Glucolipotoxicity Alters Insulin Secretion via Epigenetic Changes in Human Islets

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Type 2 diabetes (T2D) is characterized by insufficient insulin secretion and elevated glucose levels, often in combination with high levels of circulating fatty acids. Long-term exposure to high levels of glucose or fatty acids impair insulin secretion in pancreatic islets, which could partly be due to epigenetic alterations. We studied the effects of high concentrations of glucose and palmitate combined for 48 h (glucolipotoxicity) on the transcriptome, the epigenome, and cell function in human islets. Glucolipotoxicity impaired insulin secretion, increased apoptosis, and significantly (false discovery rate <5%) altered the expression of 1,855 genes, including 35 genes previously implicated in T2D by genome-wide association studies (e.g., *TCF7L2* and *CDKN2B*). Additionally, metabolic pathways were enriched for downregulated genes. Of the differentially expressed genes, 1,469 also exhibited altered DNA methylation (e.g., *CDK1*, *FICD*, *TPX2*, and *TYMS*). A luciferase assay showed that increased methylation of *CDK1* directly reduces its transcription in pancreatic β-cells, supporting the idea that DNA methylation underlies altered expression after glucolipotoxicity. Follow-up experiments in clonal β-cells showed that knockdown of *FICD* and *TPX2* alters insulin secretion. Together, our novel data demonstrate that glucolipotoxicity changes the epigenome in human islets, thereby altering gene expression and possibly exacerbating the secretory defect in T2D.

Type 2 diabetes (T2D) develops as a result of insufficient insulin release from pancreatic β-cells, often in combination with insulin resistance in liver, muscle, and fat. This leads to elevated levels of glucose and fatty acids in the circulation. Extended periods of high glucose (glucotoxicity) or fatty acid (lipotoxicity) levels, or a combination of the two (glucolipotoxicity), have detrimental effects on insulin secretion in vitro (1–3). Cell survival is also compromised under lipotoxic and glucolipotoxic conditions, depending on the duration of treatment and the type of fatty acid (1,3). The elevated levels of glucose and fatty acids found in individuals with T2D may thus further impair insulin secretion and cause a loss of functional β-cell mass. In two recent studies, we demonstrated that glucotoxicity (19 mmol/L glucose for 48 h) or lipotoxicity (1 mmol/L palmitate for 48 h) alone changes the DNA methylation pattern in human pancreatic islets, and these changes may partly explain the impaired insulin secretion in these islets (4,5). However, although most subjects with T2D experience both elevated glucose and lipid levels, the combined effects of glucolipotoxicity on DNA methylation, gene expression, and function in human pancreatic islets have not been reported.

DNA methylation in adult mammalian cells occurs mainly on cytosines followed by a guanine—so-called CpG sites—and it regulates, for example, gene transcription and mRNA splicing (6). Over the past decade, an increasing number of studies have identified altered DNA methylation levels in human islets, which seem to contribute to impaired insulin secretion and T2D (7–15). For example, case-control studies have revealed widespread methylation changes in pancreatic islets from donors with

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T2D when compared with methylation in islets from donors without diabetes (8,13). Functional follow-up experiments in β-cells showed that these alterations induce gene expression changes that impair insulin secretion.

In this study, we investigated for the first time whether 48 h of exposure to glucolipotoxic treatment (19 mmol/L glucose and 1 mmol/L palmitate) alters mRNA expression and DNA methylation patterns genome-wide in human pancreatic islets (Fig. 1). We subsequently performed functional follow-up experiments to test whether the epigenetic changes underlie islet cell defects induced by glucolipotoxicity.

RESEARCH DESIGN AND METHODS

Human Pancreatic Islets

The cohort of human pancreatic islet donors has been described previously (4,5) (Table 1). The islets were obtained from the Nordic Network for Islet Transplantation at Uppsala University, Uppsala, Sweden, through the Human Tissue Laboratory at the Lund University Diabetes Centre. Informed consent was obtained from pancreatic donors or their relatives in accordance with approval by the local ethics committee regarding organ donation for medical research.

We analyzed DNA methylation and mRNA expression in islets from 13 donors. Islets from 8 donors were analyzed by using both mRNA expression and DNA methylation arrays, and islets from an additional 10 donors were used to analyze either mRNA expression or DNA methylation. Hence the cohorts used for gene expression and DNA methylation analyses contain some unique individuals. We measured HbA_{1c} using the NGSP method.

Glucolipotoxic Treatment

A 10 mmol/L solution of BSA-conjugated palmitate was prepared as described elsewhere (5). Before analyses, human islets were cultured for 24 or 48 h in either control (5.6 mmol/L glucose) or glucolipotoxic (19 mmol/L glucose + 1 mmol/L palmitate) CMRL-1066 medium (Fig. 1*A*). In obese individuals with T2D, 1 mmol/L palmitate is at the upper limit of the circulating free fatty acid concentration (16). The molar ratio of palmitate to BSA was

