SUPPLEMENTARY DATA

Materials and data analysis

Assessment of apoptosis in human pancreatic islets and EndoC-βH1

Caspase-3 and -7 activity, as a measure for apoptosis, was measured with the Apo-ONE® Homogeneous Caspase-3/7 Assay (Promega, Madison, WI, USA) in islets from four donors. The assay contains proflourescent rhodamin 110 (Z-DEVD-R110) which serves as a substrate for both Caspase-3 and -7. Upon lysis of cells, active Caspase-3/7 in the sample will cleave Z-DEVD-R110 to fluorescent rhodamine 110, which is then measured. After islets were cultured for 48h in control or glucolipotoxic treatment, triplicates of 20 islets were hand-picked from each culture condition, washed and transferred to a plate containing HBSS and the assay reagent. Fluorescence was then measured with a Tecan Infinite® M200 PRO plate reader (Tecan Group Ltd., Männedorf, Switzerland) after a 90 minutes incubation to determine the Caspase-3/7 activity. EndoC-βH1 cells were washed in HBSS before addition of the assay reagent and measurement in a similar fashion as described above.

RNA and DNA isolation

DNA and RNA were extracted from human pancreatic islets with the AllPrep DNA/RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA quantity and quality were assessed by Nanodrop (Nanodrop, Wilmington, DE, USA) and Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The 260/280 ratios and RNA integrity number (RIN) values were >1.9 and ≥7.4, respectively, for all samples. DNA was extracted from glucolipotox-treated EndoC-βH1 with the DNeasy Blood and Tissue kit (Qiagen).

Microarray mRNA expression analysis

The Affymetrix GeneChip® Human Gene 1.0 ST whole transcript-based array (Affymetrix, Santa Clara, CA, USA), which covers 28,869 genes, was used to analyze mRNA expression (**Figure 1b**) in pancreatic islets exposed to control or glucolipotoxic treatment from 13 human donors (**Table 1**), according to the manufacturer's recommendations. The Oligo package from Bioconductor was used to compute Robust Multichip Average expression measures (1).

Gene set enrichment and pathway analysis

Enrichment of KEGG pathways within the complete mRNA expression data set was analyzed with the gene set enrichment analysis tool (http://www.broad.mit.edu/gsea/, accessed September 2016) (2). All genes were sorted by the t-statistic obtained from the analysis of mRNA expression data. Default settings for number of genes included in gene sets were used. The results were sorted on the normalized enrichment score and pathways were considered significant if the false discovery rate (FDR) was below 5% (0.05). Pathway analysis was performed on subsets of the expression data set, as described in the text, by using the Reactome pathway database at www.webgestalt.org. The results were corrected for multiple testing with the Benjamini-Hochberg method.

Genome-wide DNA methylation analysis

A total of 500 ng genomic DNA from EndoC-βH1 or human pancreatic islets of 13 donors (**Table 1**) exposed control or glucolipotoxic conditions was bisulfite-converted with the EZ DNA methylation kit (Zymo Research Corporation, Irvine, CA, USA). DNA methylation of the bisulfite-converted DNA from islets was analyzed with the Infinium HumanMethylation450K BeadChip (Illumina, San Diego, CA, USA), which contains 485,577 probes annotated to 99% of all RefSeq genes (3) according to the standard Infinium HD Assay Methylation Protocol (Part # 15019519, Illumina). The Infinium HumanMethylation450K BeadChips were then imaged with the Illumina iScan. The raw methylation score for each CpG site, which is represented as β-value, was calculated with the GenomeStudio® methylation module software. The β-values were calculated as (β = intensity of the methylated allele (M)/(intensity of the Unmethylated allele (U) + intensity of the Methylated allele (M) + 100)). All samples passed GenomeStudio® quality control steps based on built-in control probes for staining, hybridization, extension and specificity, and displayed high quality bisulfite conversion efficiency with an intensity signal above 4,000 (4). Probes were filtered for further analysis based on a mean detection P-value >0.01. β-values were then converted to M-values ($M = \log2 (\beta/(1 - \beta))$) for further bioinformatic

