A diurnal serum lipid integrates hepatic lipogenesis and peripheral fatty acid use

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Food intake increases the activity of hepatic de novo lipogenesis, which mediates the conversion of glucose to fats for storage or use. In mice, this program follows a circadian rhythm that peaks with nocturnal feeding and is repressed by Rev-erba/b and an HDAC3-containing complex during the day. The transcriptional activators controlling rhythmic lipid synthesis in the dark cycle remain poorly defined. Disturbances in hepatic lipogenesis are also associated with systemic metabolic phenotypes, suggesting that lipogenesis in the liver communicates with peripheral tissues to control energy substrate homeostasis. Here we identify a PPARα-dependent de novo lipogenic pathway in the liver that modulates fat use by muscle via a circulating lipid. The nuclear receptor PPARα controls diurnal expression of lipogenic genes in the dark/feeding cycle. Liver-specific PPARα activation increases, whereas hepatocyte-PPARδ deletion reduces, muscle fatty acid uptake. Unbiased metabolite profiling identifies phosphatidylcholine 18:0/18:1 (PC(18:0/18:1)) as a serum lipid regulated by diurnal hepatic PPARα activity. PC(18:0/18:1) reduces postprandial lipid levels and increases fatty acid use through muscle PPARα. High-fat feeding diminishes rhythmic production of PC(18:0/18:1), whereas PC(18:0/18:1) administration in db/db mice (also known as Lepr−/−) improves metabolic homeostasis. These findings reveal an integrated regulatory circuit coupling lipid synthesis in the liver to energy use by muscle by coordinating the activity of two closely related nuclear receptors. These data implicate alterations in diurnal hepatic PPARα–PC(18:0/18:1) signalling in metabolic disorders, including obesity.

PPARα promotes fatty acid (FA) synthesis in the liver. Surprisingly, hepatic PPARα overexpression (adenoviral-mediated, adPPARα) reduced circulating triglyceride (TG) and free fatty acid (FFA) levels (Fig. 1a). FA uptake and β-oxidation were increased in isolated soleus muscle, compared to control mice (adenoviral-mediated green fluorescent protein expression, adGFP) (Fig. 1b), indicating that a PPARα-dependent signal couples liver lipid metabolism to muscle FA oxidation. To identify candidate molecules, we performed untargeted liquid chromatography-mass spectrometry (LC-MS)-based metabolite profiling of hepatic lipids. Metabolite set enrichment analyses ranked acetyl-CoA carboxylase (ACACA), also known as Acc1, a rate-limiting enzyme in de novo lipogenesis, as a top altered pathway in the adPPARα/adGFP comparison (Extended Data Fig. 1a and Extended Data Table 1), consistent with a positive correlation of Acc1 (also known as ACACA) and PPARδ expression in human livers (Extended Data Fig. 1b). Transient liver-specific Acc1 knockout (LACC1KD) reduced hepatic TG content and increased serum TG and FFA levels (Fig. 1c). FA uptake was decreased in isolated soleus muscle from LACC1KD mice (Fig. 1d). In vivo FA uptake assays revealed that muscle FA uptake was decreased in LACC1KD mice in the dark/feeding cycle, when the lipogenic program is active (zeitgeber time (ZT) ZT 18 or 12 a.m.; ZT 6: lights on at 6 a.m.; ZT 12: lights off at 6 p.m.) (Fig. 1e). This defect was accompanied by slower clearance of circulating [3H]oleic acid (Fig. 1f). These results demonstrate that hepatic de novo lipogenesis is linked to muscle FA use.

Ppard expression oscillated diurnally, peaking at night, coincident with messenger RNA (mRNA) levels of the molecular clock BMAL1 (also known as Arntl) in the liver and in dexamethasone-synchronized primary hepatocytes (Extended Data Fig. 2a, b). In liver-conditioned Ppard knockout (LPPARDKO) mice, induction of hepatic Acc1 during the dark cycle was abolished; diurnal expression of Acc2, fatty acid synthase (Fasn) and stearoyl-CoA desaturase 1 (Scd1) was also altered (Fig. 2a), indicating that PPARα regulates rhythmic lipogenic gene expression in the liver. Daytime-restricted feeding reversed expression patterns of all major molecular clocks (Extended Data Fig. 2c)†. Peak mRNA levels of Ppard and lipogenic genes also shifted to the light cycle in control but not in LPPARDKO mice (Fig. 2b). The expression of diglyceride acyltransferase (Dgat1), triglyceride synthesis, choline kinase α (Chka, phosphocholine synthesis) and core circadian clock genes were unchanged in LPPARDKO mice (Extended Data Fig. 2a, c). Body weight, feeding activity and insulin sensitivity were similar between genotypes (Extended Data Fig. 2d, e and Extended Data Table 2). LPPARDKO reduced muscle FA uptake in the dark cycle in vivo (Fig. 2c), mirroring results from LACC1KD mice and demonstrating a functional consequence of this hepatic transcriptional circuitry in muscle physiology.

Products of de novo lipogenesis can exert signalling effects, for example, palmitoleate as a lipokine and 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine as an endogenous ligand of the nuclear receptor PPARγ in hepatocytes. In humans and mice, serum lipid composition closely resembles that of the liver (Extended Data Fig. 2d), suggesting that changes in hepatic de novo lipogenesis may have systemic metabolic effects. Indeed, serum or serum-derived lipid extracts — but not delipidated serum — collected in the dark cycle from wild-type mice increased FA uptake in C2C12 myotubes (versus LPPARDKO, Fig. 2e, d). Solid phase extraction of plasma lipids (Extended Data Fig. 2g) identified that the phospholipid fraction stimulated FA uptake in myotubes (Fig. 2f).

To identify phospholipids mediating functional interactions between PPARα, hepatic lipid synthesis and muscle FA use, we profiled serum lipid metabolites of samples from wild-type and LPPARDKO mice collected at six ZT points. 735 unique ion features were detected in positive and negative ionization modes (Extended Data Fig. 2f). Metabolite hierarchical clustering revealed the main differences between wild-type and LPPARDKO serum occurred during the dark cycle (Fig. 3a, b), when PPARα-controlled lipogenesis is most active. Daytime feeding led to a more pronounced discordance in serum lipidomes between these two genotypes, indicating that LPPARDKO mice were unable to adjust their lipogenic gene expression program (Extended Data Fig. 3a, b). Principal component analysis (PCA) of features in positive ionization mode, which detects phospholipids as well as mono-, di- and triacylglycerols,
These 14 lipid species were also the main drivers of the sample clustering in PCA analyses (Extended Data Fig. 3d). We focused on $m/z = 788.6$, putatively identified as PC(36:1), as its levels were decreased in both LPPARDKO and LACC1KD (versus control) serum but increased in liver tissue from PPARδ-overexpressing mice (Fig. 3d), correlating with the FA uptake data observed in each model. The extracted ion chromatogram (EIC) showed this phospholipid displayed diurnal rhythmicity peaking at night (or during the day in daytime restricted feeding) in wild-type serum, but not in LPPARDKO serum (Extended Data Fig. 3e, f). This phospholipid was also reduced in LACC1KD serum and increased in adPPARδ liver lysates (Extended Data Fig. 3e). Co-elution experiments with authentic PC(18:0/18:1) (also known as 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine, SOPC) and tandem mass spectrometry scanning confirmed this ion as PC(18:0/18:1), whereas PC(18:1/18:0) or others such as PC(16:1/20:0) were not observed (Extended Data Fig. 3g and data not shown). The concentrations of PC(18:0/18:1) in wild-type serum ranged from ~50 μM at ZT 8 (day) to ~115 μM ZT 20 (night) using deuterated d83-PC(18:0/18:0) as an internal standard. The nighttime increase in PC(18:0/18:1) levels was diminished in LPPARDKO mice (Fig. 3e). PPARδ synthetic ligand treatment (GW501516, 4 days) increased serum PC(18:0/18:1) levels in wild-type mice but not in LPPARDKO mice (Fig. 3f). These data identified PC(18:0/18:1) as a serum lipid regulated by hepatic PPARδ diurnally in three mouse models.

Intraperitoneal injection of escalating concentrations of PC(18:0/18:1) reduced serum TG and FFA levels (Extended Data Fig. 3h), with a trend of increased muscle FA uptake. Tail-vein injection of PC(18:0/18:1) (5 mg kg$^{-1}$ body weight) also reduced serum TG (Fig. 3g). Notably, PC(16:0/18:1) and PC(18:1/18:1) had no effect. In myotubes, only PC(18:0/18:1) increased FA uptake (Fig. 3h). Catheter-based, continuous infusion of PC(18:0/18:1) (25 μg min$^{-1}$ kg$^{-1}$ for 200 min) through the jugular vein also lowered circulating TG and FFA levels (Fig. 3i). As such, PC(18:0/18:1) links the hepatic PPARδ-controlled lipogenic program to serum lipid concentrations and muscle fat use.

Mechanistically, several FA use genes in the muscle, namely Cd36, Fabp3, Fabp4, Fatp1 (also known as Slc27a1), Fatp4 (also known as Slc27a4), Ppara, Cidea, and Acadm, were induced in adPPARδ and/or PC(18:0/18:1)-treated mice, but repressed in LPPARDKO and LACC1KD animals (Fig. 4a). Cd36 and Fabp3 are known mediators of lipogenic gene expression and muscle FA uptake. a. Hepatic lipogenic gene expression in wild-type (WT) and LPPARDKO mice ($n=4$ per time point). White and black bars on the x-axis represent light and dark cycles, respectively. b. Liver gene expression under daytime feeding ($n=3$). Grey bars, time when food was available. *$P<0.05$ (ANOVA), WT versus LPPARDKO. c. In vivo muscle FA uptake ($n=3$). d-f. In vitro muscle FA uptake in C2C12 myotubes treated with serum (2%) pooled from light or dark cycle samples, serum total lipids or delipidated serum (dark cycle samples) or serum lipid fractions ($n=3$). *$P<0.05$ (t-test), data shown as mean ± s.e.m.
of muscle FA uptake\(^\text{27,18}\). \(\text{Cd36}\) expression at mRNA and protein levels also oscillated in wild-type muscle peaking in the dark cycle, and shifted to the light cycle by daytime restricted feeding (Fig. 4b and Extended Data Fig. 4a). This diurnal pattern was disrupted in muscle of \(\text{LPPARDKO}\) mice. Furthermore, whereas \(\text{PPAR}\) agonist \(\text{GW501516}\) increased muscle expression of \(\text{Cd36}\) and \(\text{Fabp3}\) (Fig. 4c), enhanced

**Figure 3** | PC(18:0/18:1) links hepatic PPAR\(\alpha\) to serum lipid levels and muscle FA uptake. a, Serum lipid heat map (\(n=3\) per time point). White, light (starting at ZT 4) and black, dark cycles. b, Dendrogram from hierarchical clustering. c, Cross-comparison of changed lipids. d, Z-score plots of 14 commonly changed features. MAG, monoacylglycerol; LysoPC, lysophosphatidylcholine; SM, sphingomyelin. e, Serum PC(36:1) quantification in WT (\(n=5\)) and \(\text{LPPARDKO}\) (\(n=4\)) mice. f, Serum PC(36:1) concentrations in WT/\(\text{LPPARDKO}\) ± GW501516 (\(n=5\)). g, Serum TG changes (tail-vein injection) with phospholipids in wild-type mice (\(n=6\)). h, FA uptake in C2C12 myotubes treated with phospholipids (50 \(\mu\)M, \(n=3\)). i, Serum TG and FFA levels after PC(18:0/18:1) infusion (\(n=6\), wild-type C57BL/6J mice). *\(P<0.05\) (t-test), data presented as mean ± s.e.m.

**Figure 4** | PC(18:0/18:1) regulates muscle FA use through PPAR\(\alpha\). a, Muscle gene expression. b, Muscle \(\text{Cd36}\) protein (top) and gene expression (bottom, \(n=4\) per time point) expression. c, \(\text{Cd36}\) and \(\text{Fabp3}\) expression in wild-type and \(\text{LPPARDKO}\) muscle with or without GW501516 (\(n=5\)). d, Top, serum TG levels in wild-type and \(\text{PPAR}\alpha\)KO mice after vehicle or PC(18:0/18:1) infusion (\(n=6\), wild-type from Fig. 3i). Bottom, in vivo soleus muscle FA uptake. e, Muscle \(\text{Cd36}\) and \(\text{Fabp3}\) expression in wild-type and \(\text{PPAR}\alpha\)KO mice. f, FA uptake in C2C12 myotubes (\(n=3\)). Top, \(\text{Ppara}\) knockdown or control; bottom, wild-type \(\text{Ppara}\) or AF2m (AF2m). g, Fasting serum lipid concentrations (g), glucose tolerance test (GTT) (h), and muscle lipid content (i) in \(\text{db/db}\) mice treated with vehicle (\(n=4\)) or PC(18:0/18:1) (\(n=5\)). *\(P<0.05\) (t-test); **\(P<0.05\) (ANOVA); data presented as mean ± s.e.m.
muscle FA uptake and lowered serum TG levels in wild-type mice (Extended Data Fig. 4b), all these ligand effects were lost in LPPARDKO animals. This suggests that physiological fluctuations in levels of lipid mediators and diabetes mellitus in humans has recently been reported. Nevertheless, FA uptake: we adapted experiments with 1H-labeled oleic acid in Krebs–Ringer HEPES (KRH) buffer, 1% BSA and 100 μM oleic acid. Intracellular 1H radioactivity was determined and normalized to protein concentration. Ex vivo FA oxidation: freshly isolated soleus muscles were incubated at 37°C for 30 min with 1H-labeled acid. Supernatants were collected and the 1H radioactivity in the aqueous phase was quantified as described. In vivo FA uptake: we adapted an established protocol. Briefly, 10 μCi [1H]oleic acid in 3.5% BSA was infused through the portal vein (or in 5% intralipid through jugular vein in PC(18:0/18:1) infusion experiments). Blood samples were collected at 1, 5, 7 and 10 min. Soleus and gastrocnemius muscles were isolated at 10 min. FA uptake was calculated as described.

Data analysis. Sample handling (Extended Data Fig. 5, 6) and statistical tests are described in Methods. Data are presented as mean ± s.e.m. Information for mouse cohorts and primer sequences are described in Extended Data Tables 3, 4. For in vitro assays, the mean and s.e.m. were determined from 3–4 biological replicates for one representative experiment. Experiments were repeated at least three times. Significance was set at P < 0.05.

Extended Data Table 2. Muscle lipid contents in the PC(18:0/18:1)-treatment group trended lower (Extended Data Table 2). Glucose concentrations throughout insulin tolerance test were lower with PC(18:0/18:1) treatment (Fig. 4h), although the per cent change did not differ. Fasting insulin levels were similar (Extended Data Table 2). Muscle lipid contents in the PC(18:0/18:1)-treatment group trended lower (Fig. 4i), consistent with the notion that PC(18:0/18:1) promotes fat use in the muscle.

The data presented here reveal that intracellular oscillations of hepatic de novo lipogenesis-derived lipid metabolites coordinate metabolic functions between liver and muscle (Extended Data Fig. 4i). The finding also adds PC(18:0/18:1) to an emerging network of signalling molecules mediating inter-organ communications. The approximately twofold change in PC(18:0/18:1) concentrations is similar to other lipid mediators, including two gut-derived lipids that regulate satiety: N-acylphosphatidylethanolamine and oleylthanolamide, suggesting that physiological fluctuations in levels of lipid mediators are sufficient to stimulate specific biological functions. Specificity is further supported by data showing that systemic treatment with PC(16:0/18:1), a hepatic PPARx ligand, did not lower serum TG or stimulate FA uptake (Fig. 3g, h), nor did it activate PPARx in muscle cells (Extended Data Fig. 4j, k). An association between serum PC(36:1) levels and diabetes mellitus in humans has recently been reported. Herein, diet-induced obesity dysregulated temporal PC(18:0/18:1) production, whereas PC(18:0/18:1) treatment improved lipid and glucose metabolism in db/db mice. Although reduced ectopic fat accumulation in PC(18:0/18:1)-treated muscle would be predicted to improve metabolic homeostasis, future studies are required to determine how PC(18:0/18:1) lowers fasting glucose, how twofold fluctuations in serum PC(18:0/18:1) levels transduce physiological effects and how PC(18:0/18:1) achieves specificity towards muscle PPARx. Nevertheless, mechanisms that restore the rhythmic activity of the PPARs–PC(18:0/18:1) axis may provide new therapeutic opportunities to treat metabolic diseases.

METHODS SUMMARY

Functional assays. In vitro FA uptake: C2C12 myotubes were pre-treated with lipids complexed in 0.2% FA-free BSA overnight. Cells were washed before subjecting to a 5 min FA loading with 1 μCi [1H]oleic acid in Krebs–Ringer HEPES (KRH) buffer, 1% BSA and 100 μM oleic acid. Intracellular [1H] radioactivity was determined and normalized to protein concentration. Ex vivo FA oxidation: freshly isolated soleus muscles were incubated at 37°C for 30 min with [1H]oleic acid. Supernatants were collected and the [1H] radioactivity in the aqueous phase was quantified as described. In vivo FA uptake: we adapted an established protocol. Briefly, 10 μCi [1H]oleic acid in 3.5% BSA was infused through the portal vein (or in 5% intralipid through jugular vein in PC(18:0/18:1) infusion experiments). Blood samples were collected at 1, 2, 5, 7 and 10 min. Soleus and gastrocnemius muscles were isolated at 10 min. FA uptake was calculated as described.

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Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions S.L., A.S., J.P. and C.-H.L. designed the research. S.L. performed most of the experiments with technical assistance from K.J.S., P.B., M.R.G. and L.D.; S.L., J.D.B., E.H., M.L. and A.S. developed and performed untargeted and targeted metabolite profiling. B.H. generated adGFP and adPPARδ virus. K.I. performed metabolic cage and lipid infusion experiments. G.S.H., J.P., A.S. and J.D.B. provided critical intellectual inputs and manuscript editing. S.L. and C.-H.L. analysed the data and wrote the paper.

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Materials. Pparδ or GFP adenovirus was generated as described. The shScramble and shPpara shRNA constructs were provided by C. Newgard. Small interfering RNA sequences against Cd36, Ppara (5′-CCCTTATCGGAAATGTCTTA-3′) or luciferase (control) were cloned in the pSIREN-RetroQ vector. PparaΔF2 mutant construct was generated through site-directed mutagenesis to introduce a stop codon in front of the AF2 domain using wild-type Ppara as the template. The oligonucleotide used for mutagenesis was: 5′-GAGCATGCAGGACTGGA GTAGGTGCTACAAAGAACC-3′. Full-length Ppara or PparaΔF2 mutant complementary DNA was cloned in the pBabe retroviral vector.

Cell lines. All cell lines were obtained from ATCC: C2C12 (CRL-1772); HEK293 (CRL-1573). C2C12 myoblasts were infected with retroviral particles and selected against puromycin to generate stable lines. All stable C2C12 myoblasts were differentiable to myotubes with no apparent defects. Differentiation of C2C12 myoblasts was performed in 2% horse serum, high-glucose DMEM for 8 days.

FA uptake/oxidation assays. In vitro FA uptake. C2C12 myoblasts were pretreated with lipids complexed in 0.2% BSA (FA-free) overnight. Cells were thoroughly washed before subjecting to a 5-min FA loading with 1 μCi ml⁻¹ [1H]oleic acid in Krebs–Ringer HEPES (KRH) buffer, 1% FA-free BSA and 100 μM oleic acid. Intracellular [3H] radioactivity was determined and normalized to protein concentration.

Ex vivo FA oxidation. Freshly isolated soleus muscles were incubated at 37 °C for 30 min with 2% FA-free BSA containing KRH buffer supplemented with 0.2 mM palmitic acid and 4 μCi ml⁻¹ [1H] palmitic acid. Supernatants were collected in 2 cohorts (8–10 weeks old male, n = 6). In vivo FA uptake. We adapted an established protocol. Briefly, 10 μCi [1H] oleic acid in 3.5% FA-free BSA was infused through portal vein (or in 5% intralipid through jugular vein in C180/181 infusion experiments). Blood samples were collected at 1, 2, 5, 7 and 10 min after infusion to determine radioactivity. At 10 min, soleus and gastrocnemius muscles were isolated. FA uptake was calculated as described.

Animals. Mice used in the current study were on the C57BL/6J background, except for wild-type FVB/NJ and FVB/NJ-db/db mice used for PC180/181 tail-vein injection (see Extended Data Table 3 for detail). Liver-specific Pparδ knockout mice were generated by crossing albumin-cre transgenic mice to Pparδ ko mice (PPARδ knockout mice) (5–9 weeks old male, n = 6). For circadian gene expression, the ΔAC method was used to measure relative expression. Hierarchical clustering and heat map were generated by Cluster and Java TreeView. The primers used in this study were obtained from Primer Bank and listed in Extended Data Table 4. Protein levels of CD36 were determined by western blotting of muscle lysates using antibody against CD36 (SC-9154, Santa Cruz). For circadian studies, a pooled sample from wild-type or LPPARDKO mice (n = 4) at each time point was used. For in vivo Acc1 knockdown, the knockdown efficiency was determined by western blotting (n = 5). Two representative animals from each group were shown (Fig. 1c).

Liquid chromatography/mass spectrometry (LC-MS). A 1.2:1 chloroform: methanol solution was prepared for lipid extraction to isolate organic soluble metabolites. Following brief vortexing, samples were centrifuged at 2,500g for 4°C for 10 min. The organic layer (bottom) was transferred to a new vial and solvents were evaporated under a stream of nitrogen. Samples were then dissolved in chloroform (120 μl) to provide a mass spectrometry ready solution and stored at −80°C until LC-MS analysis (within 48 h of extraction). For both positive and negative ionization mode LC-MS runs, 20 μl of extract was injected. LC-MS analysis was performed using an Agilent 6210 Accurate-Mass time-of-flight–LC-MS system as described. For LC analysis in negative mode, a Gemini (Phenomenex) C18 column (5 mm, 4.6 × 30 mm) was used together with a pre-column (C18, 3.5 mm, 2 × 20 mm). Mobile phase A consisted of 95:5 water:methanol and mobile phase B was composed of 60:35:5 isopropanol:methanol:water. Both A and B were supplemented with 0.1% ammonium hydroxide solution (28% in water). The flow rate for each run was 0.5 ml min⁻¹. The gradient started at 0% B for 5 min and linearly increased to 100% B over 40 min, was then maintained at 100% B for 8 min before re-equilibrating for 7 min at 0% B. For the LC analysis in positive mode, a Luna (Phenomenex) C5 column (5 mm, 4.6 × 30 mm) was used together with a pre-column (C4, 3.5 mm, 2 × 20 mm). Mobile phase A and B and the gradient were the same as for negative mode, but supplemented with 0.1% formic acid and 0.1% formic acid solution for negative and positive MS analysis was performed with an electrospray source ionization (ESI) interface. The capillary voltage was set to 3.0 kV and the fragmentor voltage to 100 V. The drying gas was set to 350°C, the drying gas flow was 101 ml min⁻¹ and the nebulizer pressure was 45 p.s.i. Data were collected using a mass range from 100–1,500 Da. For wild-type and LPPARDKO serum
samples, all samples of each genotype from different time points were detected in a single consecutive run. To validate the results, samples from ZT 8, ZT 16 and ZT 24 were subject to a second run (Extended Data Fig. 5). For Scramble and LACC1KD serum or adGF and adPPARδ liver, the entire sample set was run in a single session.

**Targeted analysis of phosphocholine species.** Side-chain composition of phosphocholine species. Phosphatidylcholine fatty acyl-chain composition was analysed separately based on established conditions on an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies) by direct infusion of 1 μL of serum lipid extracts without chromatography. This approach generates lithiated phosphocholine adducts, which unlike protonated adducts, give strong signals in tandem MS spectra, and reveals the position of various acyl chains because of a stronger signal in the tandem MS coming from the loss of the acyl chain in the sn-1 position over the sn-2 position. The QQQ-MS was operated in multiple reaction monitoring mode (MRM), targeting lithium acyl adducts and product ions. The MRM transitions and parameters for PC(18:0/18:1) or PC(18:1/18:0) are listed in Extended Data Fig. 3g. Mobile phase was comprised of 98:2 methanol:water with 1 mM LiCl to facilitate the formation of lithium adducts for analysis. Samples were run in positive ionization mode with fragmentor voltage of 150 V, collision energy of 35 V and a dwell time of 25 ms.

Quantification of phosphocholine species by stable isotope dilution mass spectrometry. 200 pmol of 1,2-dioleoyl-(d70)-sn-glycerol-3-phosphocholine-1,2-13C4,2-15N-trimethyl-d9 (D83 PC(18:0/18:0)) was spiked into 50 μL of serum as the recovery standard. Serum was extracted as above. LC-MS/MS analysis was performed using an Agilent 6410 QQQ-MS in positive ionization mode equipped with an electrospray ionization source interface and an Agilent 1200 Binary Pump. For LC analysis, a Gemini Phenomenex C18 column (50 mm × 2 mm, 3-μm particle size with 100 Å pore) was used with a 50-μm steel mesh filter. Mobile phase A consisted of 95:5 water:methanol and mobile phase B consisted of 80:20 isopropanol:methanol. Both A and B were supplemented with 0.1% formic acid. The flow rate was 0.3 ml/min. The gradient started at 20% B and linearly increased to 100% B over 45 min, was maintained at 100% B for 10 min before equilibrating for 5 min at 20% B. The QQQ-MS was operated in MRM mode and PCMs were targeted using the m/z [M + H]⁺ to m/z 281,2 transition for all PCls. Capillary voltage was set to 3.0 kV, the fragmentor voltage to 200 V with a collision energy of 35 V. The drying gas temperature was 350 °C, the drying gas flow was 10 ml/min and the nebulizer pressure was 45 p.s.i. The integrated peak area for each species was normalized to the peak area of the recovery standard.

**Data analysis (Extended Data Fig. 6).** Data preprocessing. Raw data files were converted to mzXML files and subsequently aligned by XCMS. The resulting aligned features derived from wild-type, LPPARDKO, Scramble and LACC1KD serum were compared to identify common features using metaXCMS with a mass tolerance of 0.01 and retention time tolerance of 60 s. Idetical procedures were carried out to generate common features from adPPARδ and adGF liver lysates. Subsequently, these features from serum and liver lysates samples were processed by an automated workflow to identify isotopic peaks and assign putative identity with 3 p.p.m. mass tolerance. All isotopic peaks were removed and the remaining data were cutoff for features with median intensity less than 5 × 106. The reproducibility of the untargeted metabolomics platform was evaluated from two independent runs of 6 samples. The Spearman’s rank correlation coefficient was calculated and the duplicate pair with lowest correlation coefficient was plotted (Extended Data Fig. 5a).

Data normalization. We adapted methods from ref. 38. Briefly, each sample was centred by median and scaled by its inter-quartile range (IQR). The normalized distributions of samples were plotted in Extended Data Fig. 5b as box-and-whisker plot. Hierarchical clustering. Both positive and negative ionization mode features from wild-type and LPPARDKO serum around the clock were mean-centred and scaled by standard deviation on a per-feature basis. To simplify the visualization, only the mean value of each feature from every time point was used for the construction of heat map. The resulting data sets of each genotype were clustered using Euclidean distance as the similarity metric in Cluster 3.0. Heat maps were generated by Java TreeView. Heat map of LPPARDKO serum was aligned to wild type for comparison. Dendrogram of samples was plotted based on Spearman correlation with Ward linkage.

**Principal component analysis.** Auto-scaling was applied on a per metabolite basis to each biological group (wild type versus LPPARDKO and Scramble versus LACC1KD). Principal component analysis was performed in MetaBolanalyst. The three-dimensional view of the first 3 principal components was plotted in addition. Screeplot of the first and third principal components, showing the separation between sample groups and the loading plot of these two principal components were generated (Extended Data Fig. 3c, d).

Identification of significant features. The empirical P value for each pair of comparison was calculated by randomly permuting sample labels for 1,000 times to obtain the null distribution. The analysis was carried out in Multiple Experiment Viewer. False discovery rate was determined by Benjamini–Hochberg method. A feature is considered significant for downstream cross-comparison with unadjusted P < 0.05. Significantly changed features in wild-type and LPPARDKO mice serum at night (n = 6, pooled sample set from ZT 16 and ZT 20), Scramble and LACC1KD mice serum (n = 5), and adGF and adPPARδ liver lysates (n = 4) were identified by database search to identify isotopic peaks and assign putative identity. A total of 158, 418 and 54 features were significantly altered in LPPARDKO/wild-type (serum samples at ZT 16/ZT 20, P < 0.05, corresponding to 19.6% FDR, Supplementary Data), LACC1KD/scramble control (serum samples at ZT 16, P < 0.05, FDR = 17%) and adPPARδ/adGF (liver lysates, P < 0.05, FDR = 11.3%) comparisons, respectively.

**Metabolites set enrichment analysis (MSEA).** Significantly altered features in the adPPARδ/adGF liver lysate comparison were subjected to database search to assign putative identities. Among these, 26 were matched to human metabolites database (HMDB) (Extended Data Table 1). The mapped species were assigned a HMDB ID for subsequent MSEA analysis implemented in the MetaBolanalyst.

**Statistical test.** Power. Owing to the multitude of measurements on each animal cohort, it is not feasible to pre-determine a sample size that achieves the power of all subsequent measurements. Therefore, we determined the minimal number of animals required to detect a pre-defined difference in serum TG, a key readout throughout the study. Our pilot studies in wild-type mice have indicated that to detect an effect size of 50% reduction in serum TG with a power of 80%, 3–8 mice are required per group, depending on time of the day (as TG levels vary). We determined the actual number of animals used for each study based on the above sample size estimation in conjunction with the feasibility of experimental approaches. Replication. Animal experiments were performed on multiple cohorts (Extended Data Table 3). In vitro experiments were performed at least 3 times. Randomization. The randomized block design was used for all animal experiments. We identified the age, sex, body weight, cage effect and timing of experiments as blocking factors. Therefore, all animal experiments were carried out on age-matched animals of the same sex. Body weight was measured before assigning treatment groups. Cage effect was controlled in pharmacological treatment studies by randomly assigning animals to the placebo or treatment group from the same cage. To control for the timing of experiments, alternating genotypes were drawn for each measurement. Subsequent assays (gene expression, PC(18:0/18:1) concentration measurement, etc.) were performed in a blinded fashion, that is, every sample was assigned a number without genotype or treatment labelled and the assays were performed sequentially based on the sample number. Often, samples were intercalated from different groups.

Sample exclusion and statistical tests. Pre-determined sample exclusion criteria was established for technical failures. In addition, the 1.5 inter-quartile range rule was used to exclude additional outliers. Two-tailed unpaired student’s t-test was used to compare two groups/treatments for experiments considered normal distribution (for example, cultured cells). For time-series data, the two-way ANOVA procedure was used. For metabolomics data analysis, the methods are detailed in metabolomics data analysis section. Equal variance among groups was assumed.

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Extended Data Figure 1 | Analyses of liver lipid metabolites altered by PPARδ overexpression. a, Metabolite set enrichment analysis (MSEA) of lipids from adGFP and adPPARδ liver lysates (n = 4). Metabolites were identified based on database search of matching mass-charge ratio and retention time. Identified metabolites and their relative quantity were used to calculate the enrichment and statistical significance. Top 30 perturbed enzyme or pathways were shown. List of metabolites recognized by the Metaboanalyst program and subsequently used for the MSEA analysis is shown in Extended Data Table 1. b, Correlation of hepatic PPARD and ACC1 expression in human liver. Human liver gene expression microarray data was downloaded from gene expression omnibus (GSE9588) and analysed using GraphPad Prism. *P < 0.05 (t-test).
Extended Data Figure 2 | Molecular clock expression, food intake and glucose metabolism in wild-type and LPPARDKO mice. a, Liver gene expression in wild-type and LPPARDKO mice (n = 4, each time point). White bar, light cycle starting at ZT 4; black bar, dark cycle. b, Ppard and Bmal1 expression in dexamethasone-synchronized primary hepatocytes (n = 3, each time point). Circadian time, hours after dexamethasone treatment. c, Gene expression in wild-type and LPPARDKO livers under daytime restricted feeding (n = 3, each time point). Red bar, time when food was available. d, Food intake in wild-type and LPPARDKO mice measured by metabolic cages (n = 8). e, Glucose tolerance test and insulin tolerance test in wild-type (n = 6) and LPPARDKO (n = 7) mice. f, Comparison of liver and serum lipidomes. g, Column purification of serum lipids (See methods for detail). IPA, isopropyl alcohol; MeOH, methanol; HOAc, acetic acid. Data presented as mean ± s.e.m.
Extended Data Figure 3 | Identification and characterization of PC(18:0/18:1), or SOPC. a. Heat map of identified features in wild-type and LPPARDKO serum under daytime feeding (n = 3, each time point). White bar, light cycle starting at ZT 0; black bar, dark cycle; red bar, time when food was available. b. Dendrogram of serum samples under daytime restricted feeding. c. Principal component analysis (PCA) of positive mode features in wild-type, LPPARDKO, Scramble and LACC1KD serum under ad libitum feeding. Top, score plot of the first three PCs representing 53.2% of the total variation. Bottom, score plot of PC1 and PC3. Circle, 95% confidence interval. d. Loading plot of the PCA. The putative identities of 11 features identified in Fig. 3d are shown in red. Additional top features contributing to the segregation are highlighted in blue. e. Top panels, EIC of m/z = 788.6 in wild-type and LPPARDKO serum. Bottom panels, EIC of m/z = 788.6 in LACC1KD serum and adPPARδ livers. f. Normalized PC(36:1) intensity in wild-type and LPPARDKO mouse serum (n = 4) under ad libitum or daytime restricted feeding (DF). g. Top, multiple reaction monitoring (MRM) parameters for identification of acyl-chain composition of PC(36:1). Bottom left, co-elution of the PC (18:0/18:1) standard with m/z = 788.6. Bottom right, PC(36:1) acyl-chain composition determined by tandem mass spectrometry running in the MRM mode. h. Top panels, lipid levels in mice intraperitoneally injected with various doses of PC(18:0/18:1) (n = 4). Bottom, in vivo FA uptake in soleus muscle (left) and serum PC(36:1) enrichment (right) 4 h after PC(18:0/18:1) injection at 5mg kg⁻¹ body weight. *P < 0.05 (t-test), data presented as mean ± s.e.m.
Extended Data Figure 4 | Requirement of hepatic PPARδ and muscle PPARα for the inter-organ communication mediated by PC(18:0/18:1)/SOPC. a, Cd36 gene expression in muscle of wild-type and LPPARDKO mice under daytime restricted feeding (n = 3, each time point). Red bar, time when food was available. #P < 0.05 (ANOVA). b, Effects of GW501516 on serum TG and muscle FA uptake in wild-type and LPPARDKO mice (n = 5). c, Cd36 and Fabp3 gene expression in C2C12 myotubes treated with vehicle or 25 μM PC(18:0/18:1) (n = 3). d, FA uptake in control or stable Cd36 knockdown C2C12 myotubes pretreated with indicated lipids. e, The mammalian one-hybrid assay (diagram shown on the top) to determine the transactivation activity of the PPAR ligand binding domain (LBD) (n = 3). Left panel, relative luciferase unit (RLU, presented as fold change) indicative of the reporter activity regulated by Gal4 DNA binding domain (DBD)-PPARα LBD fusion protein (Gal4-PPARαLBD) in 293 cells treated with indicated phospholipids at 100 μM. Right panel, RLU of Gal4-PPARδLBD and Gal4-PPARγLBD treated with 100 μM PC(18:0/18:1). f, Heat map showing serum phospholipid changes between ZT 20 and ZT 8 in 7-month-old male C57BL/6J mice on chow (n = 3) or high-fat diet (HFD for 4 months, n = 5) from targeted metabolomics. g, Serum PC(36:1) concentrations under chow or HFD. h, Blood glucose levels of ad libitum fed db/db mice measured between ZT 0 and ZT 3 before daily lipids injections (vehicle, n = 4; PC(18:0/18:1), n = 5). i, Model for the role of PPARδ–PC(18:0/18:1)–PPARα signalling in FA synthesis and use in the liver–muscle axis. j, Top panel, in vivo fatty acid uptake in soleus and gastrocnemius muscle 4 h after vehicle or 5 mg kg⁻¹ PC(16:0/18:1) injection though the tail vein (n = 6); bottom panel, muscle Cd36 and Fabp3 gene expression after PC(16:0/18:1) injection (n = 4). k, Top panel, activities of a PPRE-containing luciferase reporter in PPARα-expressing C2C12 cells treated with vehicle, 50 μM PC(18:0/18:1) or PC(16:0/18:1) and 1 μM GW7647 (a PPARα synthetic ligand). Bottom panel, Cd36 expression in C2C12 myotubes. ∗P < 0.05, (t-test), data presented as mean ± s.e.m.
Extended Data Figure 5 | Validation of metabolomics analyses. a, The reproducibility of the untargeted metabolomics platform was validated from two separate runs of 6 serum samples. The Spearman’s rank correlations are between 0.9 and 0.94. The duplicate pair with the lowest correlation (Spearman’s $r = 0.90$) is shown. b, The raw intensity of samples was subject to normalization with median centering and inter-quartile range (IQR) scaling. The resulting data show equal distribution among different groups of samples. White bar represents samples obtained in the light cycle and black bar for those in the dark cycle.
Extended Data Figure 6 | Flow chart of metabolomics data analysis showing the positive-mode metabolites. See methods for detailed description.
Extended Data Table 1 | List of metabolites recognized by Metaboanalyst program and subsequently used for MSEA analysis

| Metabolites               | HMDB ID     |
|---------------------------|-------------|
| 1-Acylglycerol            | METPA0229   |
| eicosatrienoic acid       | HMDB02925   |
| eicosadienoic acid        | HMDB05060   |
| eicosenoic acid           | HMDB02231   |
| Glycerophosphocholine     | HMDB00086   |
| LysoPC(18:0)              | HMDB10384   |
| LysoPC(18:2)              | HMDB10386   |
| LysoPC(18:3)              | HMDB10387   |
| LysoPC(22:0)              | HMDB10398   |
| LysoPC(22:6)              | HMDB10404   |
| LysoSM(d18:1)             | HMDB06482   |
| PA(34:1)                  | HMDB07858   |
| hexadecenoic acid         | HMDB03229   |
| PC(36:1)                  | HMDB07887   |
| PC(32:0)                  | HMDB00564   |
| PC(36:2)                  | HMDB00593   |
| PE(34:1)                  | MSEA0160    |
| PE(34:2)                  | MSEA0161    |
| PE(40:6)                  | MSEA0170    |
| Phosphatidylglycerol      | MSEA0606    |
| glycerophosphoethanolamine| MSEA0611    |
| phosphatidylserine        | HMDB12330   |
| PS(36:1)                  | HMDB10163   |
| Sphingomyelin             | HMDB01348   |
| sphingosine               | HMDB00252   |
| Sphingosine 1-phosphate   | HMDB00277   |
Extended Data Table 2 | Metabolic characteristics of mouse models

|                | C57BL/6J | WT     | LPPAR-DKO |
|----------------|----------|--------|-----------|
| **Body Weight (8 wks) (g)** | 26.03±0.67 | 24.63±0.23 |
| **Fasting Insulin (ng/mL)**    | 0.35±0.10 | 0.31±0.05  |
| **Fasting Blood Glucose (mg/dL)** | 83.13±2.63 | 79.13±2.66 |
| **ALT (Unit/L)**               | 25.19±2.48 | 18.23±1.66 |
| **AST (Unit/L)**               | 102.70±25.02 | 96.78±25.02 |

|                | FVB/NJ-db/db | Control | PC(18:0/18:1) |
|----------------|--------------|---------|---------------|
| **Body Weight (g)** | 37.70±1.97   | 35.98±0.94 |
| **Fasting Insulin (ng/mL)**    | 35.70±4.96   | 42.61±5.12 |
| **Fasting Blood Glucose (mg/dL)** | 245.7±3.67   | 184.2±23.20 * |

(I) Wild-type and LPPAR-DKO mice under normal chow diet (male, 8-10 weeks old, n = 6); (II) vehicle- (n = 4) and PC(18:0/18:1)-treated (n = 5) FVB/NJ-db/db mice. *P < 0.05, two-tailed t-test. Data presented as mean ± s.e.m.
## Extended Data Table 3 | List of animal cohorts used for this study

| Cohort Name                                    | No. Cohorts | Age             | Sex     | Background | Animal No.     | Figures                        |
|------------------------------------------------|-------------|-----------------|---------|------------|----------------|--------------------------------|
| adPPARδ vs adGFP                               | 3           | 8-10 weeks      | Male    | C57BL/6J   | 4-6 genotype   | Fig. 1a,b; Fig. 3c,d; Fig. 4a; ED. Fig 1a; ED. Fig 2f; ED. Fig 3e |
| LACC1KD vs Scramble                            | 2           | 8-10 weeks      | Male    | C57BL/6J   | 5 genotype     | Fig. 1c-f; Fig. 3c-d; ED. Fig 3c-e |
| LPPARDKO vs wt, chow fed, metabolic phenotyping| 2           | 8-10 and 32-35 weeks | Male | C57BL/6J   | 6-8 genotype   | ED. Fig. 2d-e                |
| LPPARDKO vs wt, ad lib circadian study         | 4           | 8 weeks         | Male /Female | C57BL/6J   | 3-5 genotype/time point | Fig. 2a,c-f; Fig. 3a-e; Fig. 4b; ED. Fig 2a; ED. Fig 3c-f |
| LPPARDKO vs wt, daytime restricted feeding     | 1           | 8 weeks         | Male    | C57BL/6J   | 3 genotype/time point | Fig. 2b; ED. Fig. 2c; ED. Fig. 3a,b,f; ED. Fig. 4a |
| LPPARDKO vs wt, GW501516 treatment             | 1           | 10-12 weeks     | Male    | C57BL/6J   | 4 genotype/treatment | ED. Fig. 3h                 |
| wt, PC intra-peritoneal injection              | 1           | 10-12 weeks     | Male    | C57BL/6J   | 4 genotype/treatment | ED. Fig. 3f; Fig. 4c; ED. Fig. 4b |
| wt, PC tail vein injection                     | 2           | 8-10 weeks      | Male    | FVB/NJ     | 6 genotype/treatment | Fig. 3g                 |
| PPARα KO vs wt, lipid infusion                 | 1           | 10-12 weeks     | Male    | C57BL/6J   | 7 genotype/treatment | Fig. 3i; Fig. 4d-e |
| wt, db/db, PC (18:0/18:1) vs vehicle           | 1           | 8 weeks         | Male    | FVB/NJ     | 4-5 treatment   | Fig. 4g-f; ED. Fig. 4h       |
| wt, C57BL/6J, Chow vs High Fat                 | 1           | 32-35 weeks     | Male    | C57BL/6J   | 3-5 treatment/time point | ED. Fig. 4f-g |

ED, Extended Data.
Extended Data Table 4 | List of primers used for qRT–PCR and oligonucleotides for short hairpin RNA constructs

| Genes | Forward Sequence | Reverse Sequence | Accession Number |
|-------|------------------|------------------|------------------|
| Acaca/ Aco1 | CGCTGCTACGTTTCCATTG | TTCTGTCAGGTTCTCAATGC | NM_133360.2 |
| Fasn | TCCTGGAGAGCAGACAGCTT | GACGAGTGCTACTCCCTGGAAGTTG | NM_007988.3 |
| Scd1 | CTCTCTCTCTCACTGGGTTG | GAGGTGTGATGACTTCCTCCTC | NM_009127.4 |
| Dgat1 | CAGCGGTGATTATTCATTC | ACAGGTTGACATCCCGGTTAG | NM_010046.2 |
| Rev-erba | TTCCCTGGTGCTTCACTAGTA | GCCAGACCTCGTTTCCTC | