β-Cell Deficit and Increased β-Cell Apoptosis in Humans With Type 2 Diabetes

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Type 2 diabetes is characterized by impaired insulin secretion. Some but not all studies suggest that a decrease in β-cell mass contributes to this. We examined pancreatic tissue from 124 autopsies: 91 obese cases (BMI >27 kg/m^2; 41 with type 2 diabetes, 15 with impaired fasting glucose [IFG], and 35 nondiabetic subjects) and 33 lean cases (BMI <25 kg/m^2; 16 type 2 diabetic and 17 nondiabetic subjects). We measured relative β-cell volume, frequency of β-cell apoptosis and replication, and new islet formation from exocrine ducts (neogenesis). Relative β-cell volume was increased in obese versus lean nondiabetic cases (P = 0.05) through the mechanism of increased neogenesis (P < 0.05). Obese humans with IFG and type 2 diabetes had a 40% (P < 0.05) and 63% (P < 0.01) deficit and lean cases of type 2 diabetes had a 41% deficit (P < 0.05) in relative β-cell volume compared with nondiabetic obese and lean cases, respectively. The frequency of β-cell replication was very low in all cases and no different among groups. Neogenesis, while increased with obesity, was comparable in obese type 2 diabetic, IFG, or nondiabetic subjects and in lean type 2 diabetic or nondiabetic subjects. However, the frequency of β-cell apoptosis was increased 10-fold in lean and 3-fold in obese cases of type 2 diabetes compared with their respective nondiabetic control group (P < 0.05). We conclude that β-cell mass is decreased in type 2 diabetes and that the mechanism underlying this is increased β-cell apoptosis. Since the major defect leading to a decrease in β-cell mass in type 2 diabetes is increased apoptosis, while new islet formation and β-cell replication are normal, therapeutic approaches designed to arrest apoptosis could be a significant new development in the management of type 2 diabetes, because this approach might actually reverse the disease to a degree rather than just palliate glycemia. *Diabetes* 52:102–110, 2003

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FPG, fasting plasma glucose; IAPP, islet amyloid polypeptide; IFG, impaired fasting glucose.

Type 2 diabetes is characterized by a progressive decline in β-cell function and chronic insulin resistance (1,2). Obesity is a major risk factor for the development of type 2 diabetes (3,4) and is thought to confer increased risk for type 2 diabetes through the mechanism of associated insulin resistance (5). However, most people who are obese (and relatively insulin resistant) do not develop diabetes but compensate by increasing insulin secretion (6). In rodent models of obesity without diabetes there is an adaptive increase in β-cell mass (7). Although not much data are available, prior studies suggest that β-cell mass is also adaptively increased in nondiabetic obese humans (8,9). Regulation of the β-cell mass appears to involve a balance of β-cell replication and apoptosis, as well as development of new islets from exocrine pancreatic ducts (10,11). Disruption of any of these pathways of β-cell formation or increased rates of β-cell death could cause a decrease in β-cell mass. The islet in type 2 diabetes is characterized by deposits of amyloid (12) derived from the peptide islet amyloid polypeptide (IAPP), also known as amylin (13). This peptide has been shown to cause apoptosis of β-cells (14,15), particularly when it is in the form of small IAPP oligomers (16). However, it is unknown whether β-cell apoptosis is increased in type 2 diabetes.

Also, there is controversy whether β-cell mass is decreased in type 2 diabetes (9,17–24). These discrepancies are in part due to the paucity of available data in humans. It is difficult to obtain pancreatic tissue from humans, since this usually only becomes available at autopsy, when the pancreas may have undergone extensive autolysis. Also, reliable clinical information about autopsy cases is often unavailable. It is therefore not surprising that there are few studies of islet morphology in humans with diabetes, and in most of these, only small numbers of cases were included. Also, clinical data before death is minimal in many of these studies. Given the small number of cases, matching the cases for degree of obesity and age of death has not always been possible. At the Mayo Clinic some of these difficulties are overcome. Autopsies are performed within 12 h of death (usually <6 h), including weekends. The Mayo Clinic integrated medical record system allows easy access to the prior clinical records of the cases. As the Mayo Clinic tends to be the primary health care provider for people hospitalized at the medical center for their final illness, most of the autopsy cases

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TABLE 1
Characteristics of patients

	Obese			Lean	
	Type 2 diabetic subjects	IFG	Nondiabetic subjects	Type 2 diabetic subjects	Nondiabetic subjects
n	41	19	31	16	17
Sex (F/M)	17/24	9/10	15/16	7/9	7/10
Age (years)	63.3 ± 1.8	63.1 ± 2.3	66.9 ± 2.7	80.2 ± 1.9	78.1 ± 2.9

Data are means ± SE.

have had a general medical examination (including a fasting blood glucose) during the year before death.

In the present study, we took advantage of the unique autopsy material available at the Mayo Clinic to study 124 human pancreata from cases with and without diabetes and matched for obesity. All cases had a well-preserved pancreatic specimen and documented general medical exam, including a fasting blood glucose obtained during the 12 months before death. With this unique resource, we sought to address the following questions: *1*) In humans with obesity, is β-cell mass increased compared with lean nondiabetic humans? *2*) In humans with type 2 diabetes, is β-cell mass decreased compared with age-, sex-, and weight-matched nondiabetic humans? *3*) Are there measurable changes in the frequency of β-cell apoptosis or replication or new islet (ductal) formation among these groups in human pancreatic tissue obtained at autopsy that might provide insight into the mechanism subserving any deficit in β-cell mass in type 2 diabetes?

