The understanding of the etiology of type 1 diabetes (T1D) remains limited. One objective of the Diabetes Virus Detection (DiViD) study was to collect pancreatic tissue from living subjects shortly after the diagnosis of T1D. Here we report the insulin secretion ability by in vitro glucose perfusion and explore the expression of insulin pathway genes in isolated islets of Langerhans from these patients. Whole-genome RNA sequencing was performed on islets from six DiViD study patients and two organ donors who died at the onset of T1D, and the findings were compared with those from three nondiabetic organ donors. All human transcripts involved in the insulin pathway were present in the islets at the onset of T1D. Glucose-induced insulin secretion was present in some patients at the onset of T1D, and a perfectly normalized biphasic insulin release was obtained after some days in a nondiabetogenic environment in vitro. This indicates that the potential for endogenous insulin production is good, which could be taken advantage of if the disease process was reversed at diagnosis.

Our understanding of the etiology of type 1 diabetes (T1D) remains limited (1). Animal models show only partial similarities with the human disease (2), and there has been a lack of well-preserved human tissue samples (3). The functionality of islets of Langerhans at the onset of T1D in humans remains poorly characterized. Previously, in vitro studies (4,5) have shown the function of islet cells obtained several months after diagnosis, but so far there has been lack of the in vitro access to isolated islets obtained from subjects at the onset of T1D that is required to obtain an in-depth understanding. Studies demonstrating the gene expression profiles for the human pancreas and purified islets in T1D have been published (6,7), providing interesting data supporting that T1D is caused by a chronic inflammatory process with participation of...
Innate immunity. The β-cells express and release cytokines and chemokines, providing a link between the β-cell and the immune system in early T1D (8). However, these studies are based on a few cases of disease, including tissue obtained mainly from subjects with long-standing T1D (6,7).

Insulin secretion from islets obtained from nondiabetic human subjects exhibits a typical biphasic pattern when stimulated with glucose (9). At diagnosis, most patients with T1D have significant, but insufficient, insulin secretion, even though 40–50% of the β-cells may be present (10), suggesting β-cell dysfunction (11,12). It has been suggested (13) that endoplasmic reticulum (ER) stress causes β-cell dysfunction by entrapment of the protein Wolfram syndrome 1 (WFS1), inhibiting the synthesis of cAMP and thereby the secretion of insulin.

Here we report the ability of insulin secretion and explore the expression of insulin pathway genes in isolated islets of Langerhans obtained from subjects with recent onset T1D and nondiabetic control subjects.

**RESEARCH DESIGN AND METHODS**

**Patients and Pancreas Donors**

Six patients (case patients 1–6), 24–35 years of age, who gave their written informed consent were recruited to the DiViD study (14). In addition, the pancreata from two organ donors (case patients 7 and 8) who died at the onset of T1D and from three organ donors (control subjects 1–3) without pancreatic disease were included in the study. Both donors with T1D died of brain edema and total brain infarction, which were described previously (15). Clinical data regarding case patients and control subjects are shown in Supplementary Table 1. The Government’s Regional Ethics Committee in Norway and the Regional Ethics Committee in Uppsala approved the study. For details regarding the methods, see the Supplementary Data.

**RESULTS**

**Islet Function**

The mean glucose-stimulated insulin secretion (GSIS) was reduced in islets from T1D subjects (Fig. 1A). GSIS was lowest when islets were examined on day 1 after isolation but seemed to increase after 3 and 6 days of in vitro culture (Fig. 1A). Islets from individual subjects with T1D had varying levels of GSIS (Fig. 1B). When examined on day 1 after isolation, islets from all subjects except case patient 6 had very low or undetectable GSIS (Fig. 1A). After 3 and 6 days of culture, islets from case patient 1 and 2 secreted a slightly increased amount of insulin upon glucose stimulation, but did not display biphasic insulin secretion, whereas islets from case patient 4 who displayed a poor GSIS 1 day after isolation, recovered to a normal biphasic secretion after 3 and 6 days of culture. Case patient 6 already displayed a close-to-normal GSIS level 1 day after isolation, and the insulin secretion levels increased further after 3 and 6 days of culture (Fig. 1B). For the rest of the case patients (3,5,7,8), the insulin secretion remained low or undetectable after culturing. Islets from nondiabetic subjects already responded with a biphasic insulin release on day 1 and were not further stimulated.

