Residual Insulin Production and Pancreatic \( \beta \) Cell Turnover after 50 Years of Diabetes: Joslin Medalist Study.

Running title: Residual insulin production after 50 yrs T1DM

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Objective: To evaluate the extent of pancreatic β cell function in a large number of insulin-dependent diabetic patients with disease duration of 50 years or longer (Medalists).

Research Design and Methods: Characterization of clinical and biochemical parameters, and β cell function of 411 Medalists with correlation with post-mortem morphological findings of nine Medalists.

Results: The Medalist cohort, with mean disease duration and age of 56.2 ± 5.8 and 67.2 ± 7.5 years, respectively, has a clinical phenotype similar to type 1 diabetes (T1DM): mean onset at 11.0 ± 6.4 years, body mass index (BMI) at 26.0 ± 5.1 kg/m², insulin dose of 0.46 ± 0.2 u/kg, approximately 94% positive for DR3 and/or DR4, and 29.5% positive for either IA2 or GAD autoantibodies. Random serum C-peptide levels showed that greater than 67.4% had levels in the minimal (0.03-0.2 nmol/L) or sustained range (≥0.2 nmol/L). Parameters associated with higher random C-peptide were lower HbA1c, older age of onset, higher frequency of HLA DR3 genotype, and responsiveness to mix meal tolerance test (MMTT). Over half of Medalists with fasting C-peptide >0.17 nmol/L responded in MMTT by two-fold or greater rise over fasting. Post-mortem examination of pancreases from nine Medalists showed that all had insulin+ β cells with some positive for apoptosis (TUNEL), proliferation (Ki67) and insulitis (CD3).

Conclusions: Demonstration of persistence and function of insulin-producing pancreatic cells suggests the possibility of a steady state of turnover in which stimuli to enhance endogenous β cells could be a viable therapeutic approach in a significant number of patients with T1DM, even with chronic duration.

The incidence of type 1 diabetes is increasing around the world and age at onset is becoming younger (1-4). Over 90% of diabetic patients will develop significant vascular complications resulting in loss of visual acuity, kidney failure, and increased risk of cardiovascular diseases (5-7). Enhancing endogenous insulin production in diabetic patients can substantially improve glycemic control and decrease complications. However, the comparative analysis of residual pancreatic function and morphology in long term diabetic patients has not been studied. The Joslin 50-Year Medalist Study has been characterizing a large cohort of patients who have been insulin dependent for 50 years or longer (8,9). Surprisingly, preliminary screening by random serum C-peptide levels suggests that a majority of Medalists may still be producing insulin.

Studies on individuals with extreme duration of T1DM are rare, but they can be uniquely useful in identifying protective factors against the development of complications and for the preservation of endogenous insulin-producing β cells (10). Bain et al. characterized the Golden Years cohort, a population with 50 or more years of T1DM from the United Kingdom, for complication status and other clinical parameters but not residual insulin production (8). Lohr and Kloppel reported residual β cells in 46% of their 16 autopsied cases of over 21 year duration of diabetes (9). Pipeleers and Ling reported residual β cells in 40% of 43 cases of 10-30 years duration of diabetes and onset after 7 years of age (10). More recently Meier et al reported the presence of insulin-containing β cells in 88% of the pancreases from 42 T1DM patients with duration of diabetes ranging from 4-67
years of which 14 had diabetes for 32 or more years (11). Gianani et al. reported that 3/13 pancreases of childhood onset of diabetes for 10 years or longer were positive for insulin, but only one of these had either DR3 or DR4 allele (12). However, no clinical pre-mortem studies on β cell function were reported in any of these studies.

In the present study, we are reporting the clinical and physiological characterization of 411 patients with insulin-dependent diabetes of 50 years or longer, particularly with regard to their pancreatic β cell function. In addition, uniquely, multiple samples of pancreases from nine Medalists were analyzed morphologically and correlated with pre-mortem data to determine whether the clinical evidence of residual insulin production correlates with the pancreatic histological findings.

METHODS

United States residents who received the Joslin 50-Year Medal were recruited for participation. The Joslin 50-Year Medal is available to any individual who provides medical record or three other forms of documentation of 50 or more years of insulin-dependent diabetes. Information regarding the program is advertised in publications by the American Diabetes Association and the Juvenile Diabetes Research Foundation, as well as, by individual physicians, Medalists, and the general media. By September 30, 2008, 476 medals had been awarded to United States residents since the beginning of this study, 431 (90.5%) appointments were made and 411 Medalists had completed a study visit. The reasons for non-participation were death in two cases, inability to travel/poor health (8), family member illness (5), and/or work (4). Informed consent was obtained from all subjects prior to study participation. The Joslin Committee on Human Subjects approved the study. Most Medalists (88%) received routine endocrine care outside the Joslin Diabetes Center. For the data presented here, all study subjects were evaluated at Joslin by medical history, clinical exams, and blood and urine analysis. Hemoglobin Alc (HbA1C) was determined by HPLC (Tosoh G7 and 2.2, Tokyo, Japan). Lipid profiles were determined by standard enzymatic methods (kits from Roche Diagnostics, Indianapolis, IN; Denka Seiken, Tokyo, JP; and AsahiKasei, Tokyo, JP). Serum C-peptide was determined by RIA (Beckman Coulter, Inc, Fullerton, CA) and validated at the Northwest Lipid Research Laboratory at the University of Washington as previously described (13). IA2 and GAD 65 autoantibodies were assayed as described by Yu et al., both at the Joslin Diabetes Center and Barabara Davis Center for Childhood Diabetes (14).