RESEARCH DESIGN AND METHODS

Design. We obtained human pancreatic tissue at autopsy from nondiabetic subjects, patients with type 2 diabetes, and patients with impaired fasting glucose (IFG); pancreatic samples from both obese and lean patients were obtained in the nondiabetic and diabetic groups. The current study reflects the population in Minnesota during the time these patients died, which was ~98% Caucasian of Northern European origin.

Subjects. Potential cases were first identified by retrospective analysis of the Mayo Clinic autopsy database. To be included, cases were required to have *1*) had a full autopsy within 12 h of death, *2*) had a general medical examination, including at least one fasting blood glucose documented within the year before death, and *3*) pancreatic tissue stored that was of adequate size and quality. Cases were excluded if *1*) potential secondary causes of type 1 or type 2 diabetes were present, *2*) patients had been exposed to chronic glucocorticoid treatment, or *3*) pancreatic tissue had undergone autolysis or showed evidence of pancreatitis. Inclusion in the lean subgroup required a BMI <25 kg/m^2, while inclusion in the obese subgroup required a BMI >27 kg/m^2. Cases were further classified as nondiabetic (fasting plasma glucose [FPG] <110 mg/dl), IFG (FPG 110–125 mg/dl), or diabetic subjects (FPG >126 mg/dl). As a consequence of this process, we ultimately included 124 cases (Table 1).

Pancreatic tissue processing. At autopsy in all cases, pancreas was resected from the tail and, with a sample of spleen, fixed in formaldehyde and embedded in paraffin for subsequent analysis. The autopsy process does not include full dissection and removal of the retroperitoneal pancreas to determine pancreas weight (as is routinely possible in rodent studies with a more accessible pancreas). Sections were cut from these paraffin blocks and stained by Congo Red for hematoxylin/eosin and by immunohistochemistry for insulin, as previously described (25). In addition, sections were analyzed for replication by immunohistochemistry for Ki67 (anti-Human Ki-67 monoclonal antibody MIB-1, 1/25; Dako, Carpinteria, CA) (26) and for apoptosis using the TdT-FragEL Kit (cat. no. QIA 33) from Oncogene Research Products (Cambridge, MA).

Analysis of endocrine pancreatic morphology. The hematoxylin/eosin slides were examined in all cases to exclude cases with pancreatitis, autolysis, etc., as per case selection above. Slides stained for insulin (J.J. and A.E.B.), Congo Red, TUNEL, and Ki67 (A.E.B.) were then examined blinded for subgroup as detailed below. The blind was broken by P.C.B. and R.R., who performed the statistical analysis. S.B.-W. provided guidance with measurement of new islet formation and all aspects of β-cell sterology. R.A.R. coordinated availability of samples from Mayo Clinic and clinical information and overall guidance in study analysis.

Relative β-cell volume to exocrine pancreas. As the pancreas weight was not available, it was not possible to calculate the β-cell mass. The pancreas tends to be slightly larger in nondiabetic than in diabetic humans (27). Therefore, the ratio of the β-cell area/exocrine area (by convention called the relative β-cell volume) should provide a conservative estimate of any decline in β-cell mass in patients with diabetes. To measure this ratio, slides from each case were examined using an Olympus IX70 inverted system microscope (Olympus America, Melville, NY) connected to a Hewlett Packard computer with Image-Pro Plus software (Media Cybernetics, Silver Springs, MD). Slides immunostained for insulin were analyzed using the Image-Pro Plus system. The slide was scanned using ×4 objective magnification. A representative area of pancreas section was chosen for analysis and the coordinates entered into the program. The image analysis quantified total tissue area within this region, followed by the insulin-positive area to generate the ratio of insulin staining to total pancreas area. The sensitivity for the insulin-positive areas was high, so that all insulin-positive areas were included irrespective of the intensity of staining.

To ensure that any changes in the relative β-cell volume were not due to changes in the size of individual β-cells (for example due to degranulation), we also measured the density of nuclei per insulin-positive area in the obese and lean type 2 diabetic and nondiabetic groups. Five islets from each case stained for insulin were imaged to provide the insulin-positive area (μm^2) and digitally photographed at high power (×40). The resulting images were examined by A.E.B. to manually count the number of nuclei present within the insulin-stained area in order to compute the ratio of nuclei per insulin-positive area (μm^2).

Quantification of islet size and islet relative β-cell volume. To establish islet size and the islet relative β-cell volume, 20 islets larger than four cells were studied in detail from each case. First, a field at objective magnification ×20 was chosen at random, and then the islets in this field and those in the immediately adjacent fields were examined until 20 islets had been evaluated. Each of these 20 islets was evaluated to obtain the total islet cross-sectional area and the area within this islet positive for insulin. For each case, a mean

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TABLE 2
Obese and lean cases of type 2 diabetes, subdivided according to treatment (insulin, oral, or diet) to show sex, age, and BMI

	Obese type 2 diabetic subjects*			Lean type 2 diabetic subjects		
	Insulin	Oral	Diet	Insulin	Oral	Diet
n	17	16	7	3	6	7
Sex (F/M)	10/7	5/11	1/6	1/2	4/2	2/5
Age (years)	65.9 ± 1.7	61.9 ± 3.7	59.7 ± 4.7	82.0 ± 3.5	78.2 ± 3.4	81.1 ± 3.0
BMI (kg/m^2)	38.3 ± 1.5	38.5 ± 2.1	36.5 ± 2.0	23.6 ± 0.5	23.3 ± 0.8	21.2 ± 0.7

Data are means ± SE. *In one obese type 2 diabetic subject, the treatment was unknown.

