

Finding GAD: Early Detection of β -Cell Injury

Diabetes mellitus is the general terminology for a number of diseases manifesting in hyperglycemia. The two most prevalent forms, type I and type II diabetes mellitus (T1DM and T2DM), result from either a near complete loss of insulin-producing cells (T1DM, juvenile-onset diabetes, or insulin-dependent diabetes mellitus) or a relative insufficiency of insulin from combined secretory defects and target tissue resistance to the hormone (T2DM, adult-onset diabetes, or non-insulin-dependent diabetes mellitus). In 2005, nearly 16 million Americans had been diagnosed with diabetes (1), but an estimated one third of diabetes remains undiagnosed (2).

Regrettably, the diagnosis of diabetes is often made at a late stage of disease progression, once the complications of chronic hyperglycemia have emerged and well after the initial β -cell injury has taken place. Methods to detect early markers of β -cell death have clear applications for the diagnosis and prevention of diabetes as well as monitoring the engraftment or rejection of islet transplants. In this issue of *Endocrinology*, Waldrop *et al.* (3) describe the use of an assay to detect circulating glutamic acid decarboxylase 65 (GAD65) to successfully track β -cell death resulting from cell-type-specific toxins. This report marks the first time an assay of this kind has been applied *in vivo* using rats, overcoming obstacles preventing similar approaches in the past. The most remarkable finding of the study was the ability to detect β -cell death at a time point well before the onset of hyperglycemia. It is hoped that this assay, and perhaps others like it, will facilitate a better understanding of the earlier stages of diabetes as well as permitting intervention strategies to prevent or limit the severity of the disease.

The primary thrust of the paper by Waldrop and colleagues (3) was to provide proof-of-principle under controlled conditions for the application of their sensitive GAD65 assay (4) to *in vivo* experiments with selective cytotoxic compounds. Two β -cell-specific toxic compounds, alloxan and streptozotocin (STZ), were used at high doses to induce severe β -cell destruction in Wistar rats, and markers of β -cell function and integrity were monitored over time. Both toxins caused acute release of GAD65 into the peripheral circulation and concomitant induction of hyperglycemia; in the same animals, circulating insulin and C-peptide (a cosecreted peptide from insulin processing that remains in the circulation for a longer duration) were significantly depressed (3). To assess whether GAD65 release was proportional to the degree of injury, graded doses of STZ were administered to rats, and serum glycemia and GAD65 were measured at 6 and 24 h after injection. At 6 h, STZ caused a

clear concentration-dependent shedding of GAD65 into the circulation; however, at the same time, it did not produce consistent effects on glycemia, which remained relatively close to basal values. By 24 h, overt hyperglycemia (>16 mm) was observed for all STZ doses 40 mg/kg and above, whereas variable but significantly elevated serum GAD65 was observed (3).

At the low sub-diabetogenic dose of 20 mg STZ/kg, significantly elevated GAD65 was measured in the absence of hyperglycemia. This observation implies that use of GAD65 as a β -cell injury marker may be applied to detect mild insults to insulin-secreting cells. A series of experiments examining apoptosis in pancreatic sections from STZ-treated rats was used to correlate the severity of cell death to GAD65 release. Low-dose STZ was found to neither alter islet morphology nor induce programmed cell death, yet circulating GAD65 was significantly raised in these animals at 6 and 24 h. As expected, diabetogenic doses of STZ caused massive β -cell loss and rearrangement of islet architecture (3). These promising results lend support for use of this assay to study the initiation of diabetes in animal models and human subjects, potentially allowing examination of triggering events before onset of hyperglycemia.

GAD65 and the β -Cell

The GAD enzymes catalyze formation of γ -aminobutyric acid (GABA) from glutamate in neurons and islet endocrine cells (5). GABA is a well-known inhibitory neurotransmitter in the central nervous system, but less is known regarding its function in islets. Current research suggests GABA is localized to synaptic-like microvesicles, which may have differences in stimuli for regulated release, compared with insulin-containing large dense-core vesicles (6). Given the identification of both GABA_A and GABA_B receptors in islet endocrine cells (7, 8), it is largely believed that GABA functions as an autocrine and/or paracrine modulator of islet hormone release.