and statistical analyses of the methylation data (5). Background and quantile normalization was performed with the lumi package from Bioconductor (6). Background correction was performed by subtracting the median M-value of the 600 built-in negative controls and methylation data were further normalized by quantile normalization (7). ComBat was used to adjust for batch effects between runs (8). As β-values are easier to interpret biologically, M-values were reconverted to β-values when describing the DNA methylation results. The DNA methylation probes on the Infinium HumanMethylation450K BeadChip have been annotated to different genomic regions depending on their location in relation to nearby transcripts or CpG islands (3). Methylation in EndoC-βH1 was analysed with the Infinium MethylationEPIC Kit in a similar manner as described above, but probe design bias was corrected for by beta-mixture quantile (BMIQ) normalization (9).

Luciferase assay

Luciferase assay was performed as described previously (10). Briefly, a 1.5kb fragment immediately upstream of the transcription start site of the *CDK1* gene was inserted into a CpG- free firefly luciferase reporter vector (pCpGL-basic). The plasmid was then mock-methylated or methylated with three different methyltransferases, SssI, HhaI and HpaII, which methylate all CpG sites, the internal cytosine residue in GCGC sequences, or the internal cytosine residue in CCGG sequences, respectively. The unmethylated or methylated construct was then co-transfected together with a renilla luciferase plasmid (Promega, Madison, WI, USA) into the 832/13 INS-1 beta-cell line (11). Firefly and renilla luciferase luminescence, as a value of transcriptional activity, was measured for each construct with the Dual-Glo Luciferase Assay System (Promega) and an Infinite M200 PRO plate reader. Cells transfected with an empty pCpGL-vector were used as a background control for firefly luciferase results, and untransfected cells were used as a background for renilla results.

Crystal violet assay

EndoC-βH1 beta-cells (passage 76-78) were seeded and transfected in 96-well plates. 72h after the first transfection the cells were washed with PBS and stained with 0.1% crystal violet in 0.15 M NaCl. Cells were then washed with water and allowed to dry. Methanol was added to wells and absorbance was measured at 600 nm in an Infinite® M200 PRO plate reader.

References

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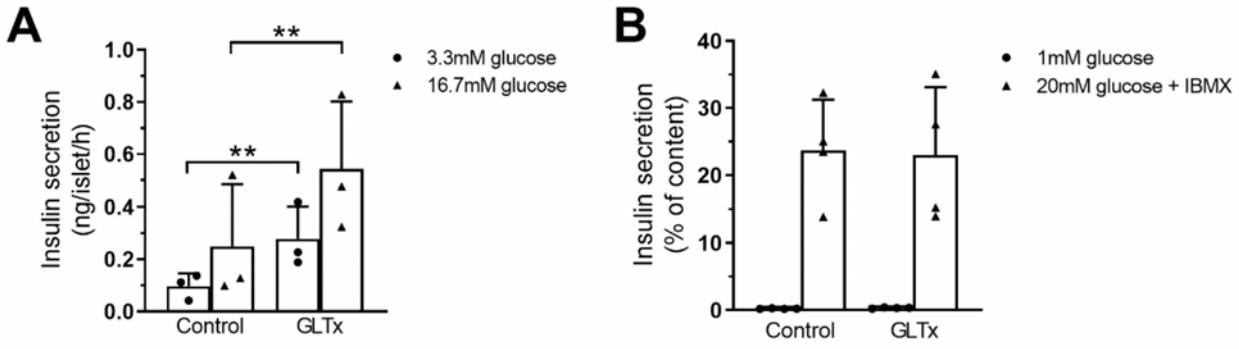
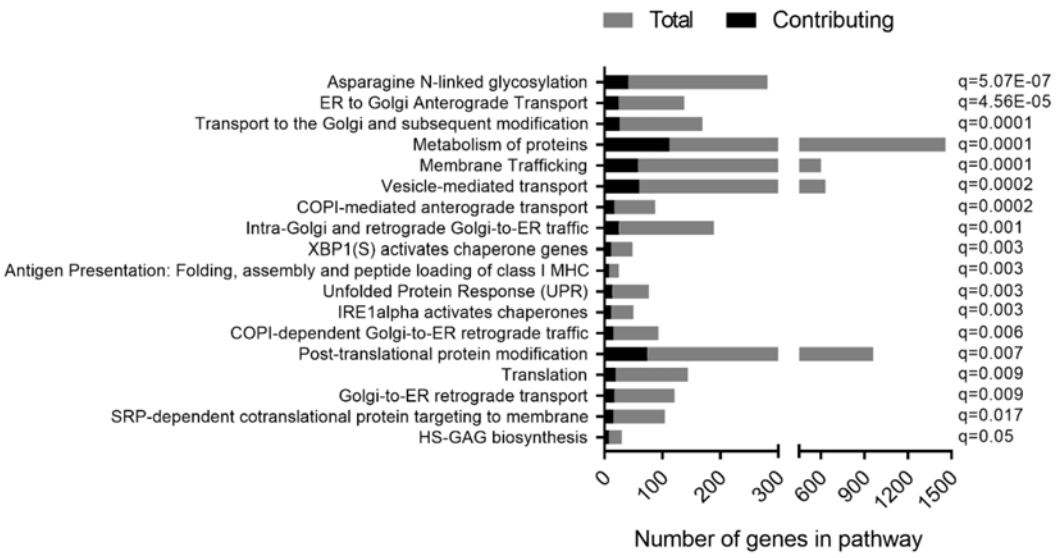
SUPPLEMENTARY DATA

