Supplemental Information

Alterations in Regulation of Energy Homeostasis in Cyclic Nucleotide Phosphodiesterase 3B-null Mice

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Methods

Construction of the *Pde3b* Targeting Vector. A 13-kb Sal*I* genomic fragment containing exon 1 of the mouse *Pde3b* gene was cloned from a 129/SvJ lambda FIXII mouse genomic library (Stratagene), using a ^{32}P-labeled probe generated from PDE3B cDNA (accession number *AF547435*) (Figure 1A). A 6.0-kb Xba*I* fragment containing exon 1 was subcloned into the Xba*I* site of pBluescript and used to construct the targeting vector (Figure 1, A and B), which consisted of the 6.0-kb Xba*I* genome fragment ligated into the multi-cloning site of pUC18 containing a 2.8-kb DT-A (diphtheria toxin) gene fragment for negative selection (Figure 1, B and C). A 600-bp Not*I*-Not*I* fragment encoding part of exon 1, including the ATG initiation codon, was removed and replaced with the 1.8-kb *Neo*^{r} (positive selection marker) derived from pMC1 neopoly A with a PGK promoter (Figure 1, B and C).

Southern Blots, Northern Blots, Genomic PCR, and Real-time RT-PCR. DNA prepared from ES cells and tail tips was digested with Bst*XI*, fractionated by agarose gel electrophoresis, transferred to nylon membranes, and hybridized with a ^{32}P-labeled probe (designated as probe 0.4) that is downstream of the 3' homology region (Figure 1, A and C). Expected sizes for WT and KO PDE3B fragments are approximately 11.5-kb and approximately 6.5-kb, respectively (Figure 1, A, C, and D).

HE and KO genotypes were detected by PCR amplification of genomic DNA using one primer [R primer, 5'-AAA GCG CCT CCC CTA CCC GGT AGA ATT GAC CTG CA-3'] derived from the sequence of the *Neo*^{r} (Figure 1B) and second primer (A primer, 5'-GTA AGG AAG CAT AGG AGG TTC AGC CAA GAT TCG T-3') derived from an upstream region of the PDE3B gene not included in the replacement vector (Figure 1, A and E). WT and HE genotypes were also detected with primers A and E (5'-CAC GTT GCA GAA GCG GCA GAG GTG GAA GAA G-3'), derived from a downstream region of exon 1, replaced by the *Neo*^{r} cassette in HE and KO mice (Figure 1, A, B, and E).

RT-PCR, Real-time RT-PCR, and Northern Blot Analysis of mRNA. Tissues were dissected from WT, HE, and KO mice, minced into small pieces, and placed into ice-cold RNAlater buffer (Ambion Inc.). TriPure mRNA isolation reagent (Boeringer Manheim) was used to isolate total RNA and mRNA from different tissues. Adiponectin mRNA was amplified from total RNA (100 ng), prepared from epididymal fat pads (age-matched [5-month-old, ± 1 day] WT and KO mice), using RNeasy Lipid Tissue Midi Kits (QIAGEN).

RT-PCR was performed on liver mRNA from WT, HE, and KO mice (Figure 1F). Primers MB5 (5'-AAC GGC TAC GTG AAG AGC TG-3', nt 94-113) and MB3 (5'-AAA GAA GTG GTC CAA GCC CA-3', nt 716-735), or Neo5 (5'-TCA TTC CTC CCA CTC A-3') and MB3 were used for PDE3B. Primers MA5 (5'- CTC ACA ACA CAG CCG ATT CC-3', nt 2620-2639) and MA3 (5'-GTT CTT TGC ATT TAG CAG GC-3', nt 3180-3199) were used for mouse PDE3A.

