Supplementary Figure 1. Specific activation of hLRH-1 by DLPC. a-c, Full length of human LRH-1 are activated by DLPC but not other PCs, lysoPCs, fatty acids, and PEs. Luciferase assays were performed with extracts of HeLa cells transfected with expression plasmids for human LRH-1 and LRH-1/SF-1 Luc reporter plasmid. Cells were treated with 100 µM of the indicated PC or 10 µM of indicated lysoPC, FA, or PE. Luciferase expression (RLU, relative light units) was normalized using an internal β-galactosidase control. PC, phosphatidylcholine; LysoPC, lysophosphatidylcholine; FA, fatty acid; PE, phosphatidylethanolamine. Error bars represent mean ± s.e.m.
Supplementary Figure 2. DUPC and DLPC specifically activate the NR5A subfamily.
Supplementary Figure 2. DUPC and DLPC specifically activate the NR5A subfamily. a. Activation of the native mouse SHP and Oct4 promoter by hLRH-1 and DUPC/DLPC. (Left) HeLa cells were transfected with a hLRH-1 expression vector along with a luciferase reporter gene containing native SHP promoter. (Right) COS-1 cells were cotransfected with a hLRH-1 expression vector and a luciferase reporter gene containing the proximal promoter region of Oct4 gene (Oct4-PP Luc) or a version (Oct4-PPmut Luc) with point mutations in the DR0 element. Cells were treated with 100 µM of the indicated compounds. b. DUPC and DLPC selectively activate LRH-1 and SF-1. HeLa cells were cotransfected with various nuclear receptors and the reporter plasmid (RE-TKluc). Cells were treated with either 100 µM DUPC or DLPC. Luciferase expression was assayed and normalized by β-galactosidase expression. Data are expressed for each reporter as fold activation of normalized luciferase activity relative to vehicle treated cells. c. DUPC/DLPC dose response for activation of a reporter gene by hLRH-1 in HeLa cells. Transfections were performed with 1, 10, 50, 100, 150, 200, or 250µM of the indicated compounds and hLRH-1 expression vector. EC50 values were estimated to be 57 µM (DUPC) and 99 µM (DLPC) for hLRH-1. d. DUPC and DLPC still activate hLRH-1 mutants lacking phosphorylation sites (S238, 243A) or the sumoylation site (K270R), but not the ligand binding pocket double mutant (F342W, I426W). C3A HepG2 cells were transfected with wild type LRH-1 or the various mutants and treated with 100 µM of the indicated compounds, and luciferase expression was normalized to fold activation relative to vehicle. Error bars represent mean ± s.e.m.

Supplementary Figure 3. DUPC and DLPC specifically activate LRH-1 target genes.
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a. C3A/HepG2 cells were treated with either vehicle, or 100mM CDCA, DPPC or DLPC. Cells were harvested either 1, 3, 6, 12, or 24 hr after treatment to isolate total RNA. Gene expression was determined by qPCR. mRNA levels were normalized by 36B4. Error bars represent mean ± s.e.m. (*P<0.05 vs 1hr treated with veh).

b. Immunoblot analysis to confirm endogenous hLRH-1 knockdown using siRNA. C3A/HepG2 cells were transfected with either non-targeting siRNA control or siRNA hLRH-1. β-actin was used for loading control.

c. (left), C3A/HepG2 cells were cotransfected with siRNA hLRH-1 (or siRNA control) and LRH-1/SF-1 Luc reporter. Cells were treated with indicated compounds 24h after transfection. Luciferase assays were performed 48h after transfection. (right) C3A/HepG2 cells were transfected with either siRNA control or siRNA hLRH-1 followed by treatment with the indicated compounds. Cells were harvested 48h after transfection to isolate total RNA. Gene expression was determined by qPCR. mRNA levels were normalized by 36B4. Error bars represent mean ± s.e.m. (*P<0.05 vs Mock treated with veh).
Supplementary Figure 4. DUPC and DLPC are LRH-1 agonists.