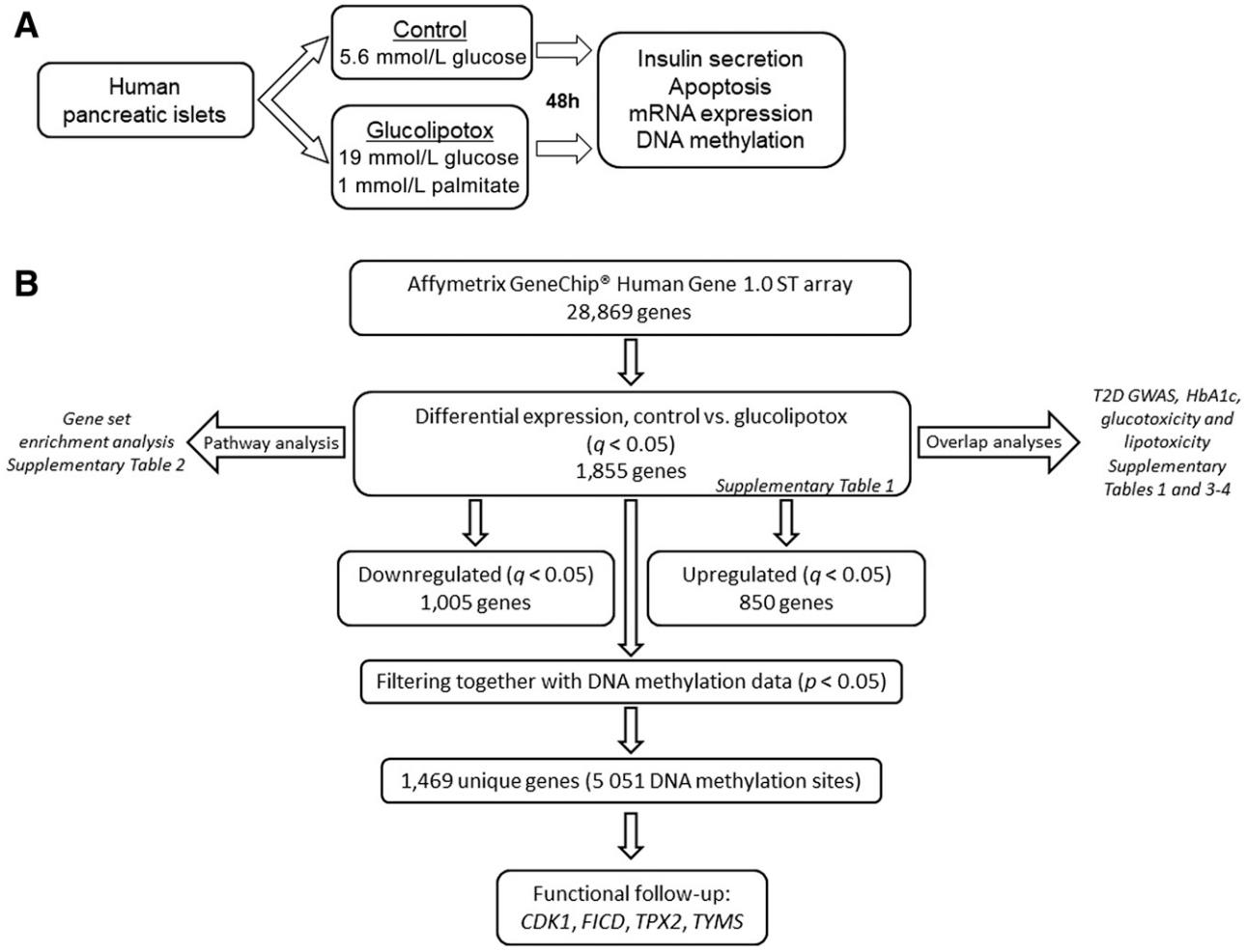

Figure 1—Study design and workflow. *A*: Design for the glucolipotoxicity study in human pancreatic islets. *B*: Workflow for the analyses of mRNA expression data and DNA methylation data in human pancreatic islets exposed to high glucose and palmitate.

Table 1—Characteristics of pancreatic islet donors

Analysis	Islet Donors (*n*)	Sex	Age (years)	BMI (kg/m^2)	HbA_{1c} (mmol/mol)	HbA_{1c} (%)
Insulin secretion	9	5 F/4 M	58.9 ± 9.2	24.3 ± 2.6	37.2 ± 2.1	5.5 ± 0.2
Apoptosis	4†	0 F/4 M	59.3 ± 6.2	25.8 ± 3.4	45.0 ± 8.5*	6.3 ± 0.8*
mRNA expression	13	6 F/7 M	53.5 ± 14.3	25.5 ± 4.3	37.5 ± 9.5**	5.6 ± 0.9**
DNA methylation	13	5 F/8 M	48.6 ± 16.4	26.4 ± 4.5	35.2 ± 7.9***	5.4 ± 0.7***

Data are mean ± SD unless otherwise indicated. F, female; M, male. †One donor had T2D. *Data missing for two donors. **Data missing for five donors. ***Data missing for three donors.

6.6:1 in the culture medium. We cultured the human EndoC-βH1 β-cell line (17) as described previously (18). Glucose (a total of 19 mmol/L) and palmitate (1 mmol/L) were added to the culture media, as indicated in RESULTS.

Insulin Secretion

We analyzed glucose-stimulated insulin secretion (GSIS) in control and glucolipotox-treated islets from nine donors and in EndoC-βH1 cells (passages 79–83). Ten replicates of 10 islets per culture condition and donor were preincubated in HEPES-balanced salt solution containing 114 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L KH_2PO_4, 1.16 mmol/L $MgSO_4$, 20 mmol/L HEPES, 25.5 mmol/L $NaHCO_3$, 2.5 mmol/L $CaCl_2$, and 0.2% BSA, supplemented with 3.3 mmol/L glucose (1.7 mmol/L glucose for one sample), pH 7.2, at 37°C for 1 h. Then we added glucose to five of the replicates, to a final concentration of 16.7 mmol/L (15 mmol/L for one sample), to study GSIS. We kept the other five replicates in 3.3 mmol/L glucose to study basal insulin secretion, and incubation continued for one more hour. The supernatant was immediately removed and the insulin concentration in the medium was measured by radioimmunoassay (Millipore, Uppsala, Sweden). We analyzed GSIS from EndoC-βH1 cells as previously described (19). Secretion in islets was normalized to the number of islets, whereas secretion in EndoC-βH1 cells was normalized to total insulin content.