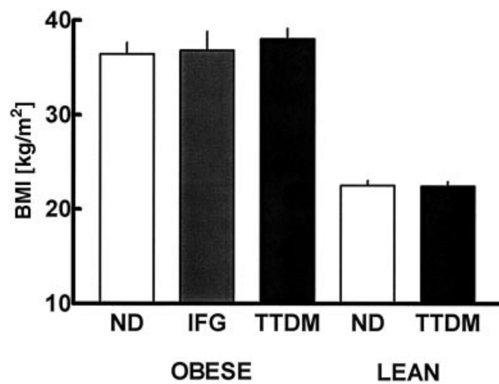

FIG. 1. The mean BMI in obese (nondiabetic [ND], IFG, and diabetic subjects [TTDM]) and lean cases (nondiabetic and type 2 diabetic subjects).

cross-sectional islet area, a mean cross-sectional insulin area within the islet, and a mean percentage area positive for insulin within the islet were therefore determined to provide the islet relative β-cell volume.

Quantification of exocrine duct cells positive for insulin for islet neogenesis estimate. To assess the relative rate of islet neogenesis from exocrine duct cells, we quantified the percentage of exocrine duct cells that were immunoreactive for insulin. A mean (±SE) of 281 ± 11 duct cells were evaluated as positive or negative for insulin from each pancreas. The resulting data were expressed as the percentage of duct cells positive for insulin in each pancreas, and the mean was calculated for each group.

Apoptosis and replication. Slides stained by the TUNEL and Ki67 techniques were each evaluated in direct comparison with those immunostained for insulin in each case. In most cases, samples of spleen were fixed and paraffin embedded with the pancreas, providing positive controls for apoptosis and replication in the splenic follicles. The number of positive cells that corresponded to an insulin-positive area in the adjacent section were counted per islet. An average of 75 islets were examined per case, and the mean number of positive cells per islet calculated for each case. TUNEL staining was occasionally present in debris that was no longer present in a distinct cell; these were not counted. Only discernible cells with TUNEL-positive nuclei were included. As the relative β-cell volume differed among groups (see RESULTS), we also computed the frequency of β-cell apoptosis and β-cell replication events divided by the relative β-cell volume to provide a comparison of these events relative to β-cell volume per case.

Islet amyloid. To quantify islet amyloid, slides from each case were stained by Congo Red and examined under polarized light for birefringence. Cases were scored for *1*) presence or absence, *2*) frequency of islet amyloid (using a scale from 0 to 3, where 0 indicated that no islets showed apple green birefringence, 1 indicated that a few islets in the sample were birefringent, 2 indicated that numerous islets in the sample were birefringent, and 3 indicated that most islets were birefringent), and *3*) extent of islet amyloid (again using a scale from 0 to 3, where 0 indicated that no islets were birefringent, 1 indicated minimal extent and weak intensity of birefringence in affected islets, 2 indicated moderate extent and intensity of birefringence in affected islets, and 3 indicated marked extent and intensity of birefringence within affected islets).

Statistics. The specific hypotheses posed were tested by use of a Student's t test. When all five groups of cases were compared, ANOVA was used. A P value <0.05 was considered statistically significant. Linear correlation was performed by regression analysis with a best-fit line and 95% CIs shown graphically.

RESULTS

Cases (Tables 1 and 2 and Fig. 1). BMI and age of death did not differ among the type 2 diabetic, nondiabetic, or IFG subgroups within the obese group. Similarly, there was no difference in age of death or BMI between lean type 2 diabetic and nondiabetic patients. As expected, the obese cases had a higher BMI than the lean cases. Also, lean cases died at a greater age than obese cases ($P < 0.0001$). The treatments used by patients with type 2 diabetes were diet, sulfonylurea pills, or insulin. No other classes of oral medications for glycemic control were available at the time these patients were living. In Table 2, we show the number of cases treated by each of these medication types before death.

Relative β-cell area to exocrine pancreas (Table 3 and Fig. 2). We report that obesity in nondiabetic humans is characterized by an ~50% increase in relative β-cell volume (2.6 ± 0.39 vs. 1.71 ± 0.28%, $P = 0.05$, obese nondiabetic vs. lean nondiabetic subjects). Since the nondiabetic obese humans died younger than the nondiabetic lean humans, the comparison in the relative β-cell volume between these groups may have been confounded by this age difference. Obese humans with IFG (1.56 ± 0.25%) and type 2 diabetes (0.96 + 0.1%) had a 40% ($P < 0.05$) and 63% ($P < 0.01$) deficit in relative β-cell volume compared with nondiabetic obese subjects. The decreased β-cell volume in patients with type 2 diabetes was due to a decreased number of β-cells rather than a decreased volume of individual cells, since the ratio of nuclei per μm^2 of insulin-positive area was no different between the nondiabetic and type 2 diabetic groups (obese $6 \pm 1 \times 10^{-3}$ vs. $6 \pm 1 \times 10^{-3}$ nuclei/μm^2, lean $5 \pm 1 \times 10^{-3}$ vs. $7 \pm 1 \times 10^{-3}$ nuclei/μm^2; P = NS). Compared with obese nondiabetic cases, obese patients with type 2 diabetes had a decreased relative β-cell volume (Fig. 3) irrespective of whether they were treated by diet alone (0.78 ± 0.13, 70% decreased; $P < 0.01$) or on oral medications (0.86 ± 0.25, 67% decreased; $P < 0.05$) or insulin (1.3 ± 0.2, 50% decreased; $P < 0.05$). There was no difference in the mean FPG values among these three treatment groups during the last 2 years of life (Fig. 3). Lean subjects with type 2 diabetes had 41% deficit in relative β-cell volume (1.0 ± 0.2 vs. 1.7 ± 0.3%, $P < 0.05$) compared with lean nondiabetic subjects. The lean diabetic subjects showed a decrease in

TABLE 3
The five patient groups (lean nondiabetic, lean type 2 diabetic, obese nondiabetic, obese IFG, and obese type 2 diabetic subjects) demonstrating β-cell and islet characteristics