Mixed Meal Tolerance Test. Any individual with a random C-peptide greater than or equal to 0.1 nmol/L was invited back for a MMTT. Subjects were instructed to fast overnight. At presentation a blood glucose (BG) level was taken via glucometer, if BG level was between 5.5 and 8.25 mmol/L the test was performed. Otherwise, individuals were given insulin or a snack to reach the appropriate range before the test was begun (15). Serum samples were assayed for C-peptide and glucose levels as detailed above at time points 0, 30, 60, 90 and 120 minutes.

Genotyping: DRB1 and DQB1 genotyping was done using linear arrays of immobilized sequence-specific oligonucleotides similar to previously described methodology, with direct sequencing of DRB1 exon 2 to differentiate DRB1*04 subtypes (16). All Medalists as of January 1, 2008 with a random C-peptide in excess of 0.1 nmol/L (n=50) were genotyped for HNF4A, GCK, TCF1, IPF1, and TCF2 (MODY 1-5, respectively) by Athena Diagnostics (Worcester, MA, USA).

Histological studies: Pancreases were
obtained through the Juvenile Diabetes Research Foundation (JDRF)-sponsored Network for Pancreatic Organ Donors with Diabetes (nPOD) program from 50-Year Medalists who consented to post-mortem organ donation. The pancreases were procurred by National Disease Research Interchange and shipped to the nPOD Pathology Core (University of Florida, Gainesville, FL) where they were breadloafed into up to 20 blocks fixed in 10% buffered formalin and processed routinely for paraffin embedding. Sections from blocks spanning the whole pancreas (6-20 blocks/pancreas, mean 15) were available for eight of the nine; only two blocks were recovered for the remaining pancreas. At Joslin Diabetes Center, paraffin sections were microwaved for antigen retrieval and immunostained with guinea-pig anti-bovine insulin (1:200, Linco), rabbit anti-bovine glucagon (1:2000 Dr. M. Appel, UMass), mouse anti-human Ki67 (1:200, BD), and rabbit anti-human CD3 (1:50, Dako) (17). CD3 positive cells in islets were only assessed in regions of the pancreas without many CD3+ cells in the parenchyma. TUNEL (Roche POD TUNEL kit) was performed on microwaved sections of those pancreases without extensive autolysis. Amyloid was detected using Thioflavin S (16). Images were taken on Olympus BH2 microscope or in confocal mode on Zeiss LSM 410 microscope.

Statistical Analysis: All variables were visually inspected and analyzed for distribution to determine appropriate statistical methods for analysis. Wilcoxon rank sum analysis was used for two-way comparisons involving independent continuous variables and Fisher’s Exact test was used to analyze the relationship of categorical variables. Analysis of variance and tests for linear trend were done using generalized linear models designating group as categorical or ordinal as appropriate. A paired t-test was used to determine if the differences between fasting and peak values during MMTT were statistically significant. P-values less than or equal to 0.05 was considered statistically significant. STATA v. 11 (College Station, TX) and SAS v. 9.2 (Cary, NC) were used to perform analyses.

RESULTS
As of September 30th 2008, 411 had participated in this study; 47% were male (Table 1). The average age was 67.2 ±7.4 years, mean age at diagnosis of diabetes was 11.0 ±6.5 years and mean duration of diabetes was 56.2 ±5.8 years. Medalists had a favorable lipid profile (HDL 1.6 ±0.58 mmol/L, LDLc 2.2 ±0.6 mmol/L, total cholesterol 4.2 ±0.9 mmol/L, triglycerides 0.9 ±0.5 mmol/L); average insulin dose per kilogram was 0.46 ±0.2 u/kg; frequency of HLA DR3 or HLA DR4 (0602 excluded) risk alleles exceeded 93% (Table 1). The prevalence of participants who were autoantibody positive was 29.7% (111) for either antigen; 14.9% (55) and 18.4% (69) for IA2 only or GAD only, respectively (Table 1).

