Coordinate Changes in Histone Modifications, mRNA Levels, and Metabolite Profiles in Clonal INS-1 832/13 β-Cells Accompany Functional Adaptations to Lipotoxicity*§

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Background: Epigenetic regulation may mediate lipotoxic effects on β-cells in type 2 diabetes.

Results: Lipotoxicity impairs insulin secretion, and alters metabolism, gene expression, and histone marks in INS-1 832/13 β-cells.

Conclusion: Perturbed insulin secretion results from epigenetic, genetic, and metabolic adaptations to increased fatty acid metabolism in β-cells.

Significance: Elucidation of the link between lipotoxicity and insulin secretion is crucial for understanding the pathogenesis of type 2 diabetes.

Lipotoxicity is a presumed pathogenetic process whereby elevated circulating and stored lipids in type 2 diabetes cause pancreatic β-cell failure. To resolve the underlying molecular mechanisms, we exposed clonal INS-1 832/13 β-cells to palmitate for 48 h. We observed elevated basal insulin secretion but impaired glucose-stimulated insulin secretion in palmitate-exposed cells. Glucose utilization was unchanged, palmitate oxidation was increased, and oxygen consumption was impaired. Halting exposure of the clonal INS-1 832/13 β-cells to palmitate largely recovered all of the lipid-induced functional changes. Metabolite profiling revealed profound but reversible increases in cellular lipids. Glucose-induced increases in tricarboxylic acid cycle intermediates were attenuated by exposure to palmitate. Analysis of gene expression by microarray showed increased expression of 982 genes and decreased expression of 1032 genes after exposure to palmitate. Increases were seen in pathways for steroid biosynthesis, cell cycle, fatty acid metabolism, DNA replication, and biosynthesis of unsaturated fatty acids; decreases occurred in the aminoacyl-tRNA synthesis pathway. The activity of histone-modifying enzymes and histone modifications of differentially expressed genes were reversibly altered upon exposure to palmitate. Thus, Insig1, Lss, Peci, Idi1, Hmgcs1, and Casr were subject to epigenetic regulation. Our analyses demonstrate that coordinate changes in histone modifications, mRNA levels, and metabolite profiles accompanied functional adaptations of clonal β-cells to lipotoxicity. It is highly likely that these changes are pathogenetic, accounting for loss of glucose responsiveness and perturbed insulin secretion.

Type 2 diabetes (T2D)³ is a multifactorial and complex disorder characterized by hyperglycaemia; this is caused by a combination of β-cell dysfunction, which leads to insufficient insulin release, and insulin resistance in target tissues (1). Pancreatic β-cells respond to glucose by metabolizing the sugar in proportion to its extracellular concentration. ATP derived from mitochondrial metabolism is the main coupling factor linking glucose metabolism with insulin secretion, whereas other factors amplify and sustain secretion of insulin (2, 3). It is widely accepted that adiposity and attendant high levels of circulating free fatty acids associate with T2D and β-cell dysfunction (4). Although fatty acids (FAs) acutely potentiate glucose-stimulated insulin secretion (GSIS) (5), chronic exposure to high levels of FA perturbs GSIS and insulin biosynthesis in β-cells (6–8). This chronic effect, referred to as lipotoxicity, also causes β-cell apoptosis (9, 10), further impairing functional β-cell mass. Alterations in multiple cellular processes have been associated with β-cell lipotoxicity. These include endoplasmic reticulum stress (11), production of reactive oxygen species (12), and toxic effects of lipid-derived signaling molecules (13). Furthermore, transcriptional regulation may be affected (14). Nonetheless, these suggested mechanisms do not fully clarify the molecular and cellular mechanisms causing impaired insulin secretion in lipotoxicity and the development of T2D.

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This article contains supplemental Tables S1–S3 and Figs. S1–S4.

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3 The abbreviations used are: T2D, type 2 diabetes; FA, fatty acid; GSIS, glucose-stimulated insulin secretion; HAT, histone acetyltransferases; HDAC, histone deacetylases; OCR, oxygen consumption rate; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; qRT, quantitative real-time; ANOVA, analysis of variance.
Metabolic and Epigenetic Adaptations in Lipotoxic β-Cells

Acutely, glucose and lipid metabolic pathways are reciprocally regulated in the pancreatic β-cell. Increased glucose metabolism results in elevated malonyl-CoA levels and inhibition of carnitine palmitoyltransferase 1, a mitochondrial enzyme transporting long chain acyl-CoA into the mitochondrion. Glucose oxidation is then favored and FAs are excluded from the mitochondrion, rendering glucose the main fuel in β-cell metabolism. The resulting increase in the cytosolic long chain acyl-CoA pool has been implicated as an amplifying process in GSIS (15). Conversely, acetyl-CoA, derived from FA oxidation, inhibits pyruvate dehydrogenase, and citrate inhibits phosphofructokinase. Consequently, mitochondrial and glycolytic metabolism of glucose will be inhibited when rates of FA oxidation are high, an effect referred to as the Randle cycle (16). Indeed, products of FA metabolism (17), cholesterol synthesis (18), and pyruvate cycling (19) have also been shown to affect GSIS.

Chronic exposure to environmental factors, such as hyperglycaemia (glucotoxicity) and/or lipotoxicity, may provide β-cells with inheritable epigenetic modifications. These are chemical alterations of the DNA that affect gene function and expression without changing the DNA sequence (20–22). Recent studies by our group and others demonstrate that epigenetic modifications, including DNA methylation and histone modifications, in human pancreatic islets influence gene expression, islet function, and most likely the pathogenesis of T2D (23–27). To our knowledge, it has not yet been shown that FAs regulate histone modifications in pancreatic β-cells.

Numerous histone-modifying enzymes are known to add acetyl and methyl groups to the N-terminal tails of histones (28). Histone acetyltransferases (HAT) add acetyl groups to the H2A, H2B, H3, and H4 histone proteins. Eight HAT isoforms, acting on different sites in histones, have been identified. Histone acetylation has been associated with activation of transcription via an open chromatin structure (28, 29). Conversely, histone deacetylases (HDAC) are associated with repression of transcription (30). Histone acetylation has been suggested to be involved in regulation of insulin secretion as well as in cellular damage associated with hyperglycaemia (20). Methylation of lysine occurs on histones H3 or H4; it is associated with either activation or repression of transcription depending on the sites of methylation. Methylation of lysine residues 4, 36, and 79 of histone H3 are generally associated with active gene expression, whereas methylation of lysine residues 9 and 27 of histone H3 are associated with silenced or inactive chromatin (20, 31). S67/9, a H3 lysine 4 methyltransferase, has been found to regulate transcription of genes necessary for GSIS in β-cells (32, 33).