	Lean		Obese		
	Nondiabetic subjects	Type 2 diabetic subjects	Nondiabetic subjects	IFG	Type 2 diabetic subjects
Relative β-cell volume/islet (% of islet)	52.0 ± 4.1	38.0 ± 3.9*	45.4 ± 2.7	43.7 ± 3.4	37.0 ± 2.3†
Islet density (islets/mm^2)	3.1 ± 0.2	3.0 ± 0.3	3.5 ± 0.3	2.8 ± 0.2‡	2.6 ± 0.2†
Mean islet size (μm^2)	7,140 ± 795	6,807 ± 526	7,187 ± 571	7,131 ± 695	7,846 ± 648
Apoptosis/islet (cells/islet)	0.07 ± 0.03	0.47 ± 0.19§	0.20 ± 0.7	—	0.31 ± 0.15
Ki67/islet (cells/islet)	0.04 ± 0.02	0.033 ± 0.01	0.06 ± 0.02	—	0.03 ± 0.01

Data are means ± SE. *$P < 0.01$ for lean nondiabetic vs. type 2 diabetic subjects; †$P < 0.05$ for obese nondiabetic vs. type 2 diabetic subjects; ‡$P < 0.05$ for obese nondiabetic vs. IFG subjects; §$P < 0.05$ for lean nondiabetic vs. type 2 diabetic subjects.

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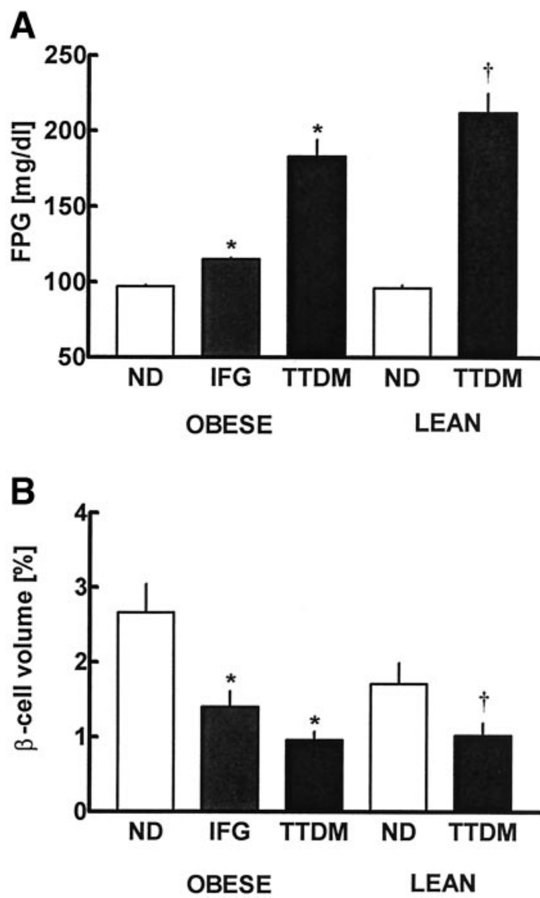

FIG. 2. The mean FPG concentration (*A*) and the mean relative β-cell volume (*B*) in obese (nondiabetic [ND], IFG, and diabetic subjects [TTDM]) and lean cases (nondiabetic and type 2 diabetic subjects).

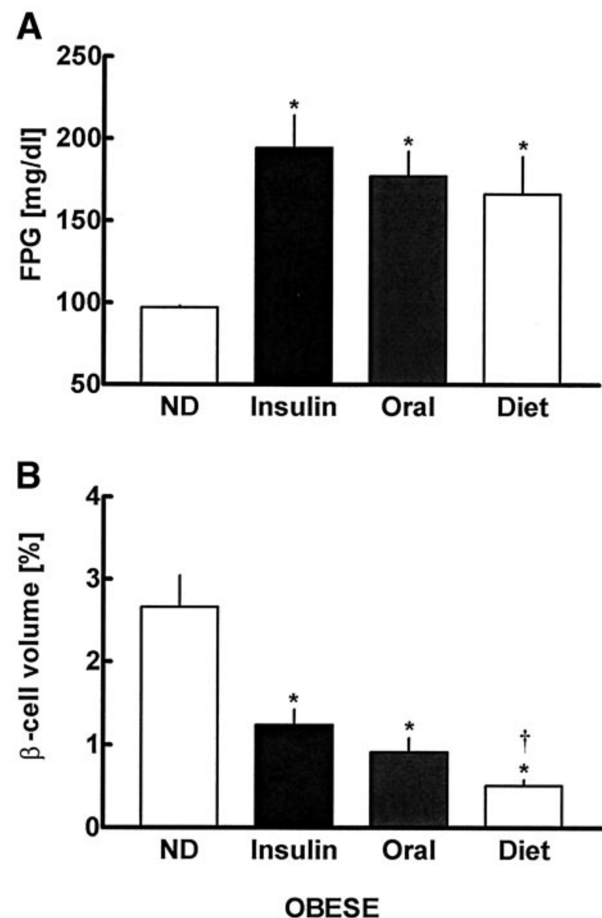

FIG. 3. The mean FPG concentration (*A*) and the relative β-cell volume (*B*) in the obese group of nondiabetic (ND) subjects or diabetic patients who were treated with insulin, sulfonylurea pills (Oral), or diet only.

relative β-cell volume irrespective of whether they were treated with diet only (1.24 ± 0.34, P = NS), oral medications (0.86 ± 0.2, $P < 0.05$), or insulin (0.82 ± 0.17, $P < 0.05$ using Welch's correction) (Fig. 4). These findings were consistent with the observations in individual islets examined from each group. The mean islet size was similar in all groups studied (Table 3). In contrast, the relative β-cell islet volume was decreased in obese cases with type 2 diabetes compared with nondiabetic obese cases ($P < 0.05$) and in lean cases with type 2 diabetes compared with nondiabetic lean cases ($P < 0.01$).