In addition to characterizing basic clinical traits we examined family history and the presence of Maturity Onset of Diabetes in the Young (MODY) polymorphisms. The family history of diabetes, both T1DM and T2DM, of all patients was reviewed. 12.9% of Medalists had at least one first-degree relative with T1DM and 29.7% had a first-degree relative with any type of diabetes (Table 1). No significant differences were observed in the frequency of first degree relatives with T1DM or any type of DM according to random C-peptide levels (Table 2). Additionally, 50 individuals with random C-peptide levels in excess of 0.1 nmol/L were genotyped for risk polymorphisms in MODY genes 1-5 ((1) HNF4A, (2) GCK, (3) TCF1, (4) IPF1, (5) TCF2). Of those typed, only four were found to have risk polymorphisms (HNF4A, IPF1 and two had TCF1). Only one
of the four identified with MODY polymorphisms had a C-peptide level greater than 0.2 nmol/L. Analyses were done excluding these individuals and there was no difference in statistical results.

To further examine the role of residual C-peptide Medalists were categorized based on their serum random C-peptide measures. These categories were derived from the Diabetes Control and Complications Trial (DCCT) criteria for examining residual C-peptide production based on response to MMTT. These categories were as follows: undetectable ≤0.03 nmol/L; minimal 0.03-0.2 nmol/L; sustained ≥0.2 nmol/L (17). Our categorization was done using the patient’s random C-peptide. We hypothesize that our means of categorization may underestimate the degree of insulin production, as random C-peptide levels are not necessarily at their maximum as production was likely not stimulated in most cases. There were 33.0% in the undetectable, 64.4% with minimal, and 2.6% with sustained random serum C-peptide levels, resulting in 67.4% (n=256) with at least “detectable” C-peptide levels (Table 2). Analyses indicated a significant difference in glycemic control as measured by glycated hemoglobin across the DCCT defined groups of random serum C-peptide levels, however, this was not linear (7.5 ±1.0%, 7.1 ± 1.1%, and 7.3 ±0.7%, respectively, p=0.005). The group with sustained levels of random C-peptide had a much higher mean of age at diagnosis (16.2 ±8.6 years) as compared to each of the other groups (mean of 10.9 years in each of the other groups, ANOVA p=0.02). MHC HLA risk alleles are also differentially distributed across these three groups of random C-peptide (Table 2). The sustained group had the highest frequency of DR3 risk alleles, while the minimal group (0.03- 0.2 nmol/L) had the highest frequency of DR4 risk alleles. Of interest is the higher frequency of the DR3 risk allele among those with sustained random C-peptide production compared to those with undetectable random levels (57.1% v 33.6% DR3). The presence of islet cell antibodies, either IA2 or GAD, was not different across the three groups; however, the frequency of IA2 autoantibodies was lower than that of GAD in all groups, with none present in the sustained group (Table 2). As shown in Table 2, there was no difference across the three groups in terms of gender, age, duration of disease, HbA1c, family history of diabetes, BMI, insulin dose, lipid profile, or prevalence of micro or macrovascular complications.

There are 14 individuals who were significantly above the rest of the majority of random C-peptide level in excess of 0.17 nmol/L, representing the top 3.5% (Figure 1). On average these individuals have an older age at diagnosis versus the rest of the cohort (17.4 ± 7.4 vs. 10.8 ± 6.3 years, p=0.0008, respectively). Trends that did not reach statistical significance comparing this group with the rest of the cohort were higher frequency of DR3 or DR4 risk alleles (100% v. 93.2%) and lower prevalence of autoantibodies (20.0% vs. 28.7%).