**Whole Transcriptome Sequencing**

We generated a total of 362 million reads and mapped those to the human genome. Both case patients and control subjects generated approximately the same amount of mappable reads. The full data set (reads) is openly available from http://dx.doi.org/10.17044/BILS/g000002.

When comparing expression similarities, using RPKM values, across all genes, islets from five of the six live subjects (case patients 1–5, but not case patient 6) are grouped together and separately from the brain-dead donors (case patients 7 and 8, and control subjects 1–3), regardless of whether the islets are from T1D or nondiabetic subjects (Fig. 2A), reflecting the major impact of brain death on the results obtained from the whole transcriptome analysis. Similarly, genes that are part of the complement system pathway also reflect the differences between islets from live and brain-dead subjects (Fig. 2B). By contrast, the insulin secretion pathway groups islet samples, whether from subjects with T1D or not, notably resulted in longer branch lengths compared with the complement system pathway (Fig. 2C).

In all case patients, except for case patient 6, the genes that (according to the KEGG database) are involved in the secretion of insulin, the insulin pathway, were less expressed when compared with those of the control subjects (Fig. 3). That comparison includes all of the genes in the insulin pathway, both the insulin gene INS itself, and the genes involved in the production and release of insulin. In case patient 6, all the genes in the pathway were upregulated compared with the nondiabetic control subjects. The expression of INS is ~10-fold lower than that in all other diabetic samples, together with lowered expression of two upstream regulators, the pancreatic and duodenal homeobox 1 (PDX1), and MAFA, a transcription factor that binds RIPE3b, a conserved enhancer element that regulates pancreatic β-cell–specific expression of INS (16). Additional genes that are consistently lower in expression include the following: 1) the adenylate cyclase–activating polypeptide (PACAP) and its receptor PACAPR; 2) FFAR1, a member of the GPR40 family of G-protein–coupled receptors and may be involved in the metabolic regulation of insulin secretion; 3) G-protein–controlled integral membrane protein and inward-rectifier–type potassium channel (KCNJ11); 4) ATP-binding cassette transporter of the MRP subfamily (ABCC8), which is involved in multidrug resistance but also functions as a modulator of ATP-sensitive potassium channels and insulin release; 5) a calcium-activated nonselective ion channel that mediates transport of monovalent cations across membranes (TRPM4); and 6) three major glucose transporters in the mammalian blood-brain barrier, which are found primarily in the cell membrane and on the cell surface, where they also function as receptors for human...
Figure 1—GSIS by isolated islets. Twenty handpicked islets were perfused with low glucose (1.67 mmol/L) for 42 min, high glucose (20 mmol/L) for 48 min, and then low glucose again, as indicated. Fractions were collected at 6-min intervals, and the secreted insulin was measured by ELISA. A: Mean insulin secretion from islets isolated from six T1D patients (case patients 1–6) and cultured for 1 (open circles), 3 (black squares), or 6 days (open squares), and from islets isolated from 15 organ donors without pancreatic disease on day 1 (open triangles). B: Individual insulin secretion from islets from two organ donors with T1D (case patients 7 and 8) and from six live patients with T1D (case patients 1–6), cultured for 1 (open circles), 3 (black squares), or 6 days (open squares). Data are observational.
T-cell leukemia virus I and II (GLUT1/2/3). By contrast, the muscarinic cholinergic receptor CHRM3, and the G-protein–coupled receptor CCKAR, which binds nonsulfated members of the cholecystokinin family of peptide hormones and acts as a major physiologic mediator of pancreatic enzyme secretion, are unchanged in expression, or are slightly more highly expressed.