There are two main forms of GAD encoded by unique genes in mammals, GAD65 and GAD67; only trace amounts of either enzyme are found in adult human tissues other than brain and endocrine pancreas (9). Expression of GAD in islets of Langerhans exhibits species differences for the two isoforms; human α -, β -, and δ -cells express only GAD65, rat islets produce both GAD65 and GAD67, whereas mouse islets synthesize GAD67 almost exclusively (9, 10). In rodent islet tissue, the GAD65 is loosely membrane associated via acylation, whereas GAD67 appears to be cytosolic (11).

The GAD65 isoform has long interested immunologists, because autoantibodies to GAD65 are detected in up to 80% of T1DM patients and can be considered predictive of the disease (12). The triggering events leading to GAD65 autoantibody formation in T1DM are still not entirely clear. One plausible hypothesis proposes an initial insult to β -cells causing the release of GAD65 into the extracellular space, thus

Abbreviations: GABA, γ -Aminobutyric acid; GAD, glutamic acid decarboxylase; STZ, streptozotocin; T1DM, type I diabetes mellitus; T2DM, type II diabetes mellitus.

Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

activating immune T cells; however, molecular mimicry of viral epitopes has also been put forth as a mechanism of autoimmunity (13).

GAD65 as a Marker of β -Cell Death

β -Cell insult, injury, and death are observed in both T1DM and T2DM. Although there is debate over the similarities and differences of mediators of β -cell death in the two subtypes of diabetes (14, 15), it is generally accepted that programmed cell death (apoptosis) underlies the loss of β -cell mass in T1DM and the type I-like pathology observed in some late-stage T2DM patients. *In vitro* studies have shown that treatment of purified rat β -cells with selective toxins, STZ or alloxan, induced the transient release of measurable GAD activity into the extracellular environment using a spectrophotometric assay (16). Similarly, STZ treatment of rat β -cells produced immunoreactive GAD65 in the culture media, as measured by RIA (17). Although these experiments were performed on purified β -cells, this compound is not toxic to other islet endocrine cells, nor does peripherally administered STZ gain access to the brain (18, 19). Hence, GAD65 release after iv or ip injection of this compound would be expected to be derived exclusively from islet β -cells. In control experiments, Waldrop and co-workers (3) examined discharge of GAD65 from toxin-treated mice and partially pancreatectomized rats; their results support the notion that plasma GAD65 originates from β -cells under these conditions.

Two main issues have hampered the use of GAD65 assays *in vivo* to learn about early stages of islet damage: 1) sensitivity of the assays available and 2) assay interference by autoantibodies. The GAD65 RIA has reached a lower limit of detection of 1–3 ng/ml (17), and time-resolved fluorescent immunoassay improved this limit 10-fold to 0.1–0.33 ng/ml (20). Waldrop *et al.* (4) recently developed a novel magnetic bead-based assay to capture GAD65 with a monoclonal antibody directed at a region of the molecule distant from known autoantibody epitopes. This assay obtained a detection limit of 31–56 pg/ml and was equally effective in plasma from GAD65 autoantibody-positive or -negative patients (3, 4).

The question that everyone is asking is whether GAD65 can be used *in vivo* to detect early islet damage. In preclinical studies, use of a GAD enzymatic assay as a marker of islet auto- and allotransplantation rejection was performed in canine models with temporary immunosuppression. Using a direct $^{14}\text{CO}_2$ -release GAD65 assay, Shapiro and colleagues (21) achieved limited success in detecting islet graft rejection but were convinced of the potential of serological detection of islet-specific proteins before the onset of hyperglycemia. Now with a much more sensitive approach to measure GAD65, Chessler's group has completed preliminary proof-of-concept experiments that seem to imply that the answer to the question is yes (3). Acute diabetogenic doses of STZ or alloxan produced rapid spikes in plasma GAD65 within 24 h; low-dose STZ, a mild insult model that can trigger progressive islet destruction, caused a gradual increase in plasma GAD65 over the course of a day. A concentration-dependent shedding of GAD65 was observed with STZ treatment at a time point before onset of hyperglycemia (3).