MMTT: The physiological characterization of the C-peptide production in the Medalists was studied through MMTT. Thirty-one individuals were invited to the Joslin for MMTT based on their random C-peptide levels being greater than 0.1 nmol/L. In addition, six non-diabetic age-matched controls were also studied. Thirteen of the 31 Medalist patients who returned for the MMTT responded with doubling of C-peptide levels over their level at time 0 minutes (Figure 2). As shown in Figure 3, the non-diabetic age-matched controls had fasting levels of 0.73 ±0.5 nmol/L and stimulated levels at 60 mins of 3.74 ±1.1 nmol/L, (p<0.004). The thirteen Medalist responders had fasting C-peptide levels of 0.14 ±0.2 nmol/L and reached a maximum of 0.45 ±0.54 nmol/L at 90 mins (p=0.03). In contrast, the 18 non-responders had fasting C-peptide level of 0.11 ±0.1
nmol/L and a maximum level of 0.15 ±0.2
nmol/L at 90 mins (p=0.33) (Figure 3).
Analyses of the responders vs. the non-
responders showed that most responders had
random C-peptide in the sustained group. It is
clear that those diabetic patients with
sustained random levels of C-peptides
(57.1%) were significantly more responsive to
MMTT, defined as at least a doubling of
baseline fasting measure of C-peptide, than
minimal group (14.2%). Additionally, the
sustained C-peptide group showed a
significantly greater response to MMTT at
36.4% compared to the minimal group as
established by random C-peptide level 15.0%,
(p<0.001).
Histology: Pancreases from nine Medalists
representing all three DCCT categories of C-
peptide production were recovered after death
for pathological analysis (Table 3). All were
DR3, DR4 or DR3/DR4 positive; three (33%)
were antibody positive to GAD or IA2
autoantibodies. None of the pancreases
studied came from patients who had a MODY
risk polymorphism; none of these have been
reported on before.
Insulin+ cells were observed in all the
pancreases as scattered single extrainsular
cells or small clusters in some lobes (Table 3).
In the seven Medalists who had onset at age
eight or younger, most islets were atrophic
without insulin staining (Figure 4A, C),
although there were often also small islets
with a few central cells that did not stain for
glucagon. While these unstained central cells
are hypothesized to be degranulated β cells, as
yet no specific β cell markers have stained
positive. However, in two of these Medalists,
insulin positive cells were found within
occasional islets.
In the pancreases from the two Medalists
(Medalists 8 and 9, Table 3) who had later
onset (23 and 30 years) and responded by
doubling their C-peptide during MMTT,
considerably more insulin+ cells were found
and these were clearly within islets (Figure
4D-I). In both, there were islets depleted of
insulin+ cells, as well as islets with
considerable proportion of insulin+ cells.
Intriguingly, in Medalist 8 (onset at 23 years),
half of the pancreas had only atrophic islets
(Figure 4C), but in a lobular pattern there
were islets with significant insulin+ cells
(Figure 4D-F) and even some with amyloid
deposits (Figure 4F). Clinical evaluations of
Medalist 8 showed her to be positive for DR3
and DR4 and well controlled with HbA1c of
6.7%. Two MMTTs in Medalist 8 confirmed
a 360% rise in C-peptide at 90 minutes.
Turnover of β cells was supported by a few
TUNEL+ insulin+ cells in islets or clusters in
two of four (50%) TUNEL-stained pancreases
(Figure 4B) and by Ki67+ islet cells in two
other pancreases; only one of which had
insulin+ Ki67+ cells (Figure 4H).
In three (33%) antibody-negative Medalists, a
few CD3+ cells were found in insulin+ islets
(Figure 4I), and in one antibody-positive
without noticeable insulin+ cells within islets,
a few islets had a CD3+ cell. There were no
other CD3 cells in the low magnification
fields of these pancreases.
DISCUSSION
This study represents the first comprehensive
analysis of the clinical characteristics
affecting β cell function in a large cohort of
insulin-dependent patients of chronic duration
(18). We have correlated these parameters
with post-mortem pancreatic pathology of
some of the same patients. The results showed
that a majority have the ability to produce
insulin endogenously suggested by random
serum C-peptide levels, response to MMTT
and confirmed by the presence of insulin+ cells in all nine available Medalist pancreases,
even in those with undetectable random C-
peptide values (19). This prevalence of
elevated C-peptide levels (67.4% >0.03
nmol/L) in the Medalist cohort is the highest
among published studies of T1DM patients
(19, 20). The DCCT reported only 11% of

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patients screened by MMTT, mean duration of 2.3±0.3 years, had this level of C-peptide (17). Although there can be differences between random C-peptide levels, which could be fasting and those resulting from a MMTT, it is likely that the random serum C-peptide levels would underestimate residual C-peptide levels, biasing our results to the null. However, this has not been studied in individuals with extreme duration of diabetes. Even with this high percentage of retained insulin production, the clinical and biochemical characteristics of this population are consistent with T1DM: low BMI (26 ±5.1kg/m²), onset of disease at 11± 6.5 years, lipid profile of high HDL (1.6 ±0.5 mmol/L) and normal LDL (2.2 ±0.6 mmol/L) and lack of insulin resistance per daily insulin dose (0.46 ±0.2 u/kg). Furthermore, this group, genetically, had high frequency of HLA diabetes risk alleles DR3 and/or DR4 (93%) (21, 22). Also consistent with T1DM, 29.5% of Medalists were positive for IA2 or GAD autoantibodies (23, 24). Thus, it is likely that most of the Medalists studied have autoimmune T1DM.

The random serum C-peptide levels in the Medalists spanned a wide range, from undetectable to over 1 nmol/L. Detailed analysis of Medalists’ clinical parameters according to their C-peptide levels provided very few clues regarding the preservation of endogenous insulin production, suggesting multiple factors contribute. Dividing the cohort into three groups according to C-peptide levels provided interesting findings, such as better glycemic control and later age of onset were associated with higher levels. A small subset of Medalists with high C-peptide levels was associated with older age at onset and more β cells. The pancreases from two of this subgroup (Table 3) had almost 10% of normal β cell mass, while neither Lohr and Kloppel (20) nor Meier et al. (11) found a correlation of later age of onset with residual beta cells, although Pipeleers and Ling did (10). It is possible that the differential preservation of insulin producing cells in the Medalist could have lead to their long term survival.