Quantification of exocrine duct cells positive for insulin (Figs. 5 and Fig. 6). The presence of islets budding from exocrine ducts were present in all pancreata irrespective of obesity or diabetic status. To quantify this process, we quantified duct cells immunoreactive for insulin. There was an increased percentage of β-cell–positive duct cells in obese versus lean cases ($P < 0.05$), but no difference between the obese nondiabetic and type 2 diabetic cases ($P = 0.60$) or lean type 2 diabetic and nondiabetic cases ($P = 0.83$).

Replication and cell death (Fig. 7 and Table 3). To further attempt to elucidate the mechanism for the deficit in β-cell mass present in type 2 diabetes, we examined the frequency of β-cell apoptosis and replication. The frequency of replication was very low in all cases examined. There was no significant difference in the frequency of replication between obese nondiabetic and type 2 diabetic subjects or lean nondiabetic and type 2 diabetic subjects (Fig. 7). There was a trend for decreased β-cell replication and new islet formation with aging (Fig. 8). Although there was a suggestion of an increased frequency of apoptotic events per islet (Table 3) in obese type 2 diabetic versus nondiabetic cases, this did not reach significance. However there was an increased frequency of apoptotic events per islet in lean type 2 diabetic versus nondiabetic cases ($P < 0.05$). Since the relative β-cell volume was decreased in type 2 diabetes, we also examined the frequency of β-cell apoptosis per islet divided by the relative β-cell volume so that a comparison could be made of the frequency of β-cell apoptosis normalized to β-cell mass. When the frequency of apoptotic events was expressed as the frequency of β-cell apoptosis divided by the relative β-cell volume, then the frequency of apoptosis was ~3-fold increased in obese cases of type 2 diabetes ($P < 0.05$) and ~10-fold increased in lean cases of type 2 diabetes ($P < 0.05$) versus their respective control groups.

Islet amyloid (Fig. 9). As expected, islet amyloid was present in the majority of obese cases with type 2 diabetes when compared with obese nondiabetic cases (81 vs. 10%, $P < 0.01$). Of interest, islet amyloid was not increased in obese cases of IFG compared with obese nondiabetic cases. In the lean cases, islet amyloid was present in 88% of type 2 diabetic subjects versus 13% of nondiabetic

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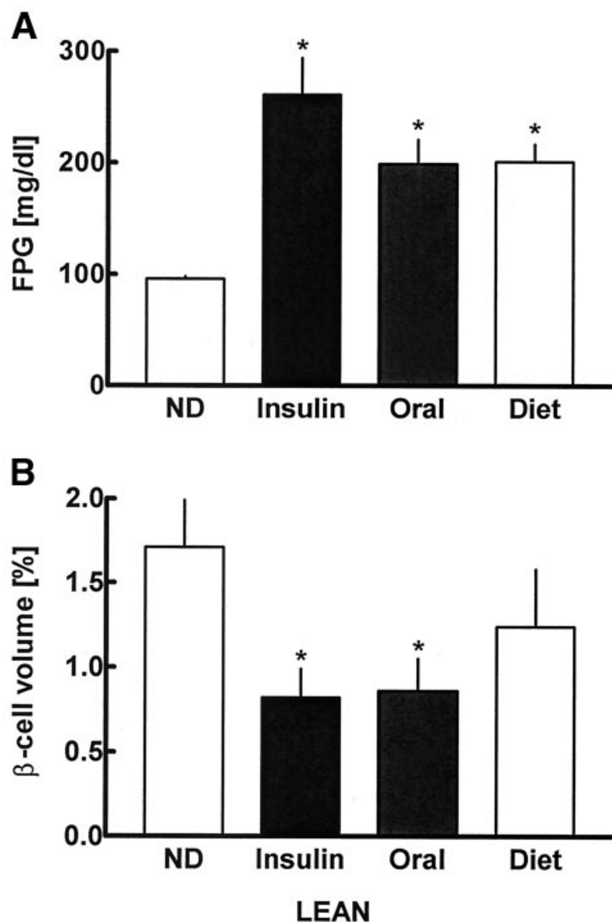

FIG. 4. The mean FPG concentration (*A*) and the relative β-cell volume (*B*) in the lean group of nondiabetic (ND) subjects or diabetic patients who were treated with insulin, sulfonylurea pills (Oral), or diet only.

subjects ($P < 0.01$). The frequency with which islets in a given sample showed positive birefringence, and the extent of birefringence in affected islets was higher in type 2 diabetic cases, both lean and obese, when compared with their nondiabetic counterparts.

DISCUSSION

We report that the relative β-cell volume in humans with both IFG and type 2 diabetes is decreased.