Potential Impact of Early β -Cell Injury Detection on Diabetes Therapy

Early detection and intensive treatment of T1DM preserves residual β -cell function and limits future disease complications (22, 23). Initial T2DM pharmaceutical regimens typically enhance tissue sensitivity to insulin or enhance secretion from the β -cell to reduce glycemic excursions; however, more recent attention has also been given to emerging therapies also capable of preserving islet mass (24). The remainder of this article describes how the sensitive GAD65 assay, or others like it, may be used to better understand the etiology of diabetes in humans and animal disease models and the impact of such tools on the treatment of human diabetes.

Little is known regarding the earliest stages of T1DM initiation; however, genetic predisposition and extrinsic influences both have roles. Insulinitis, islet infiltration by leukocytes, is commonly clinically observed in histopathological tissue analysis of autopsy and biopsy specimens from young recent-onset T1DM pancreata (25). It is theorized that cytokines, chemokines, and/or reactive oxygen species from the macrophages or damaged islet cells themselves precipitate the induction of the apoptotic program (14, 15). The destruction of the β -cell mass is accelerated by the generation of autoimmune antibodies to insulin, GAD65, IA-2, and phogrin; worsening glucose tolerance may also contribute to β -cell stress and possibly direct glucose toxicity at later stages (26, 27). At the time of diagnosis, it is common for over 70% of β -cells to have been already lost (14).

Prevention of T1DM has been the focus of several large randomized multicenter trials. For T1DM, it has been shown that intervention with immunosuppressants at the time of disease diagnosis prolonged β -cell function; however, benefits did not persist after cessation of treatment (28). Nicotinamide administration also demonstrated beneficial results toward halting progression of islet destruction in animal models of diabetes and human T1DM, but in the large-scale European Nicotinamide Diabetes Intervention Trial (ENDIT), subjects of the study showed identical disease development, regardless of treatment group (29). Preservation of residual β -cell function during intensive insulin treatment (22, 23) was the basis for a similar diabetes prevention trial (DPT-1) examining the effect of oral and parenteral insulin. Unfortunately, progression of diabetes was not able to be prevented (30). Additional interventional strategies have shown promise but have not yet been tested in larger populations (28). The scale of these prevention trials is the primary logistic concern; however, the timing of the treatment is also certainly one of the largest obstacles. Inclusion criteria based on autoantibody positivity postulates disease progression is preventable after the initial insult has occurred. Clearly, the ability to detect the initial injury would allow the earliest possible intervention and likely the greatest chance for successful prevention.

Typically, T1DM requires lifelong insulin injections for survival; the quality of life of T1DM patients is reduced because of the need for painful injections and secondary complications from inferior glycemic control. Improvements in clinical islet transplantation have offered the promise of insulin independence, if only temporarily (31). However, the

secondary effects of continuous immunosuppression necessary to prevent graft rejection may outweigh any benefit in quality of life for patients. The widespread use of this treatment is impeded not only by the availability of donor tissue but also by the high degree of apoptotic tissue loss during the isolation and engraftment of islet tissue (32). Currently, islet graft function is assessed by glycemic control and C-peptide secretion, but these parameters can be used to detect only the late stages of graft rejection (31). Here, a rapid cell viability marker specific to β -cells would allow systematic optimization of the islet isolation and transplantation procedure, focusing on preserving the amount of functional secretory tissue.