The MMTT responsiveness of the cohort indicates that the higher random or fasting C-peptide, the greater propensity to respond, suggesting some retained β cells have preserved function. It is interesting to note that in the MMTT, the fasting and maximum levels of C-peptide response of the sustained C-peptide group were approximately 10-20% of those of age-matched non-diabetic controls. For the non-responders, the C-peptide levels at fasting and maximum response were less than 2-3% of the non-diabetic control. The relative differences in the C-peptide levels among these groups corresponded to the β cell number detected by post-mortem morphological analysis of the pancreases.

Our histological studies showed that nine of nine Medalist pancreases, even those (Medalist 1 and 2) who did not have detectable random C-peptide, had residual insulin+ β cells as singlets and small clusters; several (Table 4) had insulin positive cells within islets. All of these would have been classified as pattern A by the criteria reported recently (12) since they all were DR3, DR4 or DR3/4, Caucasian, and had insulin-deficient islets.

The studies from the Medalists provide strong evidence indicating that residual β cells in T1DM patients of chronic duration are in a steady state of cellular apoptosis and proliferation even in diabetes of over 50 years duration. Meier et al. had reported both increased apoptosis and evidence of chronic inflammation in pancreas of various duration ranging from 4 to 67 years of T1DM (11). Interestingly, insulin+ β cells were co-labeled with apoptotic and proliferative markers. In addition, positive CD3 cells were detected in a few islets in all studied pancreases. Clearly more data derived from these pancreases and
of other Medalist patients will be important to solidify these conclusions. Since these are human tissues, there is no possibility to determine if the residual β cells survived the initial autoimmune destruction or formed at some later time. While rare β cell replication was seen, in most of the pancreases the majority of insulin positive cells were single cells, often within the ducts. A recent report by Thorel et al. demonstrated in a murine model that in β cell-depleted mice, α cells could differentiate into β cells in mice after prolonged duration of diabetes (i.e. 10 months). This raises the possibility that the residual C-peptide observed in Medalist represents a slow increase of β cell mass/function in this group of diabetic patients of extreme duration. It should be noted that in five of the pancreases the only insulin positive cells were singlets or small clusters and even in the four with insulin positive cells within islets there was no colocalization of insulin and glucagon. Thus, the source of residual β cells in the Medalist is unclear, but it is likely that they may be derived from multiple sources. In summary, the study of a large group of insulin-dependent diabetic patients of extreme duration characterized clinically, biochemically, and histologically has provided the surprising finding that residual functional β cells remain even after 50 years in a majority of these T1DM patients. The data presented in this study clearly support that even under prolonged autoimmune and metabolic stress pancreatic, β cells can be replenished. Thus, the amelioration of autoimmune stress together with stimulus for regeneration of endogenous β cells could be a feasible approach to improve endogenous insulin production in a substantial number of patients with T1DM.

Author contributions: HAK: Researched, collected and analyzed data and wrote and reviewed manuscript. JKS: Researched, collected and analyzed data and wrote and reviewed manuscript. JL: Collected data. AD: Reviewed and edited manuscript. LPA: Reviewed and edited manuscript. GE: Reviewed and edited manuscript. SBW: Reviewed data, reviewed and edited manuscript. GLK: Reviewed data, reviewed and edited manuscript.

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REFERENCES
1. Onkamo P, Vaananen S, Karvonen M, Tuomilehto J. Worldwide increase in incidence of Type 1 diabetes--the analysis of the data on published incidence trends. *Diabetologia* 1999;42(12):1395-403.
2. Karvonen M, Viik-Kajander M, Molchanova E, Libman I, LaPorte R, Tuomilehto J. Incidence of childhood type 1 diabetes worldwide. *Diabetes Mondiale (DiaMond)* Project Group. *Diabetes Care* 2000;23(10):1516-26.
3. Harjutsalo V, Sjoberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *Lancet* 2008;371(9626):1777-82.
4. Patterson CC, Dahlquist GG, Gyurus E, Green A, Soltesz G. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. *Lancet* 2009;373(9680):2027-33.

5. Gale EA. Glucose control in the UKPDS: what did we learn? *Diabet Med* 2008;25 Suppl 2:9-12.

6. Walsh MG, Zgibor J, Borch-Johnsen K, Orchard TJ. A multinational assessment of complications in type 1 diabetes: the DiaMond substudy of complications (DiaComp) level 1. *Diab Vasc Dis Res* 2006;3(2):84-92.

7. Soedamah-Muthu SS, Chaturvedi N, Witte DR, Stevens LK, Porta M, Fuller JH. Relationship between risk factors and mortality in type 1 diabetic patients in Europe: the EURODIAB Prospective Complications Study (PCS). *Diabetes Care* 2008;31(7):1360-6.