Supplementary Figure 4. DUPC and DLPC are LRH-1 agonists. a. In vivo coactivator recruitment was assessed in a mammalian 2-hybrid assay. HeLa cells were cotransfected with VP-16-hLRH-1 ligand binding domain (or VP-16 alone), Gal4-SRC-3 receptor interacting domain (RID), and reporter construct (G5-TK-Luc). 16h after transfection, cells were treated with the indicated compounds for 24h. Error bars represent mean ± s.e.m. b. In vitro coactivator recruitment was assessed in GST pulldown assay. Either bacterially purified GST-hLRH-1 ligand binding domain (LBD) or GST alone was incubated with 100 μM of indicated compounds. [35S]methionine-labelled coactivator SRC-3 proteins were then added to each reaction. The amount of specifically bound SRC-3 proteins was quantified by densitometry. c. LanthaScreen (Invitrogen) TR-FRET coactivator recruitment assay was used to evaluate the effects of DLPC and DPPC on the ability of full-length LRH-1 to recruit SRC2-1 peptide. 5nM His tagged full length LRH-1 was incubated in the presence of 5nM Tb-Anti-His antibody, 1mM Fluorescein-tagged SRC2-1 peptide, and decreasing concentrations of DLPC (30mM – 3nM), DPPC(30mM – 3nM) or DMSO vehicle only. After 4 hours of incubation, FRET signals were measured by excitation at 340nm and emission at 490nm and 520nm. FRET ratios were obtained by dividing the signals at 520nm/490nm and fold change over DMSO was calculated by dividing the FRET ratios from treatment with DLPC and DPPC to EtOH only. Data was graphed and IC50’s were calculated using GraphPad Prism. d. LanthaScreen TR-FRET competitive binding assay was used to evaluate the effects of DLPC on the ability of rosiglitazone to bind to PPARγ using Fluoromone green1. 5nM GST-tagged PPARγ LBD was incubated in the presence of 5nM Tb-Anti-GST antibody, 5nM Fluoromone green, and decreasing concentrations of rosiglitazone (1mM – 33pM) in the presence or absence of 10mM DPLC. After 2 hours of incubation, FRET signals and FRET ratios were measured as in c.

Supplementary Figure 5. Effects of DUPC and DLPC in C57BL/6 mice. 8-week-old male C57BL/6 mice were challenged orally with vehicle, CA, DPPC, DUPC, and DLPC for 3 days. **a.** Percentage of liver weight per body weight. **b.** Serum alanine transaminase (ALT) and aspartate transaminase (AST) levels were measured to assess the liver damages. **c.** Total adrenal gland RNA was isolated and prepared for the cDNA. The expression levels of SF-1 target genes in the adrenal gland were determined using qPCR. Error bars represent mean ± s.e.m. (n=5 animals/group).
Supplementary Figure 6. DUPC/DLPC induction of Cyp7A1 and Cyp8B1 requires Lrh-1. a, b. 4-month-old male Lrh-1<sup>f/f</sup> mice were injected with Ad-GFP or Ad-Cre through the tail vein. 2 weeks later, mice were challenged orally with vehicle, CA, DPPC, DUPC, and DLPC for 3 days. Total liver RNA was isolated and prepared for the cDNA. Hepatic Cyp7A1 and Cyp8B1 (a) and LRH-1 (b) mRNA levels were determined using qPCR. Values represent the mRNA levels normalized by the 36B4. c. Serum BA levels were measured in the same mice. Error bars represent mean ± s.e.m. (*P<0.05, **P<0.01 vs Ad-GFP infected Lrh-1<sup>f/f</sup> mice treated with veh, n=4 animals/group).
Supplementary Figure 7. Effects of DLPC in db/db mice.
Supplementary Figure 7. Effects of DLPC in db/db mice. 12-week-old male db/db mice were treated orally with vehicle or DLPC for 3 weeks. a. Glucose tolerance was assessed in overnight fasted mice. Insulin tolerance was assessed in ad libitum fed mice. In the ITT, glucose levels after insulin injection are presented as the percentage of initial glucose concentrations. b. Body weight. c. Food intake measured over the last 5 days of 3 week treatment. d. Percentage of each tissue weight normalized by body weight. RD-WAT; reproductive fat, BAT; brown fat. e. Serum insulin, BA, cholesterol, TG, and NEFA levels were measured. f. Hepatic TG, NEFA, and cholesterol levels were measured. Error bar represents mean ± s.e.m. (*P<0.05, **P<0.01 vs db/db mice treated with veh, n=5 animals/group).