Supplementary Figure 1. 24h exposure to high glucose and palmitate enhances insulin secretion from human islets (**A**, mean ± SD of experiments on islets from three donors) but has no effect on secretion from EndoC-βH1 cells (**B**, mean ± SD of four experiments with two technical replicates for each condition). ** $p < 0.01$ based on a paired t-test.



SUPPLEMENTARY DATA

Supplementary Figure 2. Pathway analysis on genes with altered expression after 48h treatment with glucose (19mM) and palmitate (1mM), but not with either nutrient alone.



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Supplementary Table 1. Differentially expressed genes in human islets exposed to high levels of glucose and palmitate compared to islets cultured under control conditions (q<0.05).
https://www.dropbox.com/s/4xb8frudxza2n4e/Supp_Table1.xlsx?dl=0

Supplementary Table 2. Significant pathways from GSEA on genes that are differentially expressed after exposure to high levels of glucose and palmitate
https://www.dropbox.com/s/xmixqz9n9ahj447/Supp_Table2.xlsx?dl=0

Supplementary Table 3. Genes that are differentially expressed after exposure to both high levels of glucose and high levels of glucose + palmitate
https://www.dropbox.com/s/670qanhrptjcmb6/Supp_Table3.xlsx?dl=0

Supplementary Table 4. Genes that are differentially expressed after exposure to both high levels of palmitate and high levels of glucose + palmitate
https://www.dropbox.com/s/qsv79geehzp3e3q/Supp_Table4.xlsx?dl=0

Supplementary Table 5. Differentially methylated CpG sites in pancreatic islets after exposure to high glucose and palmitate (p<0.05)
https://www.dropbox.com/s/wzvw3i53t396bge/Supp_Table5.xlsx?dl=0

Supplementary Table 6. Genes exhibiting both altered expression (q<0.05) and DNA methylation (p<0.05) in human islets after treatment with high levels of palmitate and glucose
https://www.dropbox.com/s/dczf9h5ovn4wuiw/Supp_Table6.xlsx?dl=0

Supplementary Table 7. Pathways enriched for genes exhibiting both altered expression (q<0.05) and DNA methylation (p<0.05) in human islets after treatment with high levels of palmitate and glucose
https://www.dropbox.com/s/trjx8y74n9wv9rb/Supp_Table7.xlsx?dl=0

Supplementary Table 8. CpG sites that are differentially methylated in EndoC-βH1 CpG sites after 48h treatment with high glucose and palmitate (p<0.05)
https://www.dropbox.com/s/9nn4hpaq80hqa8v/Supp_Table8.xlsx?dl=0