Assessment of Apoptosis in Human Pancreatic Islets and EndoC-βH1 Cells

We analyzed caspase-3 and -7 activity as a measure of apoptosis, as described previously (20). Full details can be found in the Supplementary Data.

mRNA Expression and DNA Methylation Analyses

DNA and RNA were extracted from islets by using an AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany). We used an Affymetrix GeneChip Human Gene 1.0 ST Array, based on the whole transcript (Affymetrix, Santa Clara, CA), to analyze mRNA expression and an Infinium Human Methylation 450K BeadChip (Illumina, San Diego, CA) to analyze DNA methylation in control and glucolipotox-treated pancreatic islets from 13 human donors (Fig. 1 and Table 1). Full details can be found in the Supplementary Data.

Gene Set Enrichment and Pathway Analyses

We analyzed the enrichment of KEGG pathways within the complete mRNA expression data set using the Gene Set Enrichment Analysis (GSEA) tool (http://www.broad.mit.edu/gsea/, accessed September 2016) (21). Full details can be found in the Supplementary Data. We analyzed pathways in subsets of the expression data set using the Reactome pathway database (www.webgestalt.org). The results were corrected for multiple testing by using the Benjamini-Hochberg method.

Luciferase Assay

We performed a luciferase assay as described previously (7). A brief description can be found in the Supplementary Data.

siRNA Transfection and Functional Experiments

EndoC-βH1 cells (passages 72–78) were seeded in multiwell plates and cultured overnight, and then they were transfected by using Lipofectamine RNAiMAX Reagent (Thermo Fisher Scientific, Waltham, MA) and 50 nmol/L siRNA (Thermo Fisher Scientific). The siRNAs used were s464 (*CDK1*), s21998 (*FICD*), s22745 (*TPX2*), and s14540 (*TYMS*). Silencer Select Negative Control No. 2 siRNA was used as the negative control. Cells were transfected again 24 h later. All functional experiments were performed 72 h after the first transfection. Total mRNA was extracted from transfected cells by using a GeneJET RNA Purification Kit (Thermo Fisher Scientific) and converted to cDNA with a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific), according to the manufacturer's instructions. TaqMan assays (Thermo Fisher Scientific) were then used to analyze the expression of *CDK1* (assay identifier Hs00938777_m1), *FICD* (Hs00200047_m1), *TPX2* (Hs00201616_m1), and *TYMS* (Hs00426586_m1). An assay for the housekeeping gene *HPRT1* (4326321E) was used for normalization, and knockdown efficiency was calculated with the ΔΔCt method. GSIS in transfected cells was analyzed as described in INSULIN SECRETION. The crystal violet assay is described in the Supplementary Data.

Statistics

We used paired *t* tests to analyze functional experiments in human islets and when identifying differences in mRNA expression and DNA methylation between control- and glucolipotox-treated human islets. We analyzed

experiments on clonal β-cells using the Wilcoxon matched-pairs signed rank test or Kruskal-Wallis one-way ANOVA. Data are presented as the mean ± SD. We analyzed the false discovery rate (FDR) to correct for multiple testing in the mRNA expression data (22). Genes exhibiting differential expression with an FDR <5% ($q < 0.05$) were considered significant.

Data and Resource Availability

The expression and methylation data sets generated and/or analyzed during this study are available from the corresponding author upon request. No applicable resources were generated or analyzed during the current study.

RESULTS

Insulin Secretion and Apoptosis in Human Islets After Glucolipotoxic Treatment

Control-treated islets responded with significantly increased insulin secretion when exposed to 16.7 mmol/L glucose but not with 3.3 mmol/L glucose (5.0 ± 1.3 vs. 4.0 ± 1.3; $P < 0.05$; islets from 9 donors), while glucolipotox-treated islets did not (5.1 ± 0.7 vs. 4.4 ± 0.8; $P > 0.05$; islets from 9 donors) (Fig. 2*A*). This corresponds to fold changes (secretion at 16.7 mmol/L glucose divided by secretion at 3.3 mmol/L glucose) of 1.60 ± 0.29 (control) and 1.25 ± 0.08 (glucolipotox), when calculated as the mean of the fold change values in individual experiments. We also tested the effect of 48 h of glucolipotoxic treatment on the human EndoC-βH1 β-cell line. These cells respond suboptimally to glucose alone when not starved overnight in medium with low glucose before the experiment. As starvation would interrupt the glucolipotoxic treatment, we stimulated the cells with glucose in combination with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine to ensure strong induction of insulin secretion. Like islets, glucolipotox-treated EndoC-βH1 cells responded poorly to glucose (Fig. 2*B*). Short-term glucolipotoxic treatment may stimulate insulin secretion at both basal and stimulatory glucose levels (23). Indeed, a shorter exposure (24 h) increased insulin secretion, but reduced fold change, from human islets, but it had no effect on EndoC-βH1 cells (Supplementary Fig. 1*A* and *B*). Islets exposed to glucolipotoxicity for 48 h exhibited more apoptosis than the control-treated islets (Fig. 2*C*), whereas EndoC-βH1 cells were resistant to glucolipotoxicity in terms of cell survival (apoptosis and crystal violet assays; data not shown). Our data thus demonstrate impaired insulin secretion and increased apoptosis in human islets exposed to glucolipotoxicity for 48 h, which several studies have also previously reported (1,24).