Supplementary Figure 8. Hepatic gene expression in db/db mice treated with DLPC. Total liver RNA was isolated and prepared for the cDNA. qPCR analysis of hepatic gene expression associated with a. bile acid synthesis, b. lipogenesis, c. fatty acid oxidation, and d. glucose metabolism in db/db mice treated with vehicle or DLPC for 3 weeks. Values represent the mRNA levels normalized by the 36B4. Error bar represents mean ± s.e.m. (*P<0.05, **P<0.01 vs db/db mice treated with veh, n=5 animals/group).
Supplementary Figure 9. Study of DLPC in Lrh-1<sup>fl/fl</sup> and LKO DIO mice. Liver specific Lrh-1<sup>-/-</sup> mice (LKO) were generated by crossing Lrh-1<sup>fl/fl</sup> mice with Alb-Cre transgenic mice. Null allele of Lrh-1 was confirmed in genomic DNA and mRNA levels (a). Body weight and food intake (b) in either vehicle or DLPC treated Lrh-1<sup>fl/fl</sup> and LKO DIO mice. Error bars represent mean ± s.e.m. (**P<0.01 vs Lrh-1<sup>fl/fl</sup> mice treated with veh, n=4 animals/group). c, Immunoblot intensity shown in Figure 3d was quantified using an image process program (ImageJ). Data were derived from three independent experiments. Error bars represent mean ± s.e.m. (*P<0.05, **P<0.01 vs Lrh-1<sup>fl/fl</sup> mice treated with only veh, n=4 animals/group).
Supplementary Figure 10. Hepatic gene expression in vehicle or DLPC treated Lrh-1f/f and LKO DIO mice.
Supplementary Figure 10. Hepatic gene expression in vehicle or DLPC treated Lrh-1ff and LKO DIO mice.
Supplementary Figure 10. Hepatic gene expression in vehicle or DLPC treated Lrh-1^{f/f} and LKO DIO mice. Total liver RNA was isolated and converted to cDNA, and qPCR was used to assay hepatic gene expression associated with a. bile acid metabolism, b. cholesterol metabolism, c. fatty acid synthesis, d. fatty acid oxidation, and e. glucose metabolism in Lrh-1^{f/f} and LKO DIO mice treated with vehicle or DLPC for 3 weeks. Values represent the mRNA levels normalized by the 36B4. Error bar represents mean ± s.e.m. (*P<0.05, **P<0.01 vs Lrh-1^{f/f} mice treated with veh, n=4 animals/group).
Supplementary Figure 11. Effect of DLPC in Ad-GFP or Ad-Cre infected Lrh-1\textsuperscript{fl/fl} DIO mice.
Supplementary Figure 11. Effect of DLPC in Ad-GFP or Ad-Cre infected Lrh-1f/f DIO mice. Hepatic (a), and serum (b) TG and NEFA levels were measured in Ad-GFP or Ad-Cre infected Lrh-1f/f DIO mice 3 weeks after vehicle or DLPC treatment. c. Liver sections were stained with Hematoxylin/Eosin (H & E) for general morphology or Oil Red O (ORO) for lipid accumulation in the same mice. Original magnification, X20. d. Glucose tolerance tests (GTT) were performed in the same mice. e. Fasting serum insulin levels. f. Hepatic and serum BA levels. g. Lipogenic gene expression in the liver was determined using qPCR. mRNA levels are relative to 36B4. Error bar represents mean ± s.e.m. (*P<0.05, **P<0.01 vs Ad-GFP Lrh-1f/f DIO mice treated with veh, n=5 animals/group).
Supplementary Figure 12. Working model for insulin sensitizing effects of DLPC. DLPC activation of the nuclear receptor LRH-1 sets up a positive regulatory loop in which decreased SREBP-1c expression and subsequent decreased steatosis results in increased hepatic insulin sensitivity. This leads to a decrease in serum insulin levels and a further decrease in SREBP-1c mRNA and fatty liver that continues the positive cycle. This is essentially the opposite of the lipogenic vicious cycle to insulin resistance proposed by McGarry¹.