A limitation of the present studies is use of the relative β-cell volume as a surrogate of β-cell mass. This approach will be in error to the extent that there were differences in the overall mean pancreatic weight among groups. However, available data suggest that the pancreatic weight is similar (21,22) or decreased in patients with diabetes (9,27). If pancreatic weight is decreased in type 2 diabetes, then use of the insulin-positive area as a percentage of total exocrine area should introduce, if anything, a conservative error and underestimate any decrease in β-cell mass in type 2 diabetes. Earlier reports that the β-cell mass is decreased in type 2 diabetes may have included cases of type 1 diabetes (17,18). However, more recent reports in which clinical information was better characterized conclude that β-cell mass is decreased in type 2 diabetes (9,22,24), whereas others report no decrease in β-cell mass (19,21). Another study presented in the proceedings of a recent meeting (23) reported a decreased β-cell mass in lean or obese patients with type 2 diabetes who required insulin. However, in contrast to our findings, they reported that obese type 2 diabetic cases treated by diet only or oral medications did not have a decreased β-cell mass. The reason for the discrepancy with the latter study is not known, although it is clear that type 2 diabetes is a heterogeneous disease with a polygenic basis and that there may well be differences in the islet in this disease depending on the region from which the population was sampled. A question that arises from the present study is: what is the significance of an ~60% decrease in β-cell mass in humans? Although rodents tolerate a partial pancreatectomy in which up to 80% of the β-cell mass is removed (28), primates (29) and pigs (30) develop diabetes with a decrease of β-cell mass of ~50% or greater. Furthermore, humans who have had ~50% of their pancreas removed have abnormal glucose tolerance (some developing diabetes by more recent classification) and diminished insulin secretion in response to a hyperglycemic clamp (31–33). Taken together, these findings imply that the ~60% deficit in β-cell mass observed in the present study, particularly in the context of insulin resistance encountered in type 2 diabetes, is sufficient to play an important role in causing hyperglycemia. In the current study, we report that the group with IFG, a group at high risk of developing type 2 diabetes, had a 40% deficit in relative β-cell volume compared with comparably obese control subjects with normal fasting glucose concentrations. This observation implies that the deficit in the β-cell volume (and presumptive mass) is an early process in the development of type 2 diabetes and is likely of primary importance rather than simply secondary to hyperglycemia.

β-Cell mass in rodents is dynamic and regulated with input of β-cells from new islet formation and β-cell replication within islets and output from β-cell senescence (apoptosis) (10,11). Our data support this concept and extend it to humans. Also, we support prior data reporting that obese nondiabetic humans increase β-cell mass, presumably in response to insulin resistance (8,9). However, the extent of this increase (~50%) in the markedly obese humans studied here is much lower than the increase observed in obese mice (5- to 10-fold) (34). The reported increase here may, however, be an underestimate, since the pancreatic weight in obese nondiabetic humans tends to be larger than lean nondiabetic humans (9). Again, this would introduce a conservative error in the stated conclusion that β-cell mass is increased in obese nondiabetic versus lean nondiabetic humans. We report that the mechanism of this increased β-cell mass in humans appears to be through increased new islet formation, assuming that the percentage of insulin-positive cells in exocrine ducts is related to this process. However, in contrast to rodents, it has been reported that there is almost no β-cell replication within existing islets in humans (35), as confirmed in the present work, and therefore (not surprisingly) this does not appear to be an important mechanism for regulation of β-cell mass in humans. The relatively less importance of this pathway for regulation of β-cell mass in humans compared with rodents is consistent with the observation that obese mice develop very large islets (34,36), whereas in humans, islet size is minimally altered by obesity (Table 3). The frequency of apoptosis was not different in the

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FIG. 5. Section of pancreas (×20 magnification) stained for insulin. Numerous pancreatic ducts are shown, with insulin-positive cells present in the duct walls demonstrating new islet formation from exocrine ducts.

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obese nondiabetic versus lean nondiabetic humans (Table 3). In conclusion, β-cell mass is regulated in humans, with the rate of new islet formation apparently the predominant pathway for increased β-cell mass in response to obesity.

To elucidate the underlying mechanism of the relative loss of β-cell mass in type 2 diabetes, we also examined the frequency of new islet formation as well as β-cell replication and apoptosis in islets from each group. Rodent studies have shown that increased rates of β-cell replication can contribute to compensatory increases in β-cell mass, for example in response to partial pancreatectomy (28) or short-term glucose infusions (37,38). We did not observe a decreased frequency of β-cell replication in islets of type 2 diabetic versus nondiabetic cases, although it might be argued that in the presence of hyperglycemia, there should have been an increased frequency of replication if human islets respond in the same manner as rodent islets. The capacity for increased rates of β-cell replication is diminished in the Otsuka-Long-Evans-Tokushima fatty rat model of diabetes (39). It has also been reported that genetic background influences the capacity for increased rates of β-cell replication in other animal studies (40). However, given the extremely low frequency of β-cell replication present in human islets compared with rodent islets, it appears that this important mechanism for regulation of β-cell mass in rodents is unimportant in humans.

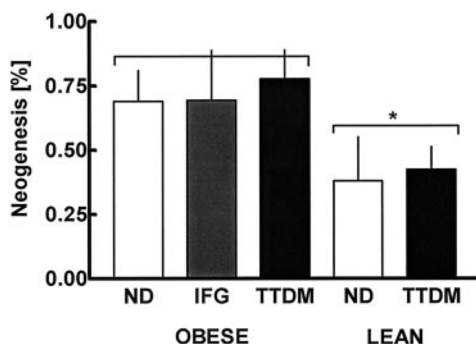

FIG. 6. The relative rate of new islet formation, estimated by fraction of duct cells positive for insulin, in the obese and lean groups. ND, nondiabetic; TTDM, type 2 diabetes.