Glucolipotoxicity Leads to Widespread Gene Expression Changes in Human Islets

To identify molecular mechanisms that may contribute to the changes we identified, we used microarrays to analyze gene expression in control- and glucolipotox-treated islets from 13 donors (Table 1). The results showed that 1,855 genes were differentially expressed in islets after glucolipotoxic treatment ($q < 0.05$; FDR <5%): 1,005 genes were downregulated and 850 were upregulated (Figs. 1*B* and 3*A* and Supplementary Table 1). We next ran a GSEA to identify cellular pathways that are affected by glucolipotoxicity. This revealed 64 significant KEGG pathways ($q < 0.05$) (Supplementary Table 2). Importantly, metabolic pathways key for islet cell function were downregulated in glucolipotox-treated islets, whereas pathways involved in protein export and exocytosis were upregulated (Fig. 3*B* and Supplementary Table 2). The latter might be a sign of compensatory changes in the islets.

We next investigated whether differentially expressed genes in human islets exposed to glucolipotoxicity overlap with candidate genes for T2D as identified by genome-wide association studies (GWAS). We used the online GWAS SNP library (http://www.genome.gov/gwastudies, accessed 2 July 2018) to acquire candidate gene lists. Of 264 T2D

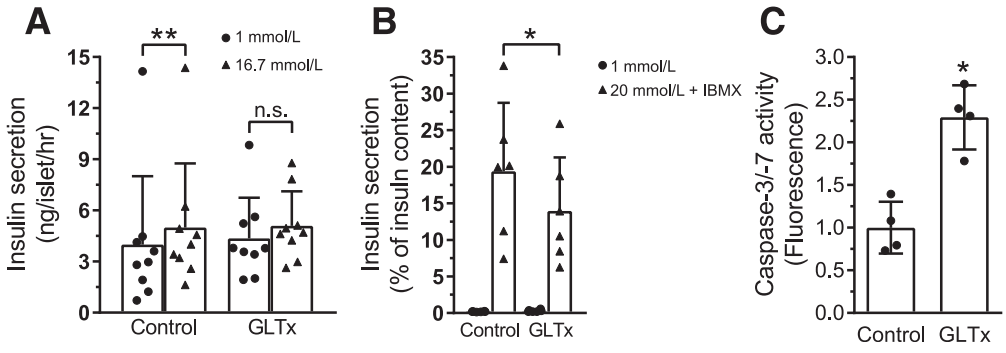

Figure 2—Glucolipotoxic treatment impairs insulin secretion and increases apoptosis in human pancreatic islets. *A*: Control-treated islets, but not glucolipotox-treated islets, respond with significantly increased insulin secretion upon stimulation with 16.7 mmol/L glucose. Data are the mean ± SD of results of experiments on islets from nine donors, with five technical replicates for each condition. $^{**}P < 0.01$, paired *t* test. *B*: GSIS is impaired in glucolipotox-treated EndoC-βH1 cells. Data are the mean ± SD of the results of six experiments, with two technical replicates for each condition. $^{*}P < 0.05$, Wilcoxon signed rank test. *C*: Glucolipotoxic treatment leads to enhanced apoptosis in cultured islets. Data are the mean ± SD of results of experiments on islets from four donors, with four technical replicates for each condition. $^{*}P < 0.05$, paired *t* test. GLTx,: glucolipotoxicity.

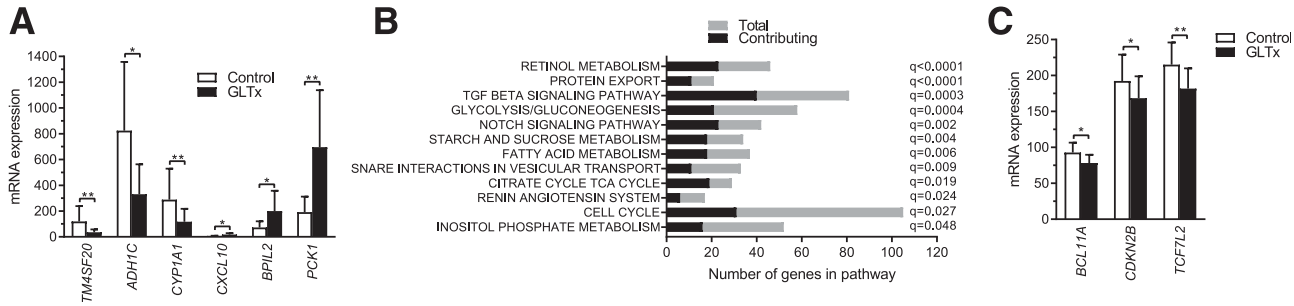

Figure 3—Glucolipotoxic treatment results in widespread gene expression changes in human pancreatic islets. *A*: Top three downregulated and upregulated genes (based on relative change) in islets cultured in glucolipotoxic conditions (islets from 13 donors). *$q < 0.05$, **$q < 0.01$, paired *t* test. *B*: GSEA of the global gene expression data reveals changes to several pathways with key roles in pancreatic islets. The gray bars indicate the total number of genes in the pathway; the black bars indicate the number of contributing genes. *C*: Expression of *BCL11A*, *CDKN2B*, and *TCF7L2* in islets cultured under control and glucolipotoxic conditions. *$q < 0.05$, **$q < 0.01$, paired *t* test. Data in *A* and *C* are presented as the mean ± SD. GLTx, glucolipotoxicity.