8. Bain SC, Gill GV, Dyer PH, Jones AF, Murphy M, Jones KE, Smyth C, Barnett AH. Characteristics of Type 1 diabetes of over 50 years duration (the Golden Years Cohort). *Diabet Med* 2003;20(10):808-11.

9. Keenan HA, Costacou T, Sun JK, Doria A, Cavellerano J, Coney J, Orchard TJ, Aiello LP, King GL. Clinical factors associated with resistance to microvascular complications in diabetic patients of extreme disease duration: the 50-year medalist study. *Diabetes Care* 2007;30(8):1995-7.

10. Pipeleers D, Int Veld P, Pipeleers-Marichal M, Gorus F. The beta cell population in type 1 diabetes. *Novartis Found Symp* 2008;292:19-24; discussion 24-31, 122-9, 202-3.

11. Meier JJ, Bhushan A, Butler AE, Rizza RA, Butler PC. Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? *Diabetologia* 2005;48(11):2221-8.

12. Gianani R, Campbell-Thompson M, Sarkar SA, Wasserfall C, Pugliese A, Solis JM, Kent SC, Hering BJ, West E, Steck A, Bonner-Weir S, Atkinson MA, Coppieters K, von Herrath M, Eisenbarth GS. Dimorphic histopathology of long-standing childhood-onset diabetes. *Diabetologia* 2010;53:690-698.

13. Mandrup-Poulsen T. Beta cell death and protection. *Ann N Y Acad Sci* 2003;1005:32-42. Erratum published online 05 June 2010.

14. Yu L, Rewers M, Gianani R, Kawasaki E, Zhang Y, Verge C, Chase P, Klingensmith G, Erlich H, Norris J, Eisenbarth GS. Antislet autoantibodies usually develop sequentially rather than simultaneously. *J Clin Endocrinol Metab* 1996;81(12):4264-7.

15. Greenbaum CJ, Mandrup-Poulsen T, McGee PF, Battelino T, Haastert B, Ludvigsson J, Pozzilli P, Lachin JM, Kolb H; Type 1 Diabetes Trial Net Research Group; European C-Peptide Trial Study Group. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. *Diabetes Care* 2008;31(10):1966-71.

16. Hull RL, Watts MR, Kodama K, Shen ZP, Utschneider KM, Carr DB, Vidal J, Kahn SE. Genetic background determines the extent of islet amyloid formation in human islet amyloid polypeptide transgenic mice. *Am J Physiol Endocrinol Metab* 2005;289(4):E703-9.

17. Steffes MW, Sibley S, Jackson M, Thomas W. Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care* 2003;26(3):832-6.

18. Gepts W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* 1965;14(10):619-33.

19. Henquin JC, Cerasi E, Efendic S, Steiner DF, Boitard C. Pancreatic beta-cell mass or beta-cell function? That is the question! *Diabetes Obes Metab* 2008;10 Suppl 4:1-4.

20. Lohr M, Kloppel G. Residual insulin positivity and pancreatic atrophy in relation to duration of chronic type 1 (insulin-dependent) diabetes mellitus and microangiopathy. *Diabetologia* 1987;30(10):757-62.

21. Sanjeevi CB, Sedimbi SK, Landin-Olsson M, Kockum I, Lernmark A. Risk conferred by HLA-DR and DQ for type 1 diabetes in 0-35-year age group in Sweden. *Ann N Y Acad Sci* 2008;1150:106-11.
22. Davies JL, Kawaguchi Y, Bennett ST, Copeman JB, Cordell HJ, Pritchard LE, Reed PW, Gough SCL, Jenkins SC, Palmer SM, Balfour KM, Rowe BT, Farrall M, Barnett AH, Bain SC, Todd JA. A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 1994;371(6493):130-6.

23. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Chase HP, Eisenbarth GS. Number of autoantibodies (against insulin, GAD or ICA512/IA2) rather than particular autoantibody specificities determines risk of type I diabetes. *J Autoimmun* 1996;9(3):379-83.

24. Orban T, Sosenko JM, Cuthbertson D, Krischer JP, Skyler JS, Jackson R, Yu L, Palmer JP, Schatz D, Eisenbarth G; Diabetes Prevention Trial-Type 1 Study Group. Pancreatic islet autoantibodies as predictors of type 1 diabetes in the Diabetes Prevention Trial-Type 1. *Diabetes Care* 2009;32(12):2269-74.