There is increasing interest in the process of new islet formation from exocrine ducts as a mechanism to replenish or increase β-cell mass (10,11,41,42). We observed new islet formation from exocrine ducts and scattered single β-cells in all cases in the present study consistent with recent (42) and some much older studies in humans (43). There was no difference in the percentage of exocrine duct cells positive for insulin in type 2 diabetic versus nondiabetic subjects, in either the lean or obese groups. This implies that the mechanism that enhances new islet formation in response to obesity is intact in humans with type 2 diabetes. In animal models, both an acute decrease in β-cell mass (partial pancreatectomy [41]) and hyperglycemia (38) induce an increase in new islet formation. As patients with type 2 diabetes had both a decreased β-cell mass and hyperglycemia, it could be argued that there is a

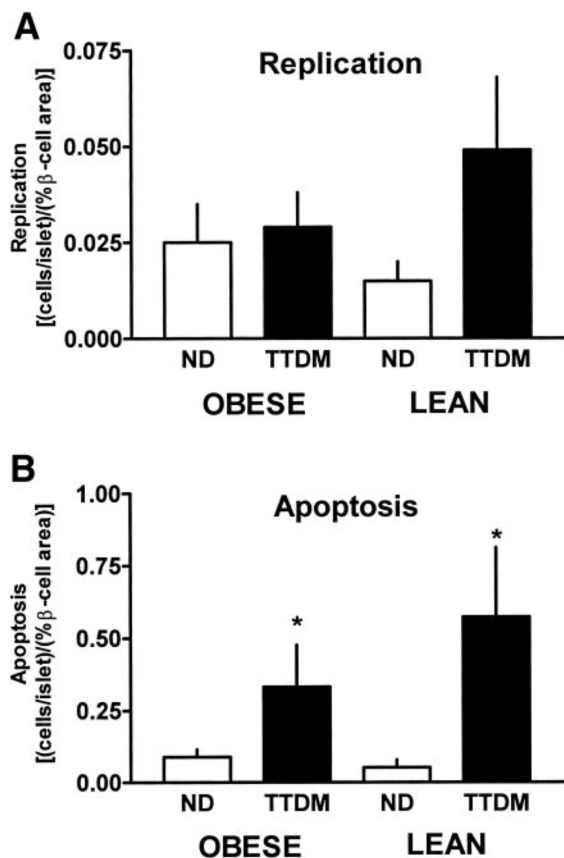

FIG. 7. The frequency of β-cell replication (*A*) and β-cell apoptosis (*B*) normalized to relative β-cell volume in each case. ND, nondiabetic; TTDM, type 2 diabetes.

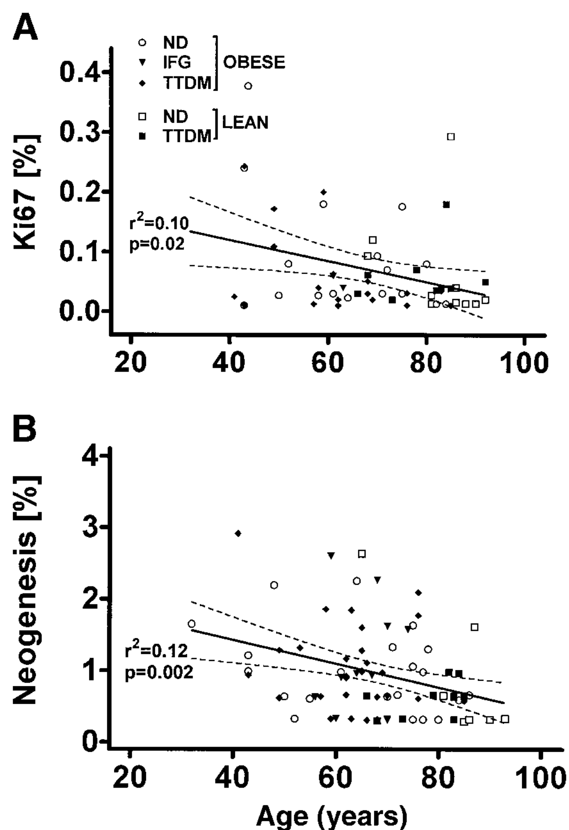

FIG. 8. Correlations of age versus β-cell replication (*A*) and insulin-positive duct cells (*B*). Solid lines are best fit, with broken lines showing 95% CIs.

failure of compensatory increased new islet formation in type 2 diabetes. However, the mechanisms that induce an increased rate of new islet formation in response to a partial decrease in β-cell mass and hyperglycemia in rodents are not known, nor is it known whether such adaptive changes occur in humans. Also, it is unknown whether these adaptive increased rates of new islet formation are sustained over years (as in humans with diabetes) as opposed to weeks (as in rodent studies of hyperglycemia). One very positive implication from the present studies is that the rate of new islet formation is sustained in patients with longstanding diabetes. In fact, there was no difference in the rate of new islet formation versus age in patients with or without type 2 diabetes. These data imply that if the increased rate of apoptosis present in type 2 diabetes (vida infra) could be inhibited, it should be possible to restore β-cell mass in these patients.

Since new islet formation, the predominant input into the β-cell mass in humans, appears to be intact in type 2 diabetes, one might predict that the mechanism for the decreased β-cell mass in type 2 diabetes is an increase in β-cell apoptosis. Consistent with this, we report a 3-fold increased frequency of β-cell apoptosis in obese cases of type 2 diabetes and a 10-fold increased frequency in lean cases of type 2 diabetes compared with the nondiabetic control cases. The increased frequency of apoptotic events per islet in type 2 diabetes is relatively low (~0.3 cells per cross section), raising the question: is this sufficient to account for the decreased β-cell mass in type 2 diabetes?

The difficulty presented by both the frequency measurements of apoptosis by the TUNEL technique as well as the replication by Ki67 is that they do not easily translate into rates. From time-lapse video microscopy studies using β-cell lines (R.R., P.C.B., unpublished observations), we have observed that apoptosis takes ~120 min from initiation to completion (disintegration of the cell). The nuclear debris might still stain positively by the TUNEL method after this period, but in the present studies, we only included TUNEL-positive events when the staining was present in an identifiable cell (see RESEARCH DESIGN AND METHODS) and did not include the staining of debris that was occasionally present. If we make the crude assumption that the ~60% loss of β-cell mass observed in type 2 diabetes in the current study takes ~5 years to evolve, the net loss of β-cells per islet per day would be ~0.8 cells per islet per day (assuming ~3,000 β-cells/islet). Given the fact that each islet cross section only observes a portion of a given islet, the frequency of an excess of ~0.3 apoptotic cells per islet seen here is potentially sufficient to account for the degree of β-cell loss.