candidate genes, 35 were significantly altered by glucolipotoxicity at the expression level (Supplementary Table 1), including *TCF7L2*, *BCL11A*, and *CDKN2B* (Fig. 3*C*). We also analyzed the overlap between the differentially expressed genes and genes for which islet expression is associated with the donor's HbA_{1c} level, a measure of long-term blood glucose levels. This showed that 187 of the differentially expressed genes in glucolipotoxicity-treated islets also associate with HbA_{1c} ($P < 0.05$) (25) (Supplementary Table 1). Finally, we compared our data with previously identified expression changes in islets treated under either glucotoxic or lipotoxic conditions (4,5,26). This showed that six of eight genes differentially expressed in glucotox-treated islets were altered also in glucolipotox-treated islets (Supplementary Table 3). Moreover, all six genes were changed in the same direction in both studies. Among the differentially expressed genes in lipotox-treated islets, 972 were also altered in glucolipotox-treated islets (Supplementary Table 4). We performed a similar comparison with previously published RNA sequencing data from lipotox-treated islets (26). We reanalyzed their data with the same statistical tools we used in the current study, thus revealing that 1,438 genes were altered by palmitate treatment ($P < 0.05$). As no genes in their data set stood for correction for multiple testing, we used the 1,438 genes for which $P < 0.05$ in comparisons with our data, and 242 of these genes were altered also in our study. The expression of three genes—*GLRA1*, *SLCO5A1*, and *LEPREL2*—was significantly altered, and altered in the same direction, by all three conditions when comparing data from the current study with data from the previous studies from our lab (4,5). We then performed a pathway analysis to investigate the role of genes specifically altered by glucolipotoxic treatment but not by either glucotoxic or lipotoxic treatment. This list contains 17 genes previously identified as T2D candidate genes through GWAS (e.g., *BCL11A* and *CDKN2B*), and the analysis revealed that 18 pathways were enriched for genes altered by glucolipotoxic treatment, including several pathways for protein metabolism, transport, and secretion, and a pathway for unfolded protein response (UPR) (Supplementary Fig. 2).

Effects of Glucolipotoxicity on DNA Methylation in Human Islets

One mechanism that may underlie the gene expression changes seen in glucolipotox-treated islets is altered DNA methylation (6). We therefore used an Illumina Human Methylation 450K BeadChip to investigate DNA methylation in control- and glucolipotox-treated islets from 13 donors. When comparing the average methylation of all investigated sites, glucolipotox treatment slightly increased DNA methylation (0.5%, $P < 0.001$; data not shown). When analyzing methylation of different genomic and CpG island (stretches of DNA with a high frequency of CpG sites) regions (27), we observed that the TSS1500 (200–1,500 base pairs upstream of transcription start sites), the 5′ and 3′ untranslated regions, gene bodies, and intergenic regions, as well as northern and southern shores and shelves and the open sea, exhibited slightly but significantly higher methylation in glucolipotox-treated islets (Fig. 4*A* and *B*).

We next analyzed methylation at individual CpG sites. This showed that 62,175 CpG sites, annotated to 16,320 unique genes, showed differential methylation ($P < 0.05$) in islets cultured under glucolipotoxic conditions (Fig. 4*C* and Supplementary Table 5). The majority of these sites (~80%) showed increased methylation after glucolipotoxic treatment. The fold change in methylation of specific sites, calculated as the ratio of methylation in glucolipotox-treated to that in control-treated islets, ranged between 0.73 and 1.65, corresponding to changes from a 27% decrease to a 65% increase in methylation.

To identify genes with both altered expression and methylation, we compared our mRNA expression ($q < 0.05$) and DNA methylation data ($P < 0.05$). We found 1,469 genes with altered mRNA expression and DNA methylation on a total of 5,051 CpG sites (Supplementary Table 6). These include well-known T2D loci such as *TCF7L2*, *CDKN2B*, and *BCL11A*. Pathway analysis for these

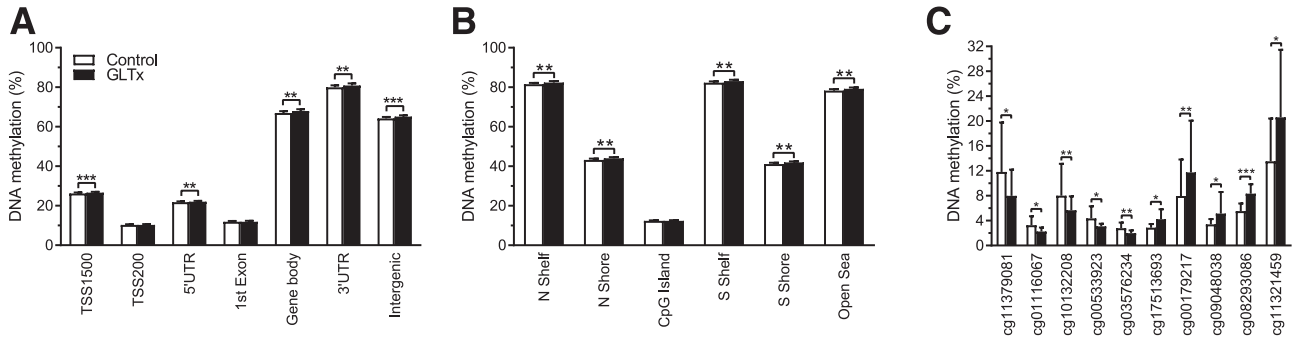

Figure 4—Glucolipotoxic treatment results in altered DNA methylation patterns in human pancreatic islets. DNA methylation in the different genomic (*A*) and CpG island (*B*) regions in pancreatic islets cultured under control and glucolipotoxic conditions (islets from 13 donors). $^{**}q < 0.01$, $^{***}q < 0.001$, paired *t* test. *C*: The 10 CpG sites with the largest relative decrease or increase in methylation in glucolipotox-treated islets (Supplementary Table 5). Data are presented as the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, paired *t* test. GLTx, glucolipotoxicity.