25. Thorel F, Népote V, Avril I, Kohno K, Desgraz R, Chera S, Herrera PL. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature* 2010;464(7292):1149-54.
Table 1. Characteristics of Medalist Study participants.

|                         | % (n); mean ± std dev |
|-------------------------|-----------------------|
| Male (%)                | 47.0% (192)           |
| HbA1c (%)               | 7.3% ±1.1             |
| Age (years)             | 67.2 ± 7.4            |
| Age at diagnosis (years)| 11.0 ± 6.5            |
| Duration (years)        | 56.2 ± 5.8            |
| BMI (kg/m^2)            | 26.0 ± 5.1            |
| C-peptide (nmol/L)      | 0.07 ± 0.12           |
| Cholesterol (mmol/L)    | 4.2 ± 0.9             |
| HDLc (mmol/L)           | 1.6 ± 0.5             |
| LDL (mmol/L)            | 2.2 ± 0.6             |
| Triglycerides (mmol/L)  | 0.9 ± 0.5             |
| Insulin dose (u/kg)     | 0.46 ± 0.2            |
| Family History          |                       |
| Any DM                  | 29.7 (122)            |
| T1DM                    | 12.9 (53)             |
| DR3\dagger               | 38.8 (116)            |
| DR4\dagger               | 52.0 (156)            |
| DR3 or DR4\dagger        | 93.7 (295)            |
| DR3/4\dagger             | 39.1 (118)            |
| IA2 or GAD\dagger        | 29.7 (111)            |
| IA2\dagger               | 14.9 (56)             |
| GAD\dagger               | 18.4 (69)             |
| Proliferative diabetic retinopathy\dagger | 55 (163) |
| Microalbuminuria (ACR<7.91)\dagger | 13.1 (45) |
| Neuropathy (MNSI >2)\dagger | 60.6 (183) |
| CVD\dagger               | 48.3 (160)            |

\dagger Percentages reflect calculations done on data available
Table 2. Characteristics of Medalist Study participants by DCCT categories of residual insulin production.

| Characteristics                     | Undetectable | Minimal       | Sustained     | P*   |
|-------------------------------------|--------------|---------------|---------------|------|
| N (%)                               | 33.0 (126)   | 64.4 (246)    | 2.6 (10)      |      |
| Male                                | 42.6 (55)    | 50.2 (123)    | 52.9 (7)      | 0.4  |
| A1c (%)                             | 7.5 ±1.0     | 7.1 ± 1.1     | 7.32 ± 0.7    | 0.005|
| Age (years)                         | 67.5 ± 8.1   | 67.0 ± 7.2    | 71.7 ± 8.3    | 0.09 |
| Age at diagnosis (years)            | 10.9 ± 6.8   | 10.9 ± 6.1    | 16.2 ± 8.6    | 0.02 |
| Duration (years)                    | 56.4 ± 6.0   | 56.1 ± 5.7    | 55.5 ± 4.1    | 0.7  |
| BMI (kg/m$^2$)                      | 26.7 ± 2.8   | 26.0 ± 4.3    | 23.8 ± 3.6    | 0.5  |
| Insulin dose (u/kg)                 | 0.47 ± 0.2   | 0.5 ± 0.2     | 0.4 ± 0.2     | 0.5  |
| Cholesterol (mmol/L)                | 4.2 ±0.9     | 4.2 ±0.9      | 4.2 ±1.1      | 0.8  |
| HDLc (mmol/L)                       | 1.6 ±0.9     | 1.6 ±0.5      | 1.7 ±0.6      | 0.7  |
| LDL (mmol/L)                        | 2.2 ±0.6     | 2.2 ±0.6      | 2.2 ±0.9      | 0.5  |
| Triglycerides (mmol/L)              | 0.92 ± 0.5   | 0.89 ± 0.51   | 0.98 ± 0.65   | 0.9  |
| Family History                      |              |               |               |      |
| Any                                 | 27.8 (35)    | 31.7 (78)     | 20.0 (2)      | 0.6  |
| Type 1                              | 11.9 (15)    | 14.2 (35)     | 10.0 (1)      | 0.8  |
| DR3                                 | 33.6 (39)    | 43.8 (84)     | 57.1 (4)      | 0.03 |
| DR4                                 | 42.2 (49)    | 57.7 (112)    | 42.9 (3)      | 0.03 |
| DR3/4                               | 44.8 (52)    | 36.6 (71)     | 14.3 (1)      | 0.14 |
| DR3 or DR4                          | 95.7 (116)   | 92.2 (177)    | 100.0 (7)     | 0.4  |
| IA2 or GAD                          | 32.8 (40)    | 27.2 (64)     | 40.0 (4)      | 0.7  |
| IA2                                 | 16.3 (20)    | 13.9 (33)     | 0             | 0.3  |
| Condition                               | 2010 (n=70) | 2015 (n=80) | 2020 (n=10) | p-value |
|-----------------------------------------|-------------|-------------|-------------|---------|
| GAD                                     | 21.1 (26)   | 16.0 (38)   | 36.4 (4)    | 0.1     |
| Proliferative DR                        | 53.9 (56)   | 52.7 (97)   | 36.4 (4)    | 0.5     |
| Microalbuminuria                        | 15.1 (18)   | 10.8 (19)   | 23.1 (3)    | 0.3     |
| Neuropathy (MNSI > 2)                   | 60.4 (67)   | 60.7 (99)   | 60.0 (6)    | 0.9     |
| CVD                                     | 46.4 (52)   | 50.0 (96)   | 50.0 (5)    | 0.8     |
| MMTT response                           | 0 (0/3)     | 14.2 (3/21) | 57.1 (4/7)  | <0.0001 |