If the cause of the decreased β-cell mass in type 2 diabetes is increased β-cell apoptosis, what is the mechanism of this? One of the features present in islets of most humans with type 2 diabetes is amyloid deposits (22,25,44–48) derived from IAPP, also known as amylin (13). The role of islet amyloid in the pathogenesis of type 2 diabetes remains controversial. Several lines of evidence support a potential role of IAPP in the pathophysiology of β-cell loss in type 2 diabetes. First, spontaneous type 2

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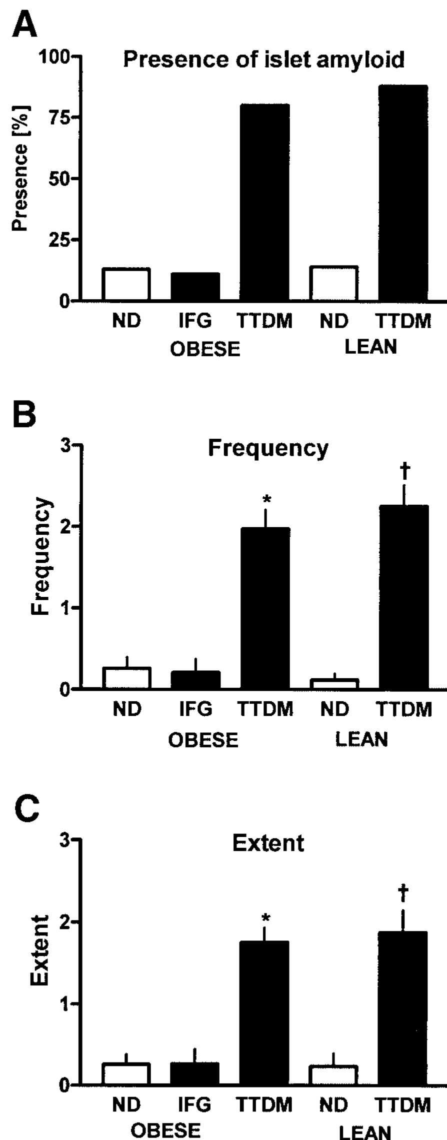

FIG. 9. The presence (*A*), frequency (*B*), and extent (*C*) of birefringence under polarized light when stained by Congo Red.

diabetes occurs in humans, monkeys, and cats, all of which share close homology in IAPP sequence and have IAPP that spontaneously forms amyloid fibrils in an aqueous environment (45–48). Species such as rats and mice that have an IAPP sequence that does not form fibrils in an aqueous solution (49) do not spontaneously develop type 2 diabetes, but instead require selective genetic manipulation (e.g., leptin-deficient mice) to develop diabetes (50). Furthermore, it has been shown that IAPP oligomers cause β-cell apoptosis (14,15), possibly by causing nonselective ion-channel leaks in cell membranes (16). Although it is not plausible to collect pancreatic tissue prospectively from humans, transgenic mice (for human IAPP) are now available which, when obese, spontaneously develop diabetes characterized by islet amyloid and decreased β-cell mass (51). Prospective studies in these mice support the hypothesis that the mechanism of the decreased β-cell mass is increased apoptosis (34).

One argument that has been made against the pathogenic importance of both islet amyloid in type 2 diabetes and cerebral amyloid in Alzheimer's disease is that not all affected cases have amyloid present. In the current study, ~10% of type 2 diabetes had no islet amyloid present in the examined sample. Furthermore, only a minority (~10%) of the cases with IFG had islet amyloid present, while they already had a deficit in presumptive β-cell mass of ~40%. These data are consistent with the previously stated hypothesis that small IAPP oligomers (not detectable by light microscopy) are the cause of β-cell loss, whereas the large extracellular amyloid deposits visible by light microscopy are inert (16,25). Alternatively, it is possible that the formation of islet amyloid is secondary to hyperglycemia and not of primary importance in the pathophysiology of type 2 diabetes.

Another factor that might contribute to the increased rate of apoptosis observed in type 2 diabetes might be hyperglycemia. Recently, it has been shown that islets exposed to high glucose concentrations have an increased rate of apoptosis (52). This mechanism could certainly compound the effects of IAPP oligomers once hyperglycemia supervenes. Since hyperglycemia and insulin resistance increase the expression rate of IAPP to a greater extent than insulin (53,54), hyperglycemia might also further enhance apoptosis due to IAPP oligomers.

In summary, we report that relative β-cell volume, and therefore the presumptive β-cell mass, is decreased in both obese and lean humans with type 2 diabetes compared with their nondiabetic age- and weight-matched counterparts. In the current study, this decrease is present whether the cases were treated by insulin, sulfonylureas, or diet. Furthermore, humans with IFG have a decreased relative β-cell volume, suggesting that this is an early process and mechanistically important in the development of type 2 diabetes. Finally, we ascribe the mechanism for the decrease in β-cell mass to an increase in the frequency of β-cell apoptosis with the rate of new islet formation being unaffected. The implication for prevention of type 2 diabetes is that strategies that avoid the increased frequency of β-cell apoptosis are most rational. Also, in people with established type 2 diabetes, inhibition of this 3- to 10-fold increased rate of apoptosis may lead to restoration of β-cell mass since islet neogenesis appears intact. To realize these goals, further studies to appreciate the mechanism of increased β-cell apoptosis are warranted.

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