Heredity also has a prominent impact on T2DM etiology, together with environmental factors, such as those brought about by a sedentary lifestyle. In health, islet mass is tightly controlled by expansion and involution to reach an equilibrium of β -cell neogenesis, replication, and apoptosis to maintain normoglycemia (33). Glucose intolerance progresses to T2DM when insulin action fails to adequately reduce blood glucose to the normal range, usually the result of defective glucose sensing by the β -cells and resistance of target tissues due to chronic hyperinsulinemia. As the disease worsens in severity, prolonged exposure to elevated circulating glucose and lipids can result in glucotoxicity and/or lipotoxicity to β -cells (33). Postmortem analysis of β -cell mass in T2DM can be as much as 60% lower than matched nondiabetic control patients (34). Although the risk of developing T2DM can be greatly diminished when diet and exercise routines are implemented (35, 36), intervention trials to prevent disease development in high-risk patients through the use of pharmaceutical drugs have also been considered (37, 38). Adequate glycemic control in T2DM can frequently be achieved with current oral drugs; however, patients eventually become refractory to these treatments, ultimately requiring insulin injections. The ability to detect islet apoptosis with a serum marker would allow tailoring the treatment regimen with the goal of preventing late-stage loss of β -cell mass in individuals diagnosed with T2DM.

Although this new sensitive GAD65 assay shows great promise, whether it will be applicable to human disease prediction and prevention remains to be seen. The circulating half-life of GAD65 will have a great impact on the potential utility of it as an early marker. Waldrop *et al.* (3) estimated a half-time of elimination of just under 3 h for recombinant GAD65, and high-dose STZ resulting in near complete β -cell destruction caused a spike within 6 h and a rapid return toward baseline but remaining statistically elevated for over 18 h. Asynchrony of β -cell death in humans is suggested by the long duration over which it occurs in T1DM and the persistence of a small number of β -cells in later stages of diagnosis (39). Mild acute STZ injury of β -cells caused a progressive appearance of circulating GAD65 in rats (3); however, it is unclear whether the prolonged shedding of GAD65 in the progression of human T1DM will be detectable.

Unfortunately, because of species differences in GAD65 expression (10), this assay cannot be used directly in all murine models of spontaneous diabetes (*e.g.* the NOD mouse) or transgenic mice with abnormal glucose homeosta-

sis. However, the next phase of preclinical experiments using this assay will provide the greatest insight into the applicability of this assay to the human state: what is the feasibility of measuring plasma GAD65 in spontaneous models of β -cell death? The BB rat model spontaneously develops a type-I like diabetic phenotype due to autoimmune islet destruction, and the ZDF Fatty Zucker rat is a genetic model of severe obesity leading to a type-II-like diabetic phenotype, with late stage gluco- and lipotoxicity (40). If the technical challenges of such a longitudinal study can be met, and the GAD65 assay proves to offer an advantage over traditional glucose tolerance, C-peptide, and insulin measurements, it will be a unique means to study the first stages of disease progression.

In conclusion, the development of an assay for an early marker of islet injury and death has clear direct applications in clinical medicine. The GAD65 assay, or others like it, may be used to better optimize islet isolation and handling during transplantation and selection of appropriate immunosuppressive drugs to maximize engraftment and minimize graft rejection. Assays of this kind could be applied to detect and limit β -cell destruction in T2DM patients as they become refractory to existing treatment regimens. This type of assay may be used to test diabetes-prone first-degree relatives of T1DM and T2DM patients to permit prevention or to allow the earliest detection and treatment of diabetes. In basic science, assay of early islet destruction could be used for selecting experimental treatments to selectively block β -cell death in diabetic models and to better understand the first stages of spontaneous diabetes in appropriate rodent strains. Although it is premature to conclude the GAD65 assay can be used directly for these studies, it represents a leap in the right direction, and very well may.

Simon A. Hinke

The Vollum Institute for Advanced Biomedical Research
Oregon Health and Science University
Portland, Oregon 97239

Acknowledgments

Received June 27, 2007. Accepted July 11, 2007.

Address all correspondence and requests for reprints to: Simon A. Hinke, The Vollum Institute, Oregon Health and Science University (MRB322/L-474), 3181 Southwest Sam Jackson Park Road, Portland, Oregon 97239. E-mail: hinkes@ohsu.edu.

S.A.H. is funded by the Canadian Institutes of Health Research and the Canadian Diabetes Association.

Disclosure Statement: The author has nothing to disclose.