\(^1\) Percentages reflect calculations done on data available
*\(^p\)-values resulted from ANOVA
Table 3. Summary of findings in 9 Medalist’s pancreases including insulin, Ki67 and TUNEL staining in cells.

| ID | Sex | Age (years) | Age dx (years) | HbA1c (%) | AB   | HLA | C-peptide (nmol/L) | Insulin+ | Ki67+ | TUNEL+ | CD3+ |
|----|-----|-------------|----------------|-----------|------|-----|-------------------|----------|-------|--------|------|
| M1 | F   | 60          | 1              | 8.8       | GAD+ | DR3/DR 4 | 0.01 | very few, scattered singlets | ND       | Insulin+ clusters | in a few islets |      |
| M2 | M   | 59          | 7              | 5.6       | Neg  | DR3     | 0.02 | in separate lobes, + in ducts | Neg      | autolysis | in a few islets |      |
| M3 | M   | 89          | 5              | 9         | Neg  | DR4     | 0.04 | in separate lobes, + in ducts | in ducts | autolysis | ND     |      |
| M4 | M   | 78          | 4              | 6.6       | IA2+ | DR3     | 0.06 | scattered, clusters up to 8 cells | + cells, but not insulin+ | ND     | ND     |      |
| M5 | F   | 71          | 8              | 5.7       | Neg  | DR3/DR 4 | 0.06 | scattered, clusters and some within islets | ND       | autolysis | ND     |      |
| M6 | F   | 73          | 7              | 7.3       | Neg  | DR3/DR 4 | 0.09 | scattered, within islets, rare cluster few scattered in small islets, rare glucagon+ in ducts | ND       | Neg    | ND     |      |
| M7 | F   | 72          | 5              | 9.8       | GAD+ | DR3/DR 4 | 0.1  | insulin+ ND     | Neg      | autolysis | ND     |      |
| M8 | F   | 79          | 23             | 6.7       | Neg  | DR3/DR 4 | 0.16 | 50% islets none; 25% normal; 25% with amyloid. All islets insulin+. Some small islets all insulin+. + in ducts | ND       | couple insulin+ | in a few islets |      |
| M9 | M   | 88          | 30             | 7.1       | Neg  | DR3     | 1.66 | Insulin+ ND     | ND       | Insulin+ | ND     | in a few islets |      |
Residual insulin production after 50 yrs T1DM
Figures
Figure 1. Distribution of the first 97% of C-peptide levels among 50-Year Medalists with inset of C-peptide values from all values. These pictures demonstrate the outlying 3% in excess of 0.17 nmol/L.

Figure 2. MMTT average response curves for responders and non-responders.

Figure 3. Mean C-peptide levels from MMTT at baseline and peak value of controls, responders, and non-responders

Figure 4. Histological findings in pancreases from 9 Medalists. In 7/9 pancreases, there were mainly atrophic islets (A) in which all or almost all cells were immunostained for glucagon, with occasional small islets that had peripheral glucagon+ cells with unstained central cells (asterix), and rare small clusters or scattered single insulin+ cells (B); all 9 pancreases had these scattered insulin+ cells. In one antibody-positive Medalist some insulin+ cells were TUNEL positive (B). In another pancreas (M8)(C-F) about half of the pancreas had only atrophic islets (C) while there were lobes within the body and tail of the pancreas with most islets with significant proportion of insulin+ cells (D and E, adjacent sections) and some with amyloid deposits (F) (Thioflavin S+). In another late onset diabetes (M9)(age 30) (G-I), every islet had at least one insulin+ cell, most had 10-20% of normal (G). In this last Medalist pancreas, rare Ki67+ insulin+ cells (Arrow indicate Ki67+ nuclei, both within the islet and near by) (H) were found. (I) In 3 pancreases, CD3 cells were rarely found in insulin+ islets while no other CD3 cells were found in the microscopic field.
Residual insulin production after 50 yrs T1DM

**Figure 2**

![Graph showing C-peptide levels over time for Responders and Non-responders.]

**Figure 3**

![Bar graph showing C-peptide levels for Controls, Responders, and Non-responders with p-values for each comparison.]

*P-value is from a paired t-test. Error bars represent standard deviation.*
Figure 4