In light of the complexity of lipid-induced β-cell dysfunction, it is important to study the phenomenon with a broad and unbiased perspective. Currently, very little is known about the comprehensive cellular response in this pathogenic condition. To this end, we examined the effects of 48 h exposure of palmitate on β-cell function, metabolite profiles, gene expression, and epigenetic regulation. We identified coordinate changes in histone modifications, mRNA levels, and metabolite profiles accompanying functional alterations in β-cells exposed to lipotoxicity. We suggest that these processes jointly serve to adapt clonal β-cells to a lipotoxic and, potentially, diabetogenic environment.

EXPERIMENTAL PROCEDURES

Development of a Lipotoxic in Vitro Model—A two-level full factorial design with a center point was generated to investigate the influence of duration (0–48 h) and palmitate concentration (0–0.5 mM) on viability and insulin secretion (basal, glucose-stimulated and fold-change) in the INS-1 832/13 β-cells. Experimental designs were created, modeled, and evaluated in MODDE 8.0.1 (Umetrics, Umeå, Sweden) as previously described in detail (34).

Cell Culture—Clonal INS-1 832/13 β-cells (35) were cultured at 37 °C in a humidified atmosphere of 95% air and 5% CO2 using RPMI 1640 culture medium (Standard medium; HyClone, Logan, UT) containing 11 mM d-glucose supplemented with 10% fetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, 10 mM HEPES, and 50 μM β-mercaptoethanol. Lipotoxicity (LipoAcute) was induced by culturing clonal INS-1 832/13 β-cells at 50% confluence in 0.5 mM palmitate conjugated with 0.5% BSA and 11.1 mM glucose for 48 h. To study recovery of lipotoxicity (LipoRecov), cells at 50% confluence were first treated with 0.5 mM palmitate conjugated with 0.5% BSA and 11.1 mM glucose for 48 h before being seeded into new plates and allowed to recover for 6 days in medium lacking the addition of palmitate (Fig. 1).

Insulin Secretion—Clonal INS-1 832/13 β-cells were cultured in 24-well plates to reach >90% confluence. Prior to glucose stimulation, the cells were washed in a HEPES-buffered saline solution (HBSS; 114 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.16 mM MgSO4, 20 mM HEPES, 2.5 mM CaCl2, 25.5 mM NaHCO3, 0.2% bovine serum albumin, pH 7.2) supplemented with 2.8 mM glucose. After incubation in 2.8 mM glucose for 2 h, cells were washed once with phosphate-buffered saline (PBS; HyClone), and fresh buffer containing either 2.8 or 16.7 mM glucose was added. After 1 h incubation, the amount of insulin secreted was determined using the Coat-a-Count kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA).

Cell Viability/Proliferation—Cell viability was measured using MTS CellTiter 96 Aqueous One Solution Assay (Promega Co., Madison, WI) according to the manufacturer’s protocol. In short, 20 μl of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium solution was transferred into each well of a 24-well plate containing adherent cells plus 200 μl of medium. Following incubation for 1 h, 100 μl were removed and absorbance was measured at 492 nm, reflecting reduced 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium produced by mitochondrial reductase activity.

Insulin Content Measurement—INS-1 832/13 β-cells were washed in PBS, and 100 μl of H2O were added per well. Following this, the cells were scraped off and sonicated. To collect total insulin, cells were centrifuged and the supernatant was collected and diluted 1:10 in acidified ethanol. The insulin content was determined by the Coat-a-Count kit (Siemens Medical Solutions Diagnostics).

Glucose Utilization—Glucose utilization was estimated from H2O produced in the reaction catalyzed by aldolase as previously described (36). In brief, clonal INS-1 832/13 β-cells were incubated for 2 h in HBSS containing 2.8 mM glucose at 37 °C.
Next, 500 μl of HBSS containing [5-3H]glucose (specific activity, 19.63 Ci/mmol; PerkinElmer Life Sciences) and glucose at a final concentration of 2.8 or 16.7 mM glucose were added and cells were incubated for 60 min at 37 °C. Subsequently, 100 μl of 35% trichloroacetic acid was added to prevent further metabolism of [5-3H]glucose. The content of [3H]OH was measured by liquid scintillation spectrometry.

**Palmitate Oxidation**—Palmitate oxidation was analyzed as previously described (37). Briefly, a reaction mixture consisting of 0.5 mM palmitic acid (Sigma) complexed to 0.5% FA-free BSA, with −10 dpm/pmol of [1-14C]palmitic acid (PerkinElmer Life Sciences) as tracer was added with glucose at a final concentration of 2.8 or 16.7 mM. The rate of palmitate oxidation was measured as released 14CO2, which was trapped by benzenthionium hydroxide, and determined by scintillation counting.

**Oxygen Consumption**—The oxygen consumption rate (OCR) was determined by the Seahorse Extracellular Flux Analyzer XF24 (Seahorse Bioscience, Billerica, MA) as previously described (36). Briefly, clonal INS-1 832/13 β-cells were seeded in an XF24 cell culture microplate at 100,000 cells/well in standard medium with or without 0.5 mM palmitate. Prior to assay, cells were preincubated in HBSS at 2.8 mM glucose for 2 h. OCR was then measured at 2.8 mM glucose for 30 min followed by a rise in glucose concentration to 16.7 mM for 60 min. Respiration driving ATP synthesis and proton leak was determined by blocking ATP synthase by the addition of oligomycin (4 μg/ml). After a further 15 min, 4 μM of the uncoupler carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) was added to determine the maximal respiratory capacity. After a further 15 min, 1 μM rotenone was added to block transfer of electrons from complex I of the respiratory chain to ubiquinone. The remaining non-mitochondria-dependent respiration was then measured for another 15 min before termination of the experiment. The following respiratory parameters were calculated according to Brand and Nicholls (38): respiratory control ratio (RCR) = maximal respiration/proton leak; coupling efficiency (CE) = total respiratory ATP/basal OCR.

**Metabolite Profiling**—Extraction of metabolites was performed as previously described (39). Briefly, clonal INS-1 832/13 β-cells were washed and incubated as described for insulin secretion for 2 h at 2.8 mM glucose followed by 1 h at 2.8 or 16.7 mM glucose. Cells were swiftly washed in ice-cold PBS and the metabolism was quenched by the addition of 300 μl of 35% trichloroacetic acid to prevent degradation of metabolites. The remaining trichloroacetic acid was added and cells were incubated for 60 min at 37 °C. Subsequently, 100 μl of 35% trichloroacetic acid was added, and cells were incubated for another 15 min before termination of the experiment. The following metabolites were measured: [1-14C]palmitate, [1-14C]glucose, [5-3H]glucose, [5-3H]hydroxybutyrate, and [5-3H]acetate. The rate of palmitate oxidation was measured as released 14CO2, which was trapped by benzethionium hydroxide, and determined by scintillation counting.