**DISCUSSION**

The results show that the expression of key regulatory elements, the insulin pathway and GSIS, was reduced in islets isolated from seven of the eight subjects with T1D compared with islets from nondiabetic organ donors. However, in one of the eight case patients, the insulin pathway expression and the GSIS were already remarkably preserved on the day of isolation. The observed GSIS improvement after 3 and 6 days of culture in four of the eight case patients suggests that when the islets are removed from the “diabetic milieu,” remaining β-cells can recover. Soluble factors released within the vicinity of the islets could induce a functional impairment of the β-cells, which could be reversed after islet isolation and in vitro culture. However, a screening of 41 cytokines and chemokines in isolated islets from the six T1D patients showed no difference from that in isolated islets from brain-dead organ donors (data not shown).

It is known that the prolonged exposure of human islets to high glucose impairs β-cell function (17), and, since even short episodes of hyperglycemia dramatically affect glucose tolerance (18), it is possible that glucotoxicity could play a role in the reduced GSIS observed in islets from these patients. In 1940, Jackson et al. (19) described that intensive insulin therapy at the diagnosis of T1D led to a long partial remission of the disease. Later, these findings were confirmed using C-peptide determinations (20). However, in an experimental transplantation model, human islets were exposed for 4–6 weeks in vivo to hyperglycemia followed by normoglycemia for 2 weeks (21). The results showed that even if the hyperglycemia-induced impairment in glucose metabolism, depletion of insulin mRNA, decreased (pro)insulin biosynthesis, increased glycogen accumulation, and depletion of insulin content were reversed by the time of the 2-week period of normoglycemia exposure, the deleterious effects of the diabetic state on human islet insulin release remained.

ER stress has been proposed (22) as an important contributor to β-cell dysfunction in T1D. In this study, the gene expression of ER stress markers (BiP, CHOP, ATF4, and XBP1) was high in islets from all subjects with T1D, but did not differ from the islets from the brain-dead nondiabetic control subjects. The process of brain death has been shown to induce ER stress (23), and the finding of a similar level of expression of these genes in islets isolated from subjects with recent onset T1D implies that the ER stress in these islets is a feature of T1D. The expression of WFS1 was lower in islets from subjects with T1D.
(both brain-dead and live subjects) than in islets from the control donors, with the exception of case patient 6, from whom the islets with almost preserved GSIS were isolated. It may be speculated that ER stress and low expression of WFS1 contribute to the reduced β-cell function in T1D.

The level of transcription of the genes in the insulin pathway was reduced in most of the diabetic case patients, compared with the nondiabetic control subjects. However, all genes in the insulin pathway were also expressed in islets from the subjects with no or very low GSIS. This is in line with the results of earlier studies (6,24) describing insulin synthesis and storage remaining in islets, even many years after T1D onset, and they show that the destruction of the β-cells is a slow ongoing process (24).

A limitation in all studies of transcriptome profiling of an unfractioned tissue is that any changes observed can be due to...
to differences in either the modulation of gene expression or from changes in its cell composition. The current analysis was conducted on handpicked islets, thereby avoiding, to a large extent, the problem with analyzing pancreatic biopsy samples in which the islets constitute only ~1%. However, the insulin-producing β-cells constitute only ~60% of the total number of cells in human islets. A reduction in the number of insulin-producing cells in the islets isolated from subjects with recent onset T1D could be in agreement with the observation of a reduction in the insulin pathway.