References

- 2005 National Health Interview Survey. Atlanta, GA: National Center for Health Statistics, Centers for Disease Control
- Cowie CC, Rust KF, Byrd-Holt DD, Eberhardt MS, Flegal KM, Engelgau MM, Saydah SH, Williams DE, Geiss LS, Gregg EW 2006 Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health And Nutrition Examination Survey 1999–2002. *Diabetes Care* 29:1263–1268
- Waldrop MA, Suckow AT, Marcovina SM, Chessler SD 2007 Release of Glutamate Decarboxylase-65 into the circulation by injured pancreatic islet β -cells. *Endocrinology* 148:4572–4578
- Waldrop MA, Suckow AT, Hall TR, Hampe CS, Marcovina SM, Chessler SD 2006 A highly sensitive immunoassay resistant to autoantibody interference for detection of the diabetes-associated autoantigen glutamic acid decarboxylase 65 in blood and other biological samples. *Diabetes Technol Ther* 8:207–218
- Sorenson RL, Garry DG, Brelje TC 1991 Structural and functional consider-

- ations of GABA in islets of Langerhans. β -Cells and nerves. *Diabetes* 40:1365–1374
6. **Braun M, Wendt A, Birnir B, Broman J, Eliasson L, Galvanovskis J, Gromada J, Mulder H, Rorsman P** 2004 Regulated exocytosis of GABA-containing synaptic-like microvesicles in pancreatic β -cells. *J Gen Physiol* 123:191–204
 7. **Brice NL, Varadi A, Ashcroft SJ, Molnar E** 2002 Metabotropic glutamate and GABA_B receptors contribute to the modulation of glucose-stimulated insulin secretion in pancreatic β -cells. *Diabetologia* 45:242–252
 8. **Yang W, Reyes AA, Lan NC** 1994 Identification of the GABA_A receptor subtype mRNA in human pancreatic tissue. *FEBS Lett* 346:257–262
 9. **Mally MJ, Cirulli V, Otonkoski T, Soto G, Hayek A** 1996 Ontogeny and tissue distribution of human GAD expression. *Diabetes* 45:496–501
 10. **Chessler SD, Lernmark A** 2000 Alternative splicing of GAD67 results in the synthesis of a third form of glutamic-acid decarboxylase in human islets and other non-neural tissues. *J Biol Chem* 275:5188–5192
 11. **Dirkx Jr R, Thomas A, Li L, Lernmark A, Sherwin RS, De Camilli P, Solimena M** 1995 Targeting of the 67-kDa isoform of glutamic acid decarboxylase to intracellular organelles is mediated by its interaction with the NH₂-terminal region of the 65-kDa isoform of glutamic acid decarboxylase. *J Biol Chem* 270:2241–2246
 12. **Lernmark A** 1996 Glutamic acid decarboxylase: gene to antigen to disease. *J Intern Med* 240:259–277
 13. **Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ** 1992 Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. *J Clin Invest* 89:283–292
 14. **Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL** 2005 Mechanisms of pancreatic β -cell death in type 1 and type 2 diabetes: many differences, few similarities. *Diabetes* 54(Suppl 2):S97–S107
 15. **Donath MY, Storling J, Maedler K, Mandrup-Poulsen T** 2003 Inflammatory mediators and islet β -cell failure: a link between type 1 and type 2 diabetes. *J Mol Med* 81:455–470
 16. **Smismans A, Ling Z, Pipeleers D** 1996 Damaged rat β cells discharge glutamate decarboxylase in the extracellular medium. *Biochem Biophys Res Commun* 228:293–297
 17. **Hao W, Daniels T, Pipeleers DG, Smismans A, Reijonen H, Nepom GT, Lernmark A** 1999 Radioimmunoassay for glutamic acid decarboxylase-65. *Diabetes Technol Ther* 1:13–20
 18. **Karunanayake EH, Hearse DJ, Mellows G** 1974 The synthesis of [¹⁴C] streptozotocin and its distribution and excretion in the rat. *Biochem J* 142:673–683
 19. **Thulesen J, Orskov C, Holst JJ, Poulsen SS** 1997 Short-term insulin treatment prevents the diabetogenic action of streptozotocin in rats. *Endocrinology* 138:62–68
 20. **Rui M, Hampe CS, Wang C, Ling Z, Gorus FK, Lernmark A, Pipeleers DG, De Pauw PE** 2007 Species and epitope specificity of two 65 kDa glutamate decarboxylase time-resolved fluorometric immunoassays. *J Immunol Methods* 319:133–143