**Microarray mRNA Expression Analysis**—RNA for microarray analysis was extracted from clonal INS-1 832/13 β-cells, using the Revert Aid First Strand cDNA synthesis kit (Fermentas, St. Leon-Rot, Germany). cDNA was synthesized from RNA extracted from the clonal INS-1 832/13 β-cells, using the Revert Aid First Strand cDNA synthesis kit (Fermentas, St. Leon-Rot, Germany). Expression analyses were performed by the Robust Multichip Average method (41) implemented in the Expression Console software version 1.0 (Affymetrix).

** Validation of the Microarray Data**—Quantitative real-time PCR (qRT-PCR) was performed for selected genes to validate the results obtained by the microarray expression analysis. cDNA was synthesized from RNA extracted from the clonal INS-1 832/13 β-cells, using the ABI Prism 7900 HT system (Applied Biosystems) using gene-specific probes and primer pairs (Assays-on-Demand, Applied Biosystems; Insig1, Rn00573480_m1; Lss, Rn00579123_m1; Peci, Rn01435330_m1; Casr, Rn00566496_m1; Rilpl1, Rn01480396_m1; Nr2c1, Rn00595744_m1; Sf3b1, Rn01437843_m1). Transcript levels were normalized to the mRNA level of hypoxanthine phosphoribosyltransferase 1 (Hprt1; Applied Biosystems; Rn01527840_m1) and quantified using the ΔΔCt method. Normfinder (42), an algorithm for identifying the optimal normalization gene, was used to test whether expression of the selected housekeeping gene, Hprt1, was stable. This was done by analysis of expression stability of all genes analyzed together with an additional housekeeping gene, cyclophilin A (Ppia; Applied Biosystems; Rn00690933_m1).

**HAT/HDAC Activity**—The HAT/HDAC activities were determined in nuclear and cytosolic fractions of the clonal INS-1 832/13 β-cells, which were obtained by the Nuclear/ Cytosol fractionation kit (BioVision, Milpitas, CA). Briefly, cells were collected by centrifugation at 600 × g for 5 min at 4 °C before fractionation. HAT activity was measured in 40 μl of the nuclear fraction. Eighty-six μl of assay mixture containing buffer, NADH-generating enzyme, and substrate were added before measuring the absorbance at 440 nm in 30-min intervals over 120 min. HDAC activity was measured in 85 μl of the nuclear fraction; 10 μl of 10× HDAC assay buffer, and 5 μl of the HDAC colorimetric substrate were added to the nuclear and cytosolic fractions, respectively, mixed, and incubated at 37 °C for 60 min before measuring absorbance at 405 nm.

**Histone (H3-K27) Methyltransferase Activity**—The histone methyltransferase activity (H3-K27) was measured in the clonal INS-1 832/13 β-cells using a kit from Epigenek (Farmingdale, NY). Briefly, the nuclear fraction was isolated and allowed to react with biotinylated substrate for 60 min at 37 °C before incubation with primary capture antibody and secondary detection antibody for 60 and 30 min, respectively. After the final washing step, the assay was developed for 2 min in room temperature before determination of absorbance at 450 nm. Quantification was performed against a standard curve ranging from 0.2 to 10 μg/ml of histone methyltransferase, prepared, and treated identically to the samples.

**Chromatin Immunoprecipitation (ChIP)**—ChIP was performed as previously described (43). Briefly, 1 × 10⁶ clonal INS-1 832/13 β-cells were cross-linked with 1% formaldehyde at room temperature for 10 min, washed once with ice-cold PBS containing a protease inhibitor mixture (Sigma), and lysed in 50 μl of lysis buffer (10 mM Tris, pH 7.5, 1 mM EDTA, 1% SDS), and then diluted with 150 μl of PBS. Then, 200 μl of the samples...
were sonicated (21 cycles, 30 s sonication and 30 s rest) in an ice bath (Bioruptor, Diagenode, Denville, NJ). The protease inhibitor mixture was included in all buffers until addition of the elution buffer. Sonicated chromatin was centrifuged 13,000 rpm at 4 °C for 5 min and 200 μl of RIPA buffer (10 mM Tris, pH 7.5, 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.1% sodium deoxycholate, 100 mM NaCl) were added to the recovered supernatants. A 1/10 volume (40 μl) was removed for input control. Antibodies to H3K4me3 (Millipore, 04-745, 1:160), H3K27me3 (Millipore, 07-449, 1:160), H3K79me2 (Millipore, 04-835, 1:121), and H3K9Ac (Abcam, ab10812, 1:133) were added to the sonicated samples and incubated at 4 °C for 2 h. A control IgG antibody (Millipore, 12-370) was added in triplicate to separate samples at the same dilution as the ChIP antibody and handled in parallel throughout the procedure. Following antibody incubations, 10 μl of protein A/G magnetic beads previously washed in RIPA buffer were added and incubated for 2 h at 4 °C. The bead-protein complexes were washed three times with RIPA buffer and once with TE (10 mM Tris, pH 7.5, 1 mM EDTA) buffer. Genomic DNA from input and ChIPs were eluted for 2 h at 68 °C in 150 μl of elution buffer (20 mM Tris, pH 7.5, 5 mM EDTA, 50 mM NaCl, 1% SDS, 50 μg/ml of proteinase K), and combined with a second elution of 150 μl of elution buffer for 10 min at 68 °C. Genomic DNA was recovered using phenol/chloroform extraction and ethanol precipitation, using linear acrylamide and glycogen carriers. DNA pellets were resuspended in 20 μl of modified TE buffer (10 mM Tris, pH 7.5, 0.1 mM EDTA).

Whole Genome Amplification—The concentration of ChIP DNA was measured using Nanodrop ND-1000. ChIP DNA was then amplified using the WGA4 Kit (Sigma) with a protocol modified for ChIP according to the manufacturer's instructions. The amplified DNA was purified using QIAquick PCR Purification kit (Qiagen) and used as input in qRT-PCR.

Analysis of ChIP—qRT-PCR was used to analyze the ChIP of the following four histone modifications: H3K4me3, H3K27me3, H3K79me2, and H3K9Ac; this was performed for 12 selected genes. 10 of these were selected based on their differential mRNA expression in clonal INS-1 832/13 β-cells exposed to lipotoxicity. Two genes, Gapdh and Ldh, were selected as positive and negative control genes based on their high and low expression in clonal INS-1 832/13 β-cells, respectively. PCR primers were designed at seven different locations per gene, including −200, −800, −250, 0, 800, 2000, and 4000 base pairs (bp) in relationship to the transcription start site of the respective gene, using NCBI Primer Blast (www.ncbi.nlm.nih.gov/tools/primer-blast). The sequence and exact positions of the primers used in qRT-PCR can be found in supplemental Table S1. Each qRT-PCR was performed using Universal Master Mix (Diagenode) with a passive reference (Rox) and SYBR Green in the ABI 7900 instrument (Applied Biosystems), using 20 ng of DNA template and 5 μg of each primer per 10 μl of reaction. ChIP fold-enrichment was calculated as enrichment of ChIP over input minus negative control (ChIP using Rabbit IgG Anti-body), using the ΔΔCt method described by the Invitrogen website.

Western Blot Analysis—Protein extraction and Western blotting were performed as described previously (40). Thirty μg of protein were loaded per lane on a mini-Protean TGX gel (Bio-Rad). Antibodies were from Santa Cruz Biotechnology, Santa Cruz, CA, unless otherwise specified; INSIG-1 (N-19) dilution 1:200, cHMGCS (D-20) dilution 1:200, and β-tubulin (ab6046, dilution 1:500, Abcam, Cambridge, UK) were detected with a primary rabbit polyclonal antibody. Horseradish peroxidase-coupled goat anti-rabbit IgG and rabbit anti-goat IgG (both 1:5000, v/v) were used as secondary antibodies. Blots were developed with enhanced chemiluminescence (ECL). Densitometry analysis was performed using ImageJ (44).

Statistical Analysis—Statistical analyses were performed in PASW Statistics 17 (IBM Corporation, Armonk, NY) unless otherwise stated. Differences between groups in assays for metabolism, enzyme activities, and metabolite profiles were assessed by one-way ANOVA followed by Tukey's multiple comparison test post hoc, when more than two groups were compared, or a Student's t test, when two groups were compared. The significance level was set at p < 0.05 and two-tailed p values were calculated. Metabolite data were mean centered, scaled to unit variance, and analyzed using principal component analysis in SIMCA P+ 12.0 (Umetrics AB). Microarray expression data were analyzed using a Multiple experiment viewer (45), and differences between control, LipoAcute, and LipoRecov clonal INS-1 832/13 β-cells were analyzed by two-tailed ANOVA followed by Tukey's multiple comparison post hoc test. The false discovery rate was used to correct for multiple comparisons for the microarray expression data; probes showing differential expression with q < 0.01 were considered to be significantly changed. Genes that were differentially expressed in the clonal INS-1 832/13 β-cells were grouped into biological KEGG pathways, using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) Bioinformatics Resources 6.7 (david.abcc.ncifcrf.gov) (46). ChIP data were analyzed by paired ANOVA followed by Bonferroni correction post hoc. Correlations between ChIP fold-enrichment and gene expression levels were calculated with Pearson 2-tailed linear regression in GraphPad Prism 5 (GraphPad Software, La Jolla, CA). All results are given as mean ± S.E. unless otherwise stated.

RESULTS

Experimental Lipotoxicity in Clonal INS-1 832/13 β-Cells—To focus the investigation on cellular effects and exclude effects of reduced viability, a two-level full factorial design with insulin secretion and viability as responses was created. Perturbed GSIS accompanied by negligible effects on viability was observed after exposure of INS-1 832/13 β-cells to 0.5 mM palmitate for 48 h (LipoAcute; Fig. 1, supplemental Fig. S1).
Importantly, insulin secretion was affected by an interaction of the concentration and duration of lipid exposure. An increase in any of these parameters alone did not impede insulin secretion. Instead, synchronous increases of both factors were required to perturb insulin secretion (supplemental Fig. S1). Thus, insulin secretion at 2.8 mM glucose increased from 8.9 ± 2.26 ng/mg of protein/h in untreated control cells to 21.2 ± 2.3 in clonal INS-1 832/13 β-cells exposed to lipotoxicity (p = 0.002; Fig. 2A). In contrast, insulin secretion at 16.7 mM glucose decreased from 78.7 ± 12.82 in control cells to 40.5 ± 4.7 ng/mg of protein/h in lipotoxic INS-1 832/13 β-cells under the same condition (p = 0.038; Fig. 2A). Accordingly, the fold-change in insulin secretion decreased from 11.5 ± 1.9 in control cells to 2.0 ± 0.3 in LipoAcute INS-1 832/13 β-cells (p = 0.0002; Fig. 2B). Thus, experimental design allowed us to establish an in vitro lipotoxic model where β-cell viability was preserved, whereas basal insulin secretion was elevated and GSIS was impaired, both recognized as hallmarks of lipotoxicity in vivo.
To investigate whether lipotoxicity was associated with inheritable epigenetic modifications, a third condition, recovery after lipotoxicity (LipoRecov, Fig. 1), was added to our study: cells were allowed to recover in normal medium for 6 days. At this point, cells were functionally recovered, as estimated by insulin secretion, which did not differ significantly from the control condition (Fig. 2A, supplemental Fig. S2A). The fold-change in GSIS in LipoRecov INS-1 832/13 β-cells was 8.4 ± 1.4 compared with 11.5 ± 1.9 in control cells (p = 0.26; Fig. 2B). Moreover, there were no differences in insulin content between Control, LipoAcute, and/or LipoRecov cells (supplemental Fig. S2B). In fact, already after a 4-day recovery, GSIS was largely restored compared with that of control cells (supplemental Fig. S2A). However, to ensure full functional recovery, cells in the recovered experimental condition were allowed to recover for an additional generation of cells (48 h doubling time) beyond this point to 6 days recovery (LipoRecov).

Glucose Utilization and Palmitate Oxidation—To examine the relationship between glucose and lipid metabolism in INS-1 832/13 β-cells, we first analyzed glucose utilization, a measure of glycolytic activity. No difference in glucose utilization was observed between LipoAcute and control cells; the response in glucose utilization to 2.8 and 16.7 mM glucose was 31.1 ± 0.7 and 156.6 ± 5.7 (p = 0.002) and 36.6 ± 3.1 and 163.7 ± 19.7 nmol/mg of protein/h (p = 0.017) in LipoAcute and control cells, respectively (Fig. 2C).

Next, we measured palmitate oxidation: we found a significant increase in palmitate oxidation at 2.8 mM glucose in LipoAcute versus control cells (26.2 ± 6.9 versus 18.5 ± 5.6 µmol/mg/h; p = 0.02, Fig. 2D). Interestingly, regardless of whether the clonal β-cells had been exposed to 0.5 mM palmitate or not, provoking the cells with 16.7 mM glucose still inhibited oxidation of exogenous palmitate by ~50% (Fig. 2D). Thus, glucose retained its regulatory role in FA oxidation in both LipoAcute and control cells.

Oxygen Consumption Rate—To evaluate the impact of FA exposure on mitochondrial function in INS-1 832/13 β-cells, we analyzed the OCR, which can be used as an overall indicator of mitochondrial metabolism. Inhibitors of respiratory chain complexes further enabled examination of flux through the respiratory chain (Fig. 2E). OCR at 2.8 mM glucose was decreased from 5.0 ± 0.3 nmol of O₂/mg prot/min in control cells to 2.9 ± 0.2 nmol of O₂/mg prot/min in LipoAcute cells (p = 0.03; Fig. 2, F and G). Glucose-stimulated respiration (16.7 mM glucose) and oligomycin-sensitive respiration were decreased from 7.7 ± 0.2 to 4.0 ± 0.4 (p = 0.003) and 5.0 ± 0.5 to 2.1 ± 0.2 (p = 0.008), respectively, in LipoAcute cells compared with control cells (Fig. 2G). The proton leak over the inner mitochondrial membrane was unaltered in LipoAcute compared with control cells but increased in LipoRecov cells compared with LipoAcute cells (3.4 ± 0.4 versus 1.8 ± 0.3 nmol of O₂/mg prot/min; p = 0.02; Fig. 2G). The respiratory control ratio, which assesses the coupling of state3/state4 respiration, and the coupling efficiency, assessing the coupling of ATP production to oxygen consumption (3B), were unaffected by lipotoxicity. OCR in LipoRecov cells did not significantly differ from that observed in control cells at any time point (Fig. 2, F and G).

Metabolite Profiling—To investigate whether lipotoxicity affected the intermediary metabolism, we profiled metabolites extracted from control, LipoAcute, and LipoRecov INS-1 832/13 β-cells by gas chromatography/mass spectrometry. Fifty-four metabolite derivatives were identified, corresponding to 51 unique metabolites. First, we used a principal component analysis to examine whether our conditions caused systematic alterations in the metabolite pattern. In the first component, describing 38% of the variation in the metabolite data, the LipoAcute condition could clearly be distinguished from the control situation (supplemental Fig. S3). In contrast, samples from control and LipoRecov cells overlapped in all components of the principal component analysis model, suggesting that the metabolite pattern in these two conditions were similar. Thus, lipotoxicity altered the metabolite pattern, which was normalized after a 6-day recovery period.

Next, we examined levels of individual metabolites in the INS-1 832/13 β-cells. Glycolytic intermediates, tricarboxylic acid cycle (TCA)-cycle intermediates, and FAs were significantly elevated by 90–140, 20–90, and 90–200%, respectively, in LipoAcute compared with control cells at 2.8 mM glucose (Fig. 3A). In contrast, levels of glutamate and GABA were significantly decreased by 30 and 69%, respectively, in LipoAcute compared with control cells at 2.8 mM glucose (Fig. 3A). At 16.7 mM glucose, levels of glycolytic intermediates and FA remained significantly elevated by 60–180 and 90–160% in LipoAcute compared with control cells, respectively (Fig. 3B). Furthermore, glutamate and GABA levels were significantly decreased by 29 and 73%, respectively, in LipoAcute compared with controls cells at 16.7 mM glucose (Fig. 3B). In contrast, challenging the cells with 16.7 mM glucose increased levels of TCA cycle intermediates to a similar extent in all three conditions (Fig. 3, A and B). Hence, no differences were observed in fold-increases of glycolytic intermediates, shuttling intermediates, or FA, regardless of whether INS-1 832/13 β-cells had been exposed to palmitate or not (Fig. 3C). In contrast, the fold-changes in the TCA cycle intermediates were significantly decreased by 31–51% in LipoAcute compared with control cells (Fig. 3C). Thus, the glucose-provoked increase in levels of glycolytic intermediates was matched by a similar increase in TCA cycle intermediates in the control and LipoRecov cells but not in the LipoAcute cells. The results from the metabolite profiling suggest that coupling of the glycolytic and TCA cycle metabolism in LipoAcute cells was perturbed.

Levels of seven FAs were increased in LipoAcute INS-1 832/13 β-cells compared with both control and LipoRecov cells (p < 0.05). Long-chain FA C16:0 (palmitate), C18:0 (stearic acid), and C18:1 (oleic acid) as well as shorter FAs C14:0 (myristic acid), 12:0 (lauric acid), 10:0 (capric acid), and 7:0 displayed increased levels in LipoAcute INS-1 832/13 β-cells at 16.7 mM glucose. In agreement with the principal component analysis, no significant differences could be found in the levels of any individual metabolites between control and LipoRecov cells at basal glucose levels, stimulated glucose levels, or in the fold-changes of metabolite regulation.

Microarray Analysis—To investigate whether the observed metabolic and functional changes were associated with changes in mRNA expression, we performed a microarray analysis.
18,896 genes of a total of 27,342 genes in the microarray gave rise to a positive signal in the INS-1 832/13/H9252-cells. An unsupervised sample hierarchical clustering analysis of the expression data, including all detected genes on the microarray, revealed that the samples were primarily clustered based on treatment (Fig. 4A). All LipoAcute samples were clustered in one group, whereas no clustering of the control and LipoRecov samples could be observed.

Furthermore, expression of 2013 genes was significantly altered at \( q < 0.01 \) after an false discovery rate analysis (Fig. 4, B and C). The full list of significantly altered genes is available online.4

With a false discovery rate of 1% of the altered genes, theoretically, 20 false positives would be expected based on 2013 significantly altered genes. Of the 2013 genes that were altered, expression of 982 was up-regulated in LipoAcute INS-1 832/13 \( \beta \)-cells compared with control cells. In contrast, expression of 1032 genes was down-regulated. The top 100 up- and down-regulated genes are listed in supplemental Tables S2 and S3, respectively. Post hoc analysis of the affected genes showed that 2011 of 2013 genes differed significantly in expression between control and LipoAcute cells, 2010 of 2013 genes differed significantly in expression between LipoAcute and LipoRecov cells, whereas expression of only 12 genes differed significantly between control and LipoRecov cells (Fig. 4B). This is in agreement with the unsupervised sample hierarchical clustering analysis, which showed clustering of LipoAcute versus control and LipoRecov cells but not in control versus LipoRecov cells.

We next performed a KEGG pathway analysis, using DAVID to identify biological pathways including genes exhibiting differential expression in INS-1 832/13 \( \beta \)-cells exposed to lipotoxic-
A total of 982 genes with increased expression and 1032 genes with decreased expression due to lipotoxicity were included in the pathway analysis. Genes with increased expression in lipotoxic INS-1 832/13 cells were enriched in the pathways of “steroid biosynthesis” (10.5-fold, \( p = 1.36 \times 10^{-6} \)), “cell cycle” (3.1-fold, \( p = 5.44 \times 10^{-4} \)), “FA metabolism” (4.4-fold, \( p = 0.021 \)), “DNA replication” (4.7-fold, \( p = 0.029 \)), and “biosynthesis of unsaturated FA” (5.7-fold, \( p = 0.049 \)) (Table 1).

**TABLE 1**

| KEGG pathway                        | Number of genes in pathway | Number of genes identified | Gene list                                                                 | Fold-enrichment | Raw \( p \) value | Bonferroni \( p \) value |
|-------------------------------------|-----------------------------|---------------------------|---------------------------------------------------------------------------|-----------------|-------------------|-------------------------|
| **Up-regulated**                    |                             |                           |                                                                           |                 |                   |                         |
| Steroid biosynthesis                | 17                          | 11                        | CYP51, TM7SF2, SQLE, DHCR7, LSS, HSD17B7, SC5DL, NSDHL, SC4M0, FBFT1, DHCR24 | 10.5            | 8.52 \times 10^{-09} | 1.36 \times 10^{-06} |
| Cell cycle                          | 122                         | 23                        | E2F1, CDC7, CDC6, E2F3, FZ1, TTR, ESPL1, SKP1, PTG1, MCM3, CDC27, MCM4, CDC2, CDC25A, CCNB1, CCNE1, MAD2L1, CCNB2, YWHAH, PCNA, BUB1, CCNA1, CCNA2 | 3.1             | 3.40 \times 10^{-06} | 5.44 \times 10^{-04} |
| Fatty acid metabolism               | 41                          | 11                        | ACA2A, ACADM, ACSL1, ACADS, ACAL, ACA2, ACSL3, PCL, DCL, CPIA1             | 4.4             | 1.31 \times 10^{-04} | 0.021                   |
| DNA replication                     | 35                          | 10                        | RPC1, POL2, LG1, PRM2, POL1, PCNA, POLA, MCM3, MCM4, RPA3                 | 4.7             | 1.84 \times 10^{-04} | 0.029                   |
| Biosynthesis of unsaturated fatty acids | 23                         | 8                         | PECR, ELOVL3, FADS1, SCD, ELOVL2, FADS2, SCD, ELOVL6                     | 5.7             | 3.14 \times 10^{-04} | 0.049                   |
| **Down-regulated**                  |                             |                           |                                                                           |                 |                   |                         |
| Aminocycl-tRNA biosynthesis         | 39                          | 12                        | IARS, WARS, YARS, NARS, DARS, SARS, FARS, LARS, EPRL, VARS, MARS, EAR5    | 6.7             | 7.89 \times 10^{-07} | 1.21 \times 10^{-04} |

**FIGURE 4.** Microarray analysis of mRNA expression in clonal INS-1 832/13 β-cells subjected to lipotoxicity. A, unsupervised hierarchical clustering analysis showing clustering primarily in palmitate-exposed (LipoAcute; to the left) versus control and LipoRecover INS-1 832/13 β-cells. B, workflow model of microarray analysis showing 2013 genes with \( q = 0.01 \) separated into up- and down-regulated genes. Post hoc analysis by Tukey’s test was used to distinguish differences between treatment groups. C, heat map showing 2013 significantly differentially expressed genes clustered on sample (top tree) and gene (left tree). All LipoAcute samples were clustered together in one group to the left, whereas the control and LipoRecov were clustered together at the right. The results were derived from five independent experiments.
HDAC activity was similar in all three groups (Fig. 6) and LipoRecov (n = 3). 8, HDAC activity measured in control (white bars), LipoAcute (black bars), and LipoRecov (gray bars) nuclear extracts (n = 3). C, H3K27 methyltransferase activity measured in control (white bars) and LipoAcute (black bars) nuclear extracts (n = 5). Bars represent mean ± S.E. *p < 0.05 LipoAcute compared with control; §, p < 0.05 LipoAcute compared with LipoRecov; **, p < 0.01; ***, p < 0.001.

Chromatin Immunoprecipitation—To further dissect whether epigenetic mechanisms are affected by lipotoxicity, four different histone modifications were analyzed in control, LipoAcute, and LipoRecov INS-1 832/13 β-cells by ChIP. Three of the analyzed histone modifications are associated with transcriptional activation (H3K9Ac, H3K79me2, and H3K4me3) and one modification is associated with transcriptional repression (H3K27me3) (28, 31, 47, 48). We then selected 10 genes from the top hits of the microarray results for quantitative analysis of histone modifications, using qRT-PCR. These included eight up-regulated genes (Insig1 (insulin-induced gene 1), 2.1-fold, p = 2.79 × 10−10), Lss (lanoster synthase, 1.4-fold, p = 6.09 × 10−10), Peci (peroxisomal D3,D2-enoyl-CoA isomerase, 1.6-fold, p = 1.96 × 10−7), Idi1 (isopentyl-diphosphatase δ-isomerase 1, 2.8-fold, p = 2.78 × 10−9), Msmo1 (methylster monooxygenase 1, 1.8-fold, p = 5.61 × 10−9), Hmgs1 (3-hydroxy-3-methylglutaryl-CoA synthase 1, 2.4-fold, p = 6.95 × 10−8), Me1 (malic enzyme 1, 1.5-fold, p = 1.47 × 10−8), and Rpa1 (replication protein A1, 1.5-fold, p = 2.27 × 10−8) and two down-regulated genes (Casr (calcium-sensing receptor, 0.2-fold, p = 3.42 × 10−8) and Rilpl1 (Rab interacting lysosomal protein-like 1, 0.7-fold, p = 5.70 × 10−9)) (Fig. 7A). The robustly transcribed Gapdh gene, encoding the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase, was used as a positive control for open chromatin. Ldha, encoding lactate dehydrogenase A, which is expressed at low levels in β-cells (49), served as a control for closed chromatin in the ChIP analysis. The histone modifications were mapped over 6000 bp, starting 2000 bp upstream of the transcription start site and finishing 4000 bp downstream of the transcription start site in the gene body, using corresponding PCR primers (supplemental Table S1).

The spatial distribution profiles of the four analyzed histone modifications (supplemental Fig. S4, A–D) were in agreement with previous ChIP studies (43, 50–53). The positive control for open chromatin, Gapdh, was substantially enriched upon ChIP for all three activating modifications.
**FIGURE 7.** mRNA expression and ChIP of H3K9Ac, H3K79me2, H4K4me3, and H3K27me3 in clonal INS-1 832/13 β-cells subjected to lipotoxicity. A, gene expression of 10 selected genes based on differential expression due to lipotoxicity in the microarray and two control genes (Gapdh and Ldha) in control (white bar), LipoAcute (black bar), and LipoRecov (gray bar) INS-1 832/13 β-cells. From the left: glyceraldehyde 3-phosphate dehydrogenase (Gapdh), lactate dehydrogenase A (Ldha), insulin-induced gene 1 (Insig1), lanosterol synthase (Lss), peroxisomal D3,D2-enoyl-CoA isomerase (Peci), isopentenyl-diphosphate β-isomerase 1 (Idi1), methylsterol monoxygenase 1 (Msma1), 3-hydroxy-3-methylglutaryl-CoA synthase 1 (Hmgcs1), malic enzyme 1 (Me1), replication protein A1 (Rpa1), calcium-sensing receptor (Casr), and Rab-interacting lysosomal protein-like 1 (Rilpl1). ***, \( p < 0.001 \) and **, \( p < 0.01 \). ChIP of four different histone modifications including (B) H3K9Ac, (C) H3K79me2, (D) H4K4me3, and (E) H3K27me3 of 10 selected genes and two control genes (Gapdh and Ldha) in control (white bars), LipoAcute (black bars), and LipoRecov (gray bars) INS-1 832/13 β-cells. Bars represent mean ± S.E. F-I, correlations examining the relationship between gene expression derived from the microarray and fold-enrichment of the following histone modifications: (F) H3K9Ac, (G) H3K79me2, (H) H4K4me3, and (I) H3K27me3. The correlation graphs show gene expression and histone modifications in control cells of the 12 genes included in A. The results were derived from five independent experiments. *, \( p < 0.05 \) LipoAcute compared with control; §, \( p < 0.05 \) LipoAcute compared with LipoRecov. *, \( p < 0.05 \); **, \( p < 0.01 \).
(H3K9Ac, H3K79me2, and H3K4me3; Fig. 7, B–D, and supplemental Fig. S4, A–C), but, as expected, there was no Gapdh enrichment upon ChIP for the repressive modification (H3K27me3; Fig. 7E and supplemental Fig. S4D). In contrast, the positive control for closed chromatin, Ldha, was substantially enriched upon ChIP for the repressive modification (H3K27me3; Fig. 7E, supplemental Fig. S4D) but there was no or low Ldha enrichment upon ChIP for the activating modifications (H3K9Ac, H3K79me2, and H3K4me3; Fig. 7, B–D, supplemental Fig. S4, A–C). Together, these two control genes confirmed that ChIP yielded the expected results for the four analyzed histone modifications.

We then examined the impact of lipotoxicity on histone modifications. Whereas H3K9Ac, H3K4me3, and H3K27me3 showed differences in fold-enrichment in the proximal promoter region 800–200 bp upstream of the transcription start site (supplemental Fig. S4, A, C, and D), H3K79me2 showed differences in fold-enrichment ~2000 bp into the gene body (supplemental Fig. S4B) in LipoAcute compared with control cells. Lipotoxicity influenced the fold-enrichment of one or more of the analyzed histone modifications in six of 10 selected genes (Fig. 7, B–E). Of these, five genes including Insig1, Lss, Peci, Idi1, and Hmgcs1 exhibited increased mRNA expression (Fig. 7A) in parallel with significantly increased fold-enrichments of histone modifications associated with transcriptional activation (Fig. 7, B and C) in LipoAcute compared with control cells. Indeed, exposure to 0.5 mM palmitate for 48 h increased the fold-enrichment of H3K9Ac in the proximal promoters of Insig1, Peci, and Hmgcs1 (Fig. 7B) and the fold-enrichment of H3K79me2 in the gene body of Insig1, Lss, and Idi1 (Fig. 7C). In contrast, Casr exhibited decreased expression (Fig. 7A) in parallel with significantly increased fold-enrichment of the histone modification associated with transcriptional repression (H3K27me3; Fig. 7E) as well as significantly decreased fold-enrichment of two histone modifications associated with transcriptional activation (H3K9Ac and H3K4me3; Fig. 7, B and D) in LipoAcute compared with control cells. Together, the significant alterations in histone modifications observed in INS-1 832/13 β-cells subjected to lipotoxicity were in agreement with the changes seen in gene expression.

Next, we examined whether there was a relationship between gene expression levels and fold-enrichment in histone modifications. To this end, we examined the correlation of the selected genes with the fold-enrichment of histone modifications from the ChIP. For these correlations, we chose data from the control condition for the 12 genes examined. Whereas fold-enrichment of the histone modifications associated with transcriptional activation correlated positively with gene expression (H3K9Ac, H3K79me2, and H3K4me3; Fig. 7, F–H), the fold-enrichment of H3K27me3, which is associated with transcriptional repression, correlated negatively with gene expression (Fig. 7F). Taken together, these data support that H3K9Ac, H3K79me2, and H3K4me3 activated transcription and that H3K27me3 suppressed transcription in INS-1 832/13 β-cells.

We finally examined whether lipotoxic β-cells exhibited similar differences in protein as in mRNA levels compared with control cells. The expression of two proteins, Insig1 and Hmgcs1, were subsequently analyzed in the β-cells by Western blot analysis. In agreement with the mRNA expression data (Fig. 7A), the protein levels of both Insig1 and Hmgcs1 increased significantly in LipoAcute compared with control β-cells (Fig. 8).

**DISCUSSION**

When β-cells are chronically exposed to free FAs, adaptive and, in some cases, pathological changes occur on several levels. The change in substrates alters metabolic fluxes, which serve to tune the genetic machinery controlling the metabolic pathways to the new situation. These changes evolve through interactions at all these levels, not one given factor or process is determining. To elucidate this complexity, we studied INS-1 832/13 β-cells undergoing reversible lipotoxic changes, but not apoptosis. Analyses of functional parameters, metabolite profiles, gene expression, and epigenetic alterations were combined to provide a more comprehensive view on the pathogenetic changes in β-cell lipotoxicity. The analysis revealed an overall shift toward lipid metabolism at the expense of glucose metabolism.

Functionally, the lipotoxic INS-1 832/13 β-cells resembled islets from patients with T2D (54) as well as primary islets and β-cell lines chronically exposed to FAs (55–57), in that basal insulin secretion was increased and GSIS was impaired. The perturbations of insulin secretion were reflected by decreases in oxygen consumption and ATP turnover (oligomycin-sensitive respiration in response to glucose). At the same time, the INS-1 832/13 β-cells adapted to the exposure to palmitate by increasing their capacity to oxidize lipids. There is some disagreement with previous studies, which have shown increased respiration upon prolonged palmitate treatment (19, 58). It should be kept in mind that we examined glucose-induced, not lipid-induced, respiration. The high rate of acetyl-CoA production from lipids is likely to inhibit pyruvate dehydrogenase (16). Consequently,
less glucose-derived carbons will enter the TCA cycle and generate NADH, which drives respiration. Interestingly, glucose utilization in INS-1 832/13 β-cells was unaffected by exposure to palmitate. This suggests that the metabolic inhibition by lipids is mainly exerted at the level of pyruvate dehydrogenase in the TCA cycle metabolism not only pertains to glucose-induced elevation of the ATP/ADP ratio, the canonical pathway for triggering of insulin secretion (59), but also the amplifying pathway. This pathway is believed to emanate from the TCA cycle, where metabolites may exit and either interact directly with the exocytic machinery or indirectly via changing, e.g., redox in the β-cells (60). Metabolite profiling showed that the responses in many of these TCA cycle intermediates and putative coupling signals were attenuated when the cells were stimulated by 16.7 mM glucose: fumarate and malate (citrate, isocitrate, and α-ketoglutarate levels were lower as well, although this did not reach statistical significance). Mitochondrial fuel shuttling was implicated by observed changes in GABA, glutamate, and aspartate levels. GABA has previously been shown to suppress GSIS from rat islets (61). However, it has also been suggested that GABA does not influence GSIS but rather has a small suppressive effect on glucagon secretion (62). Overexpression of glutamate decarboxylase, resulting in increased production of GABA, inhibits insulin secretion in a rat model (63). The substrate for this reaction, glutamate, has been proposed to act as a coupling factor in GSIS (64), although its importance is controversial (65). Independent of the potential role for glutamate as a coupling factor, glutamate and aspartate are important intermediates in the malate-aspartate shuttle. This shuttle replenishes cytosolic NAD⁺ to maintain a high glycolytic rate. Silencing (66) and overexpression (67) of the mitochondrial glutamate carrier ARALAR1, a member of the malate-aspartate shuttle, decreases and increases GSIS, respectively. Clearly, perturbations in levels of GABA, glutamate, and aspartate are likely to impact potential coupling signals, mitochondrial shuttling, and thereby insulin secretion.

We observed several pathways involved in lipid metabolism that exhibited altered expression in response to lipotoxicity. Our results are in line with previous microarray-based studies of palmitate-induced lipotoxicity in the MIN6 and INS-1 832/13 clonal β-cell lines (68, 69). We also observed up-regulation in steroid biosynthesis and in the sterol-sensitive mRNAs Insig1, Srebf-2, Lss, Hmgcs1, Hmgcr, and Ldlr. This confirms previous studies, which have suggested that the altered cholesterol metabolism may link lipotoxicity to impaired β-cell function (69–72).

Epigenetic mechanisms, e.g., DNA methylation and histone modifications, may serve as additional mechanisms to fine-tune the genetic machinery in response to fuel excess. Changes in DNA methylation patterns have been reported in T2D patients (24–27, 73). Increased DNA methylation correlated negatively with gene expression and insulin secretion in human pancreatic islets (24–26). The impact of lipotoxicity on histone modifications in β-cells has to our knowledge not been studied. Here, we demonstrated altered activity of histone-modifying enzymes in INS-1 832/13 β-cells exposed to palmitate. Accordingly, several genes with changed expression in lipotoxicity were found to also exhibit an altered degree of histone modifications: Insig1, Lss, Idi1, Hmgcs1, Peci, and Casr. Some of these genes play a key role in cholesterol synthesis; Insig1 is an insulin-induced gene that encodes an endoplasmic reticulum membrane protein regulating cholesterol concentrations in the cell (74); Lss encodes lanoster synthase catalyzing the first step in the biosynthesis of cholesterol, steroid hormones, and vitamin D (72). Idi1 encodes the peroxisomally localized enzyme isopentenyl-diphosphate δ-isomerase 1, and Hmgcs1 encodes 3-hydroxy-3-methylglutaryl-CoA synthase 1, two enzymes involved in cholesterol synthesis (75). On the other hand, Peci encodes enoyl-CoA δ-isomerase 2, a mitochondrial enzyme involved in β-oxidation of unsaturated fatty acids (76); and Casr encodes a calcium-sensing receptor (77). In the present study, exposure to palmitate significantly changed the fold-enrichment of the active chromatin marks H3K9Ac, H3K79me2, and H3K4me3 in four, three, and one gene, respectively. This suggests that H3K9Ac and H3K79me2 may be the modifications most responsive to lipids. As expected, we found that genes showing increased expression in response to lipotoxicity, e.g., Insig1, Lss, Idi1, Hmgcs1, and Peci, also exhibited an increased fold-enrichment of H3K9Ac and/or H3K79me2. In contrast, H3K27me3 is associated with a closed chromatin state (31). We identified one gene, Casr, which exhibited an increased fold-enrichment of H3K27me3 in parallel to decreased gene expression. However, Casr also exhibited decreased fold-enrichment of two active marks, H3K9Ac and H3K4me3, in response to lipotoxicity. This demonstrates how an increase in a repressive mark together with a decrease in activating marks may lead to decreased gene expression. Taken together, our epigenetic data demonstrated that lipotoxicity is associated with altered activity of histone-modifying enzymes in parallel with changes in histone modifications and expression of genes involved in cholesterol and lipid metabolism.

Due to the rapid doubling time of the INS-1 832/13 β-cells (~48 h), some caution is called for when studying epigenetics in these cells after 48 h because it may reflect inherited epigenetic modifications. Previous studies have shown that the INS-1 β-cell line is more capable of reversing effects of fuel excess on gene expression than other β-cell lines (78, 79). In the present study, very little effect on the genetic and epigenetic levels remained in the INS-1 832/13 β-cells after a 6-day recovery in medium lacking added palmitate (LipoRecov). Accordingly, GSIS, metabolic processes, and metabolite profiles were largely normalized. Nonetheless, effects on the epigenetic level were apparent in the acute lipotoxic state. These alterations were paralleled by changes in expression of corresponding genes.
The regulation of histone modifications in INS-1 832/13 β-cells provided us with important information about epigenetic control of gene expression in β-cells responding to fuel excess. Such alterations may be more persistent in a post-mitotic cell, i.e. the primary β-cell. Also, the duration of lipotoxicity in an obese individual will greatly exceed the 48 h incubation period used here. On the other hand, the reversibility of the alterations suggests that even deleterious effects of elevated free FAs might be reversible.

In conclusion, this study demonstrated how lipid-induced β-cell dysfunction is associated with coordinate changes in mitochondrial function, metabolite profiles, gene expression, and histone modifications. Our data support a causal link between a metabolic challenge of β-cells and epigenetic modifications associated with differential expression of genes involved in cholesterol and FA metabolism. As a result, β-cell function, assessed as GSIS, was impaired. Ultimately, these changes may be disadvantageous for β-cells and subsequently play a role in the pathogenesis of T2D.

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