1,469 genes showed that 38 pathways were enriched. This again included protein and vesicle transport pathways as well as protein folding and UPR pathways (Supplementary Table 7). Next, we used a luciferase assay to test whether methylation changes induced by glucolipotoxic treatment can regulate transcription directly and thereby may cause differential expression in treated islets. Here we chose to study *CDK1*, which is known to be important for β-cell function (28) and has four significant methylation sites, of which three are located in the 1,500 base pairs upstream of the transcription start site, in islets cultured under glucolipotoxic conditions. *CDK1* expression was also reduced after the treatment (Fig. 5*A*). Our data show that increasing methylation with the methyltransferases SssI and HhaI (which methylate 31 and 6 methylation sites, respectively) strongly inhibits transcription from the *CDK1* promoter, whereas methylation with HpaII (two methylation sites) had no significant effect (Fig. 5*B*). These results support a role for DNA methylation in the glucolipotoxicity-induced changes in gene expression.

We next tested whether epigenetic changes induced by glucolipotoxic treatment are reversible. Here we used EndoC-βH1 cells exposed to glucolipotoxicity for 48 h (GLTx_acute). Some cells were then allowed to recover for 48 h under normal culture conditions (GLTx_recov). Methylation of 37,382 CpG sites had changed in GLTx_acute cells when compared with control-treated cells ($P < 0.05$) (Supplementary Table 8). Methylation of 5,045 of the 37,382 sites (13.5%) changed during recovery ($P < 0.05$, GLTx_acute vs. GLTx_recov). Among sites that changed during recovery, 98% reversed toward baseline while 2% continued to change in the same direction as during the treatment. This means that ~87% of the methylation changes induced by the treatment persisted or were further enhanced after the 48-h recovery period. Of note, 10,762 genes contain CpG sites whose methylation was affected by the glucolipotoxic treatment in both islets and EndoC-βH1 cells (Supplementary Tables 5 and 8).

Genes Exhibiting Differential Methylation and Expression Regulate β-Cell Function

To strengthen the functional relevance of our findings, we functionally investigated four genes displaying both differential methylation and differential expression: *CDK1*, *FICD*, *TPX2*, and *TYMS*. These are among the top 100 upregulated and downregulated genes, as determined on the basis of fold change, and have previously been reported to have cellular functions with potential importance in insulin secretion and β-cell survival (28–31). They were also altered by treatment with palmitate alone (5). *CDK1*, *TPX2*, and *TYMS* were downregulated in glucolipotox-treated islets, whereas *FICD* was upregulated (Fig. 5*A* and *C*). We knocked these genes down in EndoC-βH1 cells (Fig. 5*D*) and assessed the effects on insulin secretion. Knockdown of *FICD* and *TPX2* increased GSIS. Knockdown of *TPX2* also slightly increased basal secretion (Fig. 5*E*). *CDK1*-deficient cells had slightly less insulin content (negative control siRNA 458.3 ± 78.7 vs. *CDK1* siRNA 389.4 ± 64.8 mU/mg protein; $P = 0.03$), whereas knockdown of the other genes had no significant effect on insulin content (data not shown). Furthermore, because glucolipotoxic treatment resulted in increased apoptosis in human islets, we tested whether knockdown of the four genes resulted in β-cell loss. Just as with their resistance to the glucolipotoxic treatment, the number of EndoC-βH1 cells was not reduced and apoptosis was not increased after any of the four genes were silenced (per crystal violet and caspase activity assays; data not shown).

DISCUSSION

Circulating levels of glucose and lipids are generally elevated and islet function is impaired in subjects with T2D. In this study we examined the effects of treatment with high levels of glucose and palmitate on islet function, gene expression, and DNA methylation. Our data show that glucolipotoxic conditions impair insulin secretion and increase apoptosis in human islets. Furthermore, we show that gene expression changes extensively