Real-time RT-PCR Analysis: One hundred ng of liver total RNA from 4-month-old WT, HE, and KO mice (n = 4 for each genotype) were subjected to real-time quantitative 1-step RT-PCR (7900HT; Applied Biosystems), using the QuantiTect SYBR Green RT-PCR kit (QIAGEN) according to manufacturer's protocols. Triplicate RNA samples were used for real-time RT-PCR reactions. Primers for amplification of mouse PDE3A (from exons 1 and 2), PDE3B (from exons 2 and 3), or cyclophilin A (Cyc), were PDE3A-1145 (5'-CGA GGA CCA AGG AAG AGA TTC-3'), PDE3A-1280 (5'-ATG AGC TGC TCC CTG GGT AT-3'), PDE3B-1042 (5'-AGT ACC GCG GAG GAA AAA GT-3'), PDE3B-1186 (5'-AAG TCC CAG TCC CAG AGG AT-3'), Cyc-598 (5'- AAG GTG AAA GAA GGC ATG AAC-3') and Cyc-695 (5'- AGT TGT CCA CAG TCG GAA ATG-3'), respectively. Transcripts of Cyc were quantified as endogenous controls. Relative abundances of PDE3A and 3B were calculated on the basis of Ct (threshold cycle) value ($2^{-\Delta\Delta Ct}$), with $^{\Delta\Delta}$Ct equal to the normalized signal level in a sample relative to the normalized signal level in the corresponding calibrated sample. Primers for amplification of other mouse genes are as follows; Adiponectin-6814 (5'-GTC TCA GCT GTC GGT CTT CC-3'), Adiponectin-7029 (5'-CCT GGC TTT ATG CTC TTT GC-3'), PGC-1α-585 (5'-CCG AGA ATT CAT GGA GCA AT-3'), PGC-1α-713 (5'-TTT CTG TGG GTT TGG TGT GA-3'), G6Pase-61 (5'-AGG AAG GAT GGA GGA AGG AA-3'), G6Pase-222 (5'-TGG AAC CAG ATG GGA AAG AG-3') PEPCK-1934 (5'-TCA ACA CCG ACC TCC CTT AC-3'), PEPCK-2169 (5'-CCC TAG CCT GTT CTC TGT GC-3'), TRB3-257 (5'-GAT GCC AAG TGT CCA GTC CT-3'), TRB3-416 (5'-CTT GCT CTC GTT CCA AAA GG-3'), IL-1-222 (5'-TCA CAG CAG CAC ATC AAC AA-3'), IL-1-333 (5'-TGT CCT CAT CCT GGA AGG TC-3'), IL-6-64 (5'-AGT TGC CTT CTT GGG ACT GA-3'), IL-6-222 (5'-TCC ACG ATT TCC CAG AGA AC-3'). TNF-α-1045 (5'-CTG GGA CAG TGA CCT GGA CT-3'), TNF-α-1248 (5'-GCA CCT CAG GGA AGA GTC TG-3'), SOCS-3-1420 (5'-TGC AAG GGG AAT CTT CAA AC-3'), SOCS-3-1543 (5'-GCC AGC ATA AAA ACC CTT CA-3'), PAI-1-891 (5'-GAC ACC CTC AGC ATG TTC ATC-3'), and PAI-1-1108 (5'-AGG GTT GCA CTA AAC ATG TCA G-3'), respectively.

Northern blots: Analyses were performed with mRNA purified from fat pad, liver, and heart tissues of WT and KO mice (Figure 1, H and I). Primers MB1 (5'-ATG AGG AAA GAC GAG CGC GA-3') and MB2 (5'-CGG CAG AGG TGG AAG AAG AA-3') for the mouse fat *Pde3b* were synthesized from nt 271-437 (in the portion of the *Pde3b* gene replaced by the *Neo*^{r}) to produce a 167-bp PCR fragment (probe B). Primers MA1 (5'-ACT CAT GCC AGG AAA ATG GG-3') and MA2 (5'-TCA CAG GTC TGG CTG TGG AG-3') for the mouse cardiac *Pde3a* were derived from nt 3381-3747, to yield a 367-bp fragment (probe A). These PCR products were labeled and used as probes for Northern blots. Probes were hybridized to Northern blots at 55 °C (for probe B) or 58 °C (for probe A) overnight in ULTRAhyb buffer (Ambion Inc.). Blots were washed (15 min, room temp) in Low Stringency Wash Solution #1 (Ambion Inc.) or in 2X SSC, 0.1% SDS, and then (15 min, 55 °C) in High Stringency Wash Solution #2 (Ambion Inc.) or in 0.1X SSC, 0.1% SDS.

