In a recent article, it was found that when type 2 diabetic islets were incubated with metformin for 24 h, insulin content and insulin granule amount increased significantly (Fig. 2), which was accompanied by partial restoration of glucose responsiveness (24). Metformin also improved β-cell survival (24). Recently, the role of incretins (GLP-1, glucose-dependent insulinotropic polypeptide [GIP], and some of their analogs) in the therapy of diabetes has received much attention (43). In vitro and laboratory animal models have shown that these molecules can protect the β-cell from apoptosis and promote β-cell differentiation and proliferation. In a study from our laboratory (44), pancreatic islets were prepared from the pancreas of nondiabetic and type 2 diabetic donors and then incubated in the presence of 5.5 mmol/l glucose, with or without the addition of exendin-4 (a long-acting GLP-1 mimetic). Insulin secretion from the type 2 diabetic islets improved after incubation with exendin-4, which also induced a significantly higher expression of insulin, GLUT2, glucokinase, and some β-cell regeneration and differentiation factors, including pancreas duodenum homeobox-1 (Pdx-1).

Amelioration of insulin release per se may have direct beneficial actions on β-cells (45). Insulin receptor phosphorylation leads to activation of phosphoinositide 3 (PI-3) and mitogen-activated (MAP) kinases, protecting the β-cell from apoptosis (46). In addition, several data have demonstrated that pro-insulin–derived C-peptide can affect the function and survival of a number of cell types, including β-cells (47). When isolated human islets were cultured in the presence of 50 ng/ml C-peptide, islet cell apoptotic rate decreased significantly, which was accompanied by increased mRNA and protein expression of the anti-apoptotic molecule B-cell CLL/lymphoma 2 (Bcl-2), without changes in the expression of the pro-apoptotic protein Bax (48).

CONCLUSIONS — Pancreatic β-cell dysfunction is key to the development and progression of type 2 diabetes. Both altered β-cell function and decreased β-cell mass are likely to contribute to the

defects in insulin release typical of diabetes. These defects cause a progressive increase of glucose levels, with deterioration of glycemic control over the years. Interestingly, however, some evidence is emerging to show that the onset of diabetes may be delayed and the progression of glucose control deterioration slowed by certain therapies. These beneficial effects are associated, at least in part, to better maintained β-cell function. For these approaches to be more effective, strategies should be developed to deliver potentially useful drugs to the β-cell at the desired concentrations and for the necessary duration. In addition, efforts should be made to better understand which alterations at the level of β-cell microenvironment are present and may impede the complete success of therapeutic interventions.

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