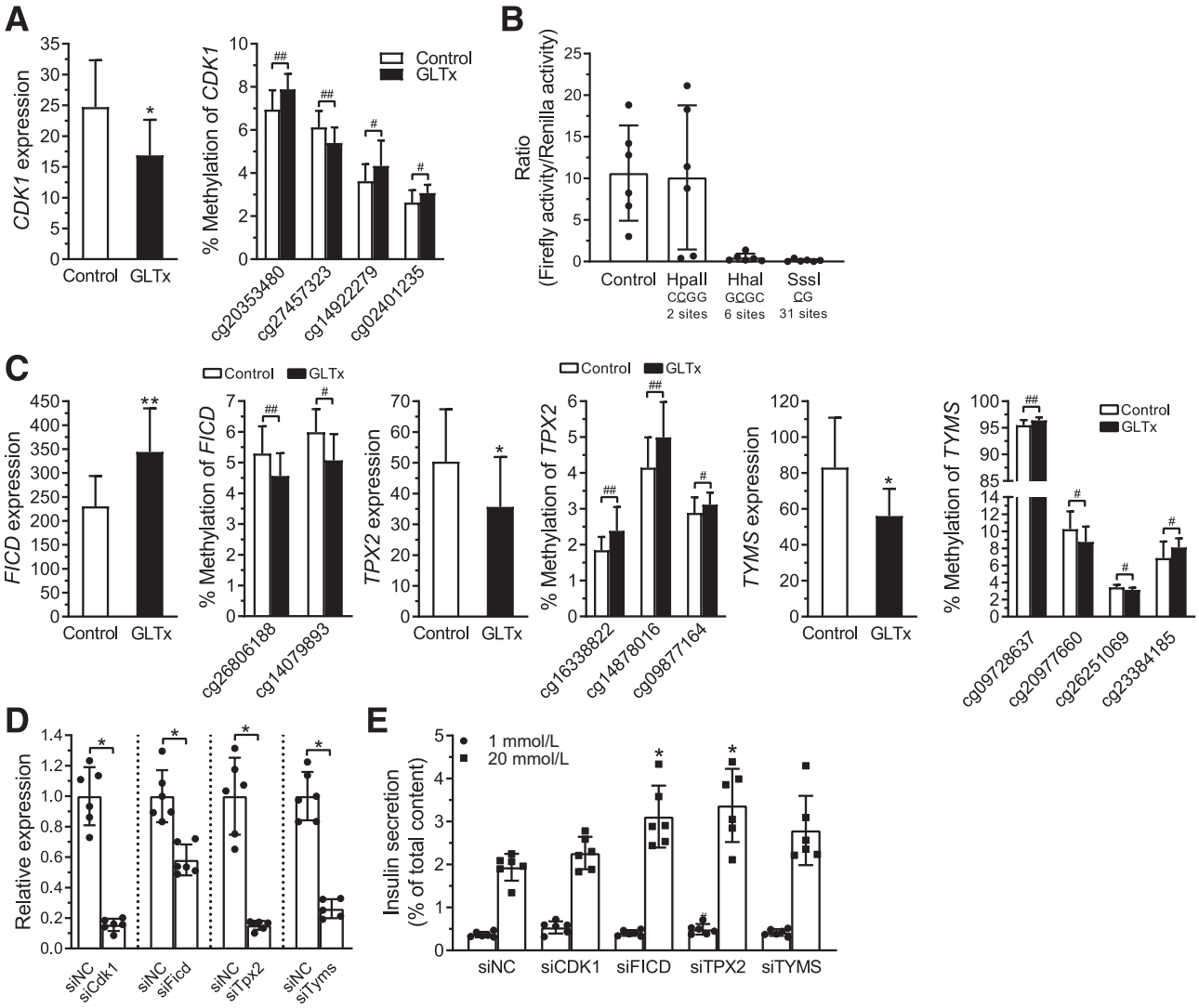

Figure 5—Functional follow-up revealed a role for glucolipotoxicity (GLTx)-induced expression changes in insulin secretion. *A*: Gene expression (left) and DNA methylation (right) for *CDK1* in human islets exposed to glucolipotoxic conditions (islets from 13 donors). $^{*}q < 0.05$; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, paired *t* test. *B*: A luciferase assay showed that the transcriptional activity of the *CDK1* promoter was greatly inhibited after methylation of the promoter with SssI or HhaI, whereas methylation with HpaII had no effect ($n = 6$). $P = 0.001$, Kruskal-Wallis one-way ANOVA. *C*: Expression and methylation of *FICD* (left), *TPX2* (middle), and *TYMS* (right) in human islets exposed to glucolipotoxic conditions (islets from 13 donors). $^{*}q < 0.05$, $^{**}q < 0.01$; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, paired *t* test. *D*: Quantitative PCR analysis of siRNA-mediated *CDK1*, *FICD*, *TPX2*, and *TYMS* knockdown in EndoC-βH1 cells. Data are the mean ± SD of six experiments. $^{*}P < 0.05$, Wilcoxon matched-pairs signed rank test. *E*: GSIS in siRNA-transfected β-cells. Data are the mean ± SD of six experiments, with two or three technical replicates for each condition. $^{*}P < 0.05$ vs. negative control siRNA (siNC) at 20 mmol/L (Wilcoxon matched-pairs signed rank test).

in glucolipotox-treated islets, which in many cases may be due to altered DNA methylation. Finally, follow-up in clonal β-cells shows that these changes affect insulin secretion, thus supporting a contributing role for the induced epigenomic and transcriptomic changes in T2D.

Recent studies show that dietary intake affects epigenetic patterns, which seem to play an important role in T2D development. For example, a randomized controlled trial of high intake of saturated or polyunsaturated fatty acids resulted in distinct epigenetic changes in adipose tissue (32). Short-term treatment of islets or β-cells with high levels of glucose or fatty acids increases insulin secretion, often at both basal and stimulatory glucose levels (23). Longer treatment periods, however, impair insulin secretion (33,34). Moreover, treating human islets with high glucose or the saturated fatty acid palmitate altered DNA methylation (4,5). Also, other epigenetic modifications are affected by nutrients. For example, culturing β-cells in high glucose and palmitate for 48 h resulted in altered histone modifications (33), and nutrient-induced changes of miRNAs and long noncoding RNAs have also been reported (35). The current study supports a role for glucolipotoxicity-induced methylation and expression changes in T2D. Several metabolic pathways of