The most obvious limitation of the current study is the small number of case patients examined. The number of recruited patients was limited by the unexpectedly high frequency of complications arising from the biopsy procedure (14). Therefore, we considered it unethical to continue to enroll patients. A further important consideration is the degree of matching between the tissues obtained from the patients with T1D and those used as control subjects. This study did not include pancreatic biopsy samples from healthy individuals, and we therefore chose to use tissue harvested by alternative methods. Nondiabetic organ donors were chosen as their clinical characteristics are well defined, all having normal HbA1c levels and being negative for four autoantibodies. The islets from the control subjects were not cultured and stimulated with glucose on days 3 and 6, but it has previously been shown (25) that the culturing of normal β-cells does not improve their insulin secretion ability. Due to the limited number of islets available from each of the case patients, we chose to stimulate with glucose only, knowing that additional secretagogues would potentially provide more complete information.

In summary, our findings illustrate the importance of β-cell dysfunction, and not only the loss of the number of insulin-producing cells, at the onset of T1D. The restoration of specific function of the isolated islets removed from the diabetogenic milieu should encourage further characterization of the underlying mechanisms of this functional impairment. Hopefully, this would allow the initiation of clinical intervention trials specifically aiming to restore β-cell function alone or combined with drugs targeting the ongoing injurious processes within the pancreas at the onset of T1D.

Acknowledgments. The authors thank specialist nurse Trine Roald (Oslo University Hospital, Oslo, Norway), who has provided invaluable efforts in the coordination of the study; Sofie Ingvast (Uppsala University, Uppsala, Sweden), for excellent technical assistance; the nurses and doctors at the local hospitals, for providing contact with the patients; and, finally, the patients who participated in this study. The authors also thank the Uppsala Genome Center and UPPMAX for providing assistance in massive parallel sequencing and computational infrastructure.

Funding. The project was founded by grants from South-Eastern Norway Regional Health Authority (to K.D.-J.); The Novo Nordisk Foundation (to O.K. and K.D.-J.), through the PEVNET Study Group founded by the European Union Seventh Framework Programme [FP7/2007-2013] under grant agreement 261441 PEVNET (http://www.uta.fi/med/pevnet/publications.html); Barndiabetesfonden (Swedish Child Diabetes Foundation); the Swedish Medical Research Council (grants 65X-12219-15-6 and K2015-54X-12219-19-4); Diabetes Wellness Sweden; the Swedish Diabetes Foundation; and the Family Emfors Fund. O.K. is in part supported by the National Institutes of Health (grant U01-AI-065192-06). Human pancreatic islets were obtained from The Nordic Network for Clinical Islet Transplantation, which is supported by the Swedish national strategic research initiative EXODIB (Excellence of Diabetes Research in Sweden) and the Juvenile Diabetes Research Foundation. The Uppsala Genome Center and UPPMAX provided assistance in massive parallel sequencing and computational infrastructure. Work performed at the Uppsala Genome Center has been funded by the RFVVR (Council for Research Infrastructures/Swedish Research Council) Swedish National Infrastructure for Large Scale Sequencing and Science for Life Laboratory (SNISS), Uppsala, Sweden.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. L.K. was responsible for clinical coordination, the recruitment of patients, and data collection, analysis, and interpretation and drafted the manuscript. O.S. performed the GSIS analyses, analyzed and interpreted the data, and drafted the manuscript. G.S. performed the whole transcriptome sequencing analysis, interpreted the data, and drafted the manuscript. B.E. and T.B. performed the surgery and participated in the writing of the article. K.F.H., J.L., M.G., and O.K. contributed to the study design, data analysis and interpretation, and writing of the manuscript. K.D.-J. was the principal investigator of the study; had the initial idea for the DiViD study; participated in study design, funding, regulatory issues, international collaboration, and data collection, analysis, and interpretation; and participated in the writing of the manuscript. L.K. and K.D.-J. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

3. Coppeliers KT, Roep BO, von Herrath MG. Beta cells under attack: toward a better understanding of type 1 diabetes immunopathology. Semin Immunopathol 2011;33:1–7
13. Fonseca SG, Urano F, Weir GC, Gromada J, Burcin M. Wolfram syndrome 1 and adenylyl cyclase 8 interact at the plasma membrane to