cAMP PDE Assay. Samples (usually 0.1 ml) for assay were incubated (usually 10 min) at 30 ^oC in a total volume of 0.3 ml containing 50 mM HEPES, pH 7.5, 8.3 mM $MgCl_2$, 0.1 mM EDTA, and 0.1 μM [^{3}H]-cAMP (25,000-35,000 cpm) as substrate (1). After dephosphorylation of [^{3}H]-5-AMP with *Crotalus atrox* venom (Sigma-Aldrich), [^{3}H]-adenosine product was separated from [^{3}H]-cAMP substrate by ion-exchange chromatography (QAE-Sephadex; Amersham Phamacia Biotech) and quantified by scintillation counting. Substrate hydrolysis was usually less than 20%. PDE activity data are presented as mean ± SEM of at least three independent experiments. Within each experiment, values were usually mean of at least three individual determinations for each experimental condition. PDE3 activity is that fraction of total activity inhibited by 1 μM cilostamide (a selective PDE3 inhibitor), and PDE4 activity, that inhibited by 10 μM rolipram (a selective PDE4 inhibitor).

Tissue Fractionation. Dissected tissues were chopped with scissors and homogenized (20 strokes with a Teflon pestle) in 1:5 (wt/vol) ice-cold homogenization buffer (50 mM HEPES, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$, 3 mM benzamidine, 0.5 mM Pefabloc SC (Roche Diagnostics Corp.), 10 μg/ml each of pepstatin, leupeptin, and aprotinin). Homogenates were then sonified (in ice, 20 pulses, 40% duty cycle, output scale 4) and centrifuged (5,000 *g*, 10 minutes). The supernatants were centrifuged (100,000 *g*, 60 minutes) to separate cytosol and membrane fractions. The latter were solubilized in homogenization buffer containing 1% NP-40. As indicated elsewhere, slightly different methods for tissue fractionation were used in some experiments.

Partial purification of PDE3A and PDE3B in liver cytosolic fractions. Econo-pac polypropylene columns (1.5 x 12 cm, 20 ml bed volume) (Bio-Rad) were packed with 4.0 ml of DEAE Sephacel Fast Flow (Amersham Biosciences) preequilibrated with Buffer A (50 mM HEPES, 250 mM sucrose, 1 mM EDTA, 10 mM sodium pyrophosphate, 5 mM NaF, 1 mM Na_3VO_4, Roche protease inhibitor cocktail, pH 7.5). Cytosolic fractions (50 mg protein) from WT and KO livers were passed twice through the DEAE columns, which were washed twice with Buffer A (10 ml x 2). Fractions containing PDE activity were eluted with Buffer A containing 500 mM NaCl (10 ml, passed twice through the column), and further concentrated via Centricon (10 kD cut-off, Millipore) to approximately 2-3 ml. For partial purification of microsomal membrane fractions, microsomal fractions from WT and KO livers were resuspended in Buffer A containing 1% NP-40 detergent, homogenized and sonicated. Detergent-solubilized membrane fractions were recentrifuged (100,000 *g*, 30 minutes) to remove insoluble material. Solubilized membrane and partially purified cytosolic fractions were then subjected to gel filtration **(Supplemental Figure 1)**.

Western blots. After SDS-PAGE [10% Tris-glycine gels (for FAS, 7% gels were used)], proteins were electro-transferred to PVDF membranes (Millipore). Membranes were blocked (overnight, 4 °C) with 5% non-fat dried milk (NFDM) (Bio-Rad) in PBS, incubated with primary antibodies in 0.5% NFDM in PBS for 2 hours at RT, and washed three times with PBS, 10 minutes each. Membranes were incubated with HRP-labeled second antibody (Santa Cruz Biotechnology Inc.) in 0.5% NFDM in PBS for 1 hour at RT and washed three times with PBS (10 minutes each).

Commercial sources of antibodies: Monoclonal anti-β-actin antibody, against N-terminal sequence, (1:10,000, Sigma-Aldrich); anti-FAS antibody (1:1,000, Santa Cruz Biotechnology Inc.); anti-PKA substrates antibody (1:400; Cell Signaling Technology); anti-phospho-CREB (pS^{133}; 1:1,000; Cell Signaling Technology); anti-CREB (1:1,000; Cell Signaling Technology); anti-PGC-1α (1:200; Santa Cruz Biotechnology Inc.); anti-PEPCK (1:400; PCK1 C-terminal, Abgent); anti-TRB3 (1:4,000; Calbiochem); anti-SOCS-3 (1:200; Santa Cruz Biotechnology Inc.); anti-actin (1:10,000; Sigma-Aldrich); anti-phospho-Tyr (1:400; BD Biosciences); anti-IRS (1:500; Cell Signaling Technology); anti-insulin receptor (IR) (1:400; BD Biosciences); anti-phospho-PKB (1:400; Cell Signaling Technology); anti-PKB (1:400; Cell Signaling Technology); anti-phospho-FKHRL1 (pS^{253}; 1:400; Cell Signaling Technology); anti-FKHRL1 (1:400; Cell Signaling Technology); anti-phospho-GSK-3 (pY^{216}; 1:500; BD Biosciences); anti-GSK-3 (1:500; BD Biosciences); anti-phospho-IRS (pS^{612}; 1:400; Cell Signaling Technology); anti-phospho-IRS (pS^{307}; 1:400; Cell Signaling Technology); anti-phospho-JNK (1:500; R&D Systems); anti-JNK (1:500; R&D Systems); anti-phospho-Erk (1:500; BD Biosciences); anti-Erk (1:400; BD Biosciences).

PDE3B N- or C-terminal specific antibodies were raised by Lofstrand Labs Limited. Antibody recognizing PKB phosphorylated on serine 473 was kindly provided by L. Rönnstrand, Lund University, Malmö, Sweden.

Adipocyte preparation, adipocyte size, lipolysis, analysis of FAS and PKB. To measure lipolysis (glycerol accumulation), 400 µl of 5% (vol/vol) adipocytes in Krebs-Ringer-HEPES (KRH) buffer (25 mM HEPES, pH 7.4, 125 mM NaCl, 5 mM KCl, 1 mM KH_2PO_4, 2.5 mM $CaCl_2$, 2.5 mM $MgCl_2$, and 2 mM glucose) with 3% FFA-free BSA were incubated with indicated additions at 37 ^oC for 60 minutes with shaking. Reactions were stopped by placing the tubes on ice and 200 µl from each tube was removed for determination of glycerol (Trinder kit; Sigma-Aldrich). For determination of FAS expression, adipocytes (10% cell suspension) were washed and homogenized in 50 mM Tris buffer, pH 7.5, containing 1 mM EDTA, 1 mM EGTA, 270 mM sucrose and 10 µg/ml each of antipain and leupeptin and 1 µg/ml pepstatin. After centrifugation (13,000 *g*, 5 minutes), the fat cake was removed and the infranatants were used for FAS western blot analysis. In other experiments, adipocytes (10% cell suspension) were stimulated with or without 1 nM insulin, washed, and homogenized in 40 mM HEPES, 10 mM NaF, 1 mM DTT, 1 mM PMSF, 0.25 mM sodium orthovanadate, 10 µg/ml each of antipain and leupeptin and 1 µg/ml pepstatin. After centrifugation (16,600 *g*, 15 minutes, 4 °C), and removal of the fat cake, the infranatants were used for analysis of PKB/phospho-PKB by western blots. The chemiluminescent signals were developed with Supersignal West Pico kit (Pierce Biotechnology, Inc.), followed by detection by LAS-3000 or LAS-1000 Plus systems.

Glucose uptake. 2-deoxy-D-[1-^{3}H]-glucose uptake was measured as previously described (2, 3) One hundred microliters of 30% (vol/vol) adipocytes in Krebs-Ringer bicarbonate-HEPES (KRBH) buffer (120 mM NaCl, 4 mM KH_2PO_4, 1 mM $MgSO_4$, 0.75 mM $CaCl_2$, 10 mM $NaHCO_3$, 30 mM HEPES, pH 7.4) with 1% BSA was added to 100 µl of KRBH buffer with different concentrations of insulin and incubated for 10 minutes at 37 ^oC. Fifty microliter aliquots of KRBH buffer containing 0.5 mM 2-deoxyglucose, 0.5 µCi 2-deoxy-D-[1-^{3}H]-glucose (Amersham Biosciences) and either 60 µM cytochalasin β in DMSO or DMSO alone were added. After 10

minutes reactions were stopped with 10 μl of 1.5 mM cytochalasin β. Cells were separated from the buffer by centrifugation (6000 *g*) through dinonyl phthalate for 1 minute. 2-deoxy-D-[1-^{3}H]-glucose uptake was determined by scintillation counting.

Lipogenesis. Lipogenesis was measured as previously described (2, 4). One-milliliter aliquots of 2% (vol/vol) adipocytes in modified KRH buffer (5 mM HEPES, pH 7.4, 119 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.2 mM adenosine, and 0.55 mM glucose) with 3.5% BSA were added to vials containing 0.4 μCi D-[6-^{3}H]-glucose (Amersham Biosciences) and incubated with different concentrations of insulin for 3 hours at 37 ^{o}C. Reactions were stopped with a toluol-based scintillation liquid containing 0.3 g/l POPOP (1, 4-bis[5-phenyl-2-oxazolyl]benzene, 2,2'-p-phenylene-bis[5-phenyloxazole]) and 5 g/l PPO (2,5-diphenyl oxazole). Incorporation of D-[6-^{3}H]-glucose into cellular lipids was measured by scintillation counting.

Liver TG content. Liver pieces (100 mg each) were homogenized in 1 ml buffer (0.5 M Tris, and 1% Triton X-100, pH 7.4). Portions (50 μl) were transferred into a glass tube containing 3 ml of a 2:1 chloroform:methanol solution and stored under N_2 at 4 ^{o}C overnight. After addition of 1.5 ml of water, the samples were mixed and centrifuged at 200 *g* for 10 minutes. After removal of the upper layer, water (0.75 ml) was added and the procedure was repeated. The samples were dried with N_2 and resuspended in 200 μl chloroform. Ten microliter aliquots were transferred into eppendorf tubes and air dried first without and then with 2 μl thesit (20% vol/wt in chloroform). After addition of water (10 μl), the samples were incubated at 37 ^{o}C for 10 minutes. A standard curve was prepared with the use of different concentrations of triolein. Three hundred microliters of triglyceride reagent (Trace/DMA, Thermo Electron Corp.) was

added to the tubes containing samples and standards. After 5 minutes incubation at 37 ^{o}C the samples were transferred to a microtiter plate and absorbance at 540 nm was measured.

Preparation of liver extracts from nonfasting and fasting (6 hours) mice. Livers were immediately dissected and washed thoroughly with PBS to remove blood. Two hundred milligrams of liver were taken, weighed, chopped with scissors, and homogenized (20 strokes with a Teflon pestle) in 1:20 (wt/vol) ice-cold homogenization buffer (50 mM HEPES, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$, 10 mM sodium pyrophosphate, 5 mM NaF, 0.1 μM okadaic acid, 0.1 μM calyculin A, 3 mM benzamidine, 2 tablets of Roche proteinase inhibitor cocktail, and 1% NP-40). Homogenates were sonified on ice (Branson Sonifier, 20 pulses, 40% duty cycle, output scale 4) and centrifuged (1,000 *g*, 5 minutes). Resulting supernatants (total extracts) were aliquoted and stored at -80 °C until the future use. Protein concentration was determined using MicroBCA protein assay kit (Pierce Biotechnology Inc.).

Isolation of mouse islets. Groups of three islets were distributed in 96-well plates and incubated in 200 µl of modified KRBH buffer (10 mM HEPES, pH 7.2-7.4, 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5 mM $NaHCO_3$, and 0.2% BSA) with the indicated concentrations of glucose and GLP-1, for 1 hour at 37 °C. Buffer was withdrawn for quantification of insulin using commercial RIA kits (Linco Research, Inc.). For Western blotting, isolated islets were washed with PBS and homogenized by passing through a syringe in buffer containing 50 mM N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid, pH 7.4, 250 mM sucrose, 1 mM EDTA, 0.1 mM EGTA, 10 µg/ml leupeptin, 10 µg/ml antipain, and 1 µg/ml pepstatin (Peptide Institute Inc.). Western blots of homogenates (40 islets/lane, 4-month-old WT and KO mice) were reacted with polyclonal PDE3B antibodies (raised against a peptide corrsponding to a part of the regulatory domain of the rat PDE3B).

Pancreas Immunocytochemistry. Pancreases were dissected, fixed overnight in Stefanini's solution (2% paraformaldehyde and 0.2% picric acid in 0.1 M PBS, pH 7.2), rinsed thoroughly in Tyrode solution containing 10% sucrose, and frozen on dry ice. Sections (10 μm thick) were cut and thaw-mounted on slides. Alternatively, specimens were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (6 μm thick) were mounted on coated slides, deparaffinized, and hydrated before further handling.

Hyperinsulinemic-Euglycemic Clamp. The procedure, performed essentially as described by Chen et al. (5), is based on that developed by Kim, Shulman, and collaborators (6-10). The clamps were performed 4–6 d after catheter placement as described in (11). Mice were fasted overnight (food was removed at 5:00 pm the day before an experiment) and experiments were started at 7:30 am. Each mouse was immobilized in a restrainer (522-BSRR; PlasLabs), and the catheter was externalized. The tip of the tail was cut before starting the first infusion, and all subsequent blood samples were from this site. Blood was collected into heparinized microcapillary tubes (Fisher Scientific) and centrifuged for 10 seconds to obtain plasma. Basal endogenous glucose production rate was determined by continuously infusing [3-^{3}H]glucose (3 μCi bolus, then 0.02 μCi/min, 740 GBq/mmol; NET 331C; Perkin Elmer). Samples for determination of plasma [3-^{3}H]glucose concentration were taken after 90 and 115 minutes of basal infusion. After 120 minutes of basal [3-^{3}H]glucose infusion, the hyperinsulinemic-euglycemic clamp was begun with a priming bolus (300 mIU/kg) of human insulin over 3 min, then followed by continuous infusion of 15 pmol/kg/min (Humulin R; Eli Lilly). Plasma glucose was measured at 20- and 30-min intervals, respectively, during the first and second hour of the clamp, and 20% glucose was infused at a rate adjusted to keep plasma glucose at approximately 110 mg/dl.

Insulin-stimulated whole-body glucose flux was estimated using a primed-continuous infusion of HPLC-purified [3-^{3}H]glucose (10 μCi bolus, 0.1 μCi/min; Perkin Elmer) throughout the clamps.

To estimate insulin-stimulated glucose transport activity in individual tissues, 2-deoxy-D-[1-^{14}C]glucose (2-[^{14}C]DG; Perkin Elmer) was administered as a bolus (10 μCi) at 40 minutes before the end of clamps. Blood samples (20 μL) were taken at 80, 85, 90, 100, 110, and 120 minutes after the start of the insulin infusion for determination of plasma [^{3}H]glucose, $^{3}H_2O$, and 2-[^{14}C]DG concentrations. Additional blood samples (10 μL) were collected before the start and at the end of clamps for measurement of plasma insulin concentrations. Microdialysis pumps (model CMA 102, CMA/Microdialysis).were used for all infusions. Gastight syringes (10 μl; Hamilton Co.) were used for bolus injections. At the end of clamps, mice were anesthetized with ketamine (100 mg/kg; Fort Dodge Animal Health) and xylazine (10 mg/kg; Phoenix Scientific) anesthesia. Within 5 min, four muscles (soleus, gastrocnemius, tibialis anterior, and quadriceps) from both hindlimbs, epididymal adipose tissue, and liver were taken. Each tissue, once exposed, was dissected out within 2 sec, frozen immediately using liquid N_2-cooled aluminum blocks, and stored at -70 °C for later analysis.

In vivo glucose flux analysis. For the determination of plasma [3-^{3}H]glucose and 2-deoxy-D-[1-^{14}C]glucose concentrations, plasma was deproteinized with $ZnSO_4$ and $Ba(OH)_2$, dried under vacuum at room temperature to remove $^{3}H_2O$, resuspended in water, and counted in BioSafe II scintillation fluid (Research Products International) using a Beckman LS60.001C (Beckman Coulter, Inc.) with correction for background, counting efficiency, and channel cross-over. For determination of tissue 2-deoxy-D-[1-^{14}C]glucose-6-phosphate, tissue samples were homogenized in distilled water (50 mg tissue/500 μl water) and an aliquot was counted to determine totals ^{14}C. The remainder of the homogenate was subjected to anion-exchange chromatography (model 731-6211, Bio-Rad Laboratories) to separate nonmetabolized 2-deoxyglucose (neutral ^{14}C counts eluted with 6 ml distilled water) from 2-deoxyglucose-6-phosphate (anionic ^{14}C counts eluted with 6 ml of 0.2 M formic acid/0.5 M ammonium acetate).

Calculations. Basal endogenous glucose production was calculated as the ratio of the preclamp [3-^{3}H]glucose infusion rate (dpm/min) to the specific activity of the plasma glucose (mean of the 90- to 120-min values of preclamp period in dpm/μmol). Clamp whole-body glucose uptake was calculated as the ratio of the [3-^{3}H]glucose infusion rate (dpm/min) to the specific activity of plasma glucose (dpm/μmol) during the last 20 minutes of the clamp (mean of the 100- to 120-min samples). Whole-body glycolysis was determined from the rate of increase in plasma $^{3}H_2O$ determined by linear regression using the 80- to 120-min points. Plasma $^{3}H_2O$ concentrations were calculated from the difference between plasma ^{3}H counts before and after drying. Clamp endogenous glucose production was determined by subtracting the average glucose infusion rate in the last 30 minutes of the clamp from the whole-body glucose uptake. Whole-body glycogen and lipid synthesis were estimated by subtracting the whole-body glycolysis from the whole-body glucose uptake, which assumes that glycolysis and glycogen/lipid synthesis account for the majority of insulin-stimulated glucose uptake. Tissue glucose uptake was calculated from the plasma 2-deoxy-D-[1-^{14}C] glucose concentration curve (using plasma ^{14}C counts at 80–120 min, the area under the curve was calculated by trapezoidal approximation) and tissue 2-deoxy-D-[1-^{14}C] glucose-6-phosphate content as described (12).

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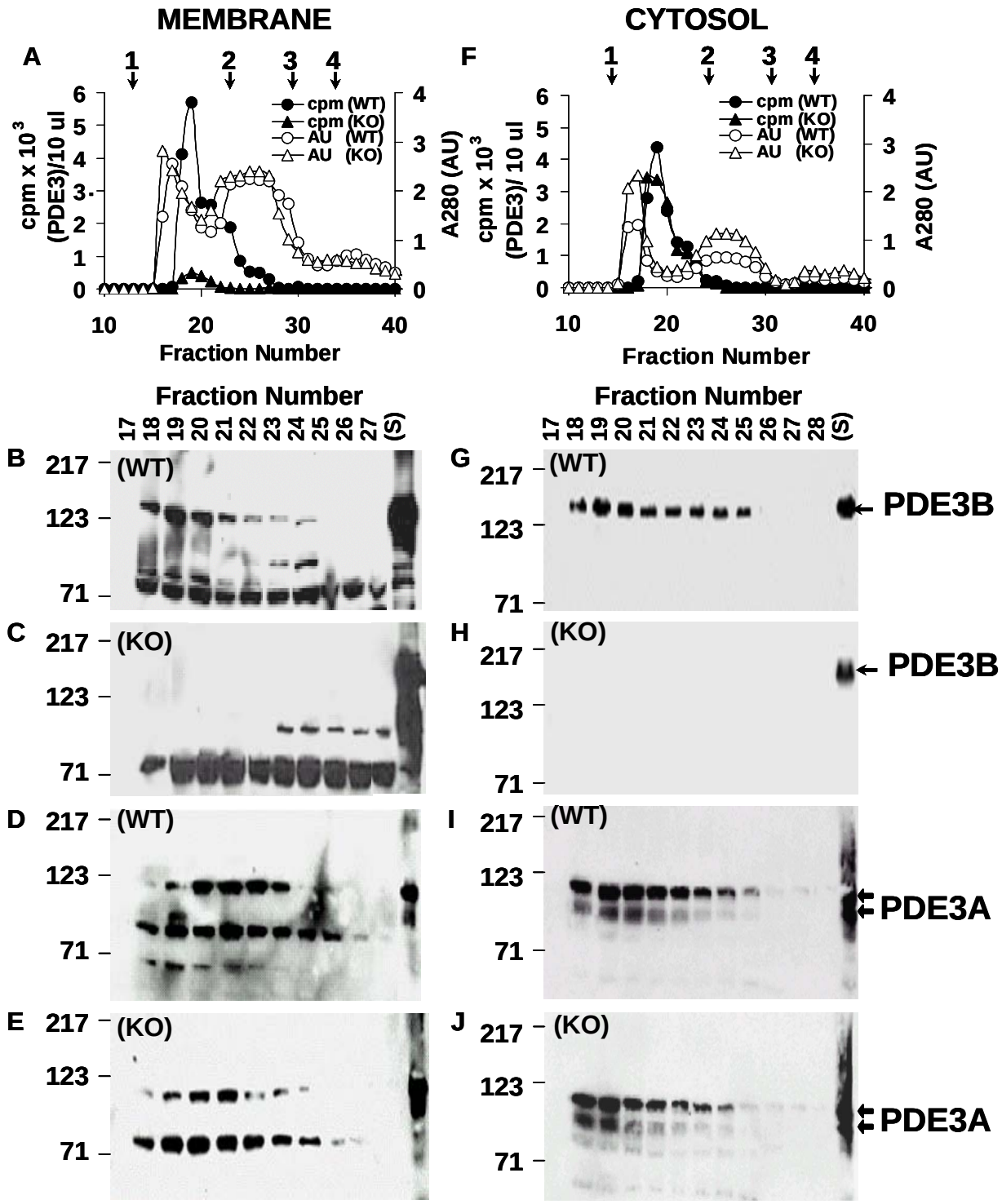
Supplemental Figure 1. Gel filtration chromatography of solubilized liver membrane fractions and partially purified cytosolic fractions (**after DEAE chromatography**)**.**