importance for β-cell function, such as glycolysis and citrate cycle pathways, which are key for proper insulin secretion (36), were downregulated in glucolipotox-treated islets. Also, several candidate genes for T2D were differentially expressed, including *TCF7L2*, which in GWAS shows the strongest and most consistently replicated association with the disease (37). Genes known to associate with HbA_{1c} level were also altered in glucolipotox-treated islets (25). Finally, our follow-up experiments support not only that glucolipotoxicity-induced methylation changes may cause the identified expression changes, but also that the changes in gene expression may alter insulin secretion and thereby contribute to the secretory defects in glucolipotox-treated islets. Knockdown of *FICD* and *TPX2* in clonal β-cells affected insulin secretion. *FICD* expression was increased in glucolipotox-treated islets. The protein encoded by *FICD* is involved in endoplasmic reticulum homeostasis because it both AMPylates and de-AMPylates—and thereby regulates—the endoplasmic reticulum chaperone BiP (38). As knockdown of *FICD* improved GSIS in EndoC-βH1 cells, its increased expression in glucolipotox-treated islets could contribute to the observed impairment of GSIS. TPX2 is involved in nucleating microtubules (30). Knockdown of *TPX2* increased insulin secretion, indicating that the reduction of *TPX2* expression may be part of a compensatory effort in glucolipotox-treated islets. Microtubules have been reported to be involved in sustained insulin secretion (39,40), although not all studies support this finding (41). TPX2 has also been shown to inhibit kinesin 11/EG5 (42). Although kinesin 11 has not been studied in relation to insulin secretion, it is involved in protein secretion (43), and its inhibition in islets could affect insulin secretion. TPX2 may also be involved in epigenetic regulation, as it interacts with SIRT1 and impacts histone acetylation (44). Our functional experiments thus suggest that expression changes induced by glucolipotoxic treatment alter GSIS, but more experiments are needed in order to dissect the mechanisms behind these effects. However, none of the studied genes affected cell number or apoptosis when silenced in EndoC-βH1 cells. CDK1 is a known cell cycle regulator, and one may expect that silencing this gene would affect cell number (45). However, EndoC-βH1 cells divide very slowly (about once per week), and it might take longer than the 72 h we used for an effect on cell number, survival, or both to manifest in CDK1-deficient cells.

About half of the genes altered by glucolipotoxic treatment were also altered in palmitate-treated islets in a study from our laboratory (5). The overlap was smaller when comparing to data from a similar lipotoxicity study by Cnop et al. (26). Several reasons potentially explain this difference in overlap. Cnop et al. used islets from a smaller number of donors, meaning that some genes may have been missed because of power issues. Furthermore, palmitate concentration (it was higher in our studies) and culture conditions differed. Finally, whereas we used a microarray to assess gene expression, Cnop et al. used RNA sequencing. The genes altered in islets treated with high glucose and palmitate, but not either nutrient alone, were enriched in pathways involved in protein metabolism, transport, and secretion—that is, pathways of obvious importance in insulin-secreting β-cells. A UPR pathway was also enriched for these significant genes. The UPR is known to play a role in T2D and β-cell function (46). Together, these findings suggest that changes induced by high glucose and palmitate in combination may play a role in the deterioration of β-cell function in vivo, which does not occur when only glucose or palmitate level is elevated. The list of genes with altered expression in glucolipotox-treated islets contains 35 genes with genetic variants that previous GWAS have associated with altered T2D risk. More than half of these gene variants or proteins encoded by the genes, such as *TCF7L2*, *GCKR*, and *HNF1B*, have been suggested to directly or indirectly affect islet cell development, function, or both (47–65). Several of the remaining genes (e.g., *FAF1* and *MAP3K1*) have been shown to affect cell proliferation, apoptosis, or both—some specifically in β-cells (66–71). This further supports the hypothesis that expression changes induced in islets by high levels of palmitate and glucose contribute to the increased risk for T2D in obese individuals or further impair β-cell function in individuals with T2D.

Many methylation changes identified in this study are modest. However, T2D is a polygenic, multifactorial disease (37) in which each genetic variant contributes a small part of the overall risk. The same can probably be said about epigenetic changes. Additionally, several changes in the same biological pathway could increase the impact on cellular function (72). And, importantly, the exposure to elevated nutrient levels in vivo extends for years or decades, rather than for 48 h. The epigenetic effects in vivo may thus be much larger than what we found in this study. Also, most of the epigenetic changes persisted even after the cells were removed from the glucolipotoxic environment. Thus, normalizing blood nutrient levels through treatment and/or lifestyle changes may not fully reverse already established epigenetic effects. Interestingly, a large proportion of the CpG sites affected by glucolipotoxicity in both human islets and EndoC-βH1 cells were annotated to the same genes. When instead looking at the exact sites, the overlap was smaller. The reasons for this may be lower power in the EndoC-βH1 experiments than in the islet experiments. Also, EndoC-βH1 cells are of fetal origin, and as such they exhibit DNA methylation patterns that are different from those in mature β-cells (73). Treatments that alter DNA methylation may hence have some different effects in EndoC-βH1 cells and mature β-cells. Nevertheless, the EndoC-βH1 cell line is the best existing in vitro model for human β-cells.

In conclusion, our data show that glucolipotoxicity induces methylation and expression changes in human pancreatic islets that may contribute to impaired insulin secretion and increased cell death. Together, these effects

further support a role for glucolipotoxicity-induced changes in the development and/or exacerbation of T2D.

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