 21. **Shapiro AM, Hao EG, Lakey JR, Yakimets WJ, Churchill TA, Mitlianga PG, Papadopoulos GK, Elliott JF, Rajotte RV, Kneteman NM** 2001 Novel approaches toward early diagnosis of islet allograft rejection. *Transplantation* 71:1709–1718
 22. **The Diabetes Control and Complications Trial Research Group** 1993 The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *The Diabetes Control and Complications Trial Research Group. N Engl J Med* 329:977–986
 23. **Shah SC, Malone JL, Simpson NE** 1989 A randomized trial of intensive insulin therapy in newly diagnosed insulin-dependent diabetes mellitus. *N Engl J Med* 320:550–554
 24. **Baggio LL, Drucker DJ** 2006 Therapeutic approaches to preserve islet mass in type 2 diabetes. *Annu Rev Med* 57:265–281
 25. **Itoh N, Hanafusa T, Miyazaki A, Miyagawa J-i, Yamagata K, Yamamoto K, Waguri M, Imagawa A, Tamura S, Inada M, Kawata S, Tarui S, Kono N, Matsuzawa Y** 1993 Mononuclear cell infiltration and its relation to the expression of major histocompatibility complex antigens and adhesion molecules in pancreas biopsy specimens from newly diagnosed insulin-dependent diabetes mellitus patients. *J Clin Invest* 92:2313–2322
 26. **Mathis D, Vence L, Benoist C** 2001 β -Cell death during progression to diabetes. *Nature* 414:792–798
 27. **Pihoker C, Gilliam LK, Hampe CS, Lernmark A** 2005 Autoantibodies in diabetes. *Diabetes* 54(Suppl 2):S52–S61
 28. **Skyler JS** 2007 Prediction and prevention of type 1 diabetes: progress, problems, and prospects. *Clin Pharmacol Ther* 81:768–771
 29. **Gale EA, Bingley PJ, Emmett CL, Collier T** 2004 European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. *Lancet* 363:925–931
 30. **Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, Cuthbertson D, Rafkin-Mervis LE, Chase HP, Leschek E** 2005 Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial–Type 1. *Diabetes Care* 28:1068–1076
 31. **Ryan EA, Paty BW, Senior PA, Bigam D, Alfidhli E, Kneteman NM, Lakey JR, Shapiro AM** 2005 Five-year follow-up after clinical islet transplantation. *Diabetes* 54:2060–2069
 32. **Emamaullee JA, Shapiro AM** 2006 Interventional strategies to prevent β -cell apoptosis in islet transplantation. *Diabetes* 55:1907–1914
 33. **Prentki M, Nolan CJ** 2006 Islet β -cell failure in type 2 diabetes. *J Clin Invest* 116:1802–1812
 34. **Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC** 2003 β -Cell deficit and increased β -cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110
 35. **Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Hu ZX, Lin J, Xiao JZ, Cao HB, Liu PA, Jiang XG, Jiang YY, Wang JP, Zheng H, Zhang H, Bennett PH, Howard BV** 1997 Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care* 20:537–544
 36. **Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukkaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M** 2001 Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 344:1343–1350
 37. **Gerstein HC, Yusuf S, Bosch J, Pogue J, Sheridan P, Dinccag N, Hanefeld M, Hoogwerf B, Laakso M, Mohan V, Shaw J, Zinman B, Holman RR** 2006 Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial. *Lancet* 368:1096–1105
 38. **Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM** 2002 Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346:393–403
 39. **Pipeleers D, Hoorens A, Marichal-Pipeleers M, Van de Castele M, Bouwens L, Ling Z** 2001 Role of pancreatic β -cells in the process of β -cell death. *Diabetes* 50(Suppl 1):S52–S57
 40. **Hui H, Dotta F, Di Mario U, Perfetti R** 2004 Role of caspases in the regulation of apoptotic pancreatic islet β -cells death. *J Cell Physiol* 200:177–200

Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.