Solubilized liver membranes (3 mg protein) and partially purified liver cytosolic fractions (after DEAE columns, 3 mg), were prepared as described in Methods, and subjected to gel filtration chromatography (FPLC-Superose 12, AKTA FPLC System, Amersham-Pharmacia). **LEFT**, Membranes, **RIGHT**, Cytosol. Protein content (AU 280 nm) (○, ∆) and PDE3 activity (PDE3 cpm/10 μl) (●, ▲) were measured in indicated fractions from WT (○, ●) and KO (∆, ▲) liver membranes (**A**), and cytosol (**F**); ~90% of the applied PDE3 activity from membrane and cytsosol fractions was recovered in indicated fractions. molecular weight standards: 1, thyroglobulin, 2, γ-globulin, 3, ovalbumin, 4, myoglobin, 5, Vit B_{12}. (**B, C, G, and H**), Western blots of recombinant (r)PDE3B (equivalent to 5 pmol PDE3 enzyme activity) as positive control and indicated fractions (20 μl) from WT and KO membranes (**B** and **C**), and rPDE3B (0.5 pmol PDE3 activity) and indicated fractions from WT and KO cytosol (**G** and **H**) were reacted with rabbit anti-PDE3B-CT antibody. (**D, E, I, and J**), Western blots of lung homogenates (0.5 pmoles PDE3 activity) as a positive control (for PDE3A), and indicated fractions (20 μl) from WT (**D**) and KO (**E**) membranes; and lung homogenates (2 pmol PDE3 activity) and indicated fractions (20 μl) from WT (**I**) and KO (**J**) cytosol were immunoblotted with rabbit anti-PDE3A-CT antibody.

Supplemental Figure 1.



Supplemental Table 1. Body Weight and Serum Chemistries

	Male		Female	
	WT	KO	WT	KO
Body weight (g)	35.6 ± 1.6	39.7 ± 0.8[A]	31.3 ± 0.8	33.5 ± 0.6[A]
Liver weight (g)	1.7 ± 0.1	1.9 ± 0.1	1.5 ± 0.1	1.4 ± 0.0
FFA (mM)	1.6 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	1.9 ± 0.1
Glycerol (mg/dL)	15.4 ± 1.1	16.5 ± 1.6	18.3 ± 1.0	21.4 ± 1.2
Triglycerides (mg/dL)	89.4 ± 4.7	95.4 ± 7.0	68.8 ± 6.8	62.8 ± 3.2
Glucose (mg/dL)	144 ± 6	147 ± 13	144 ± 8	139 ± 9
Insulin (ng/ml)	1.8 ± 0.3	2.0 ± 0.2	1.2 ± 0.1	1.0 ± 0.1
Leptin (ng/ml)	12.6 ± 1.1	13.9 ± 0.6	9.7 ± 0.7	10.3 ± 0.8

Serum chemistries of WT and KO mice. Analyses were carried out in age-matched, 6-month-old (± 5 days) mice fed normal chow. Results are presented as mean ± SEM, *n* = 6-8 mice in each group. [A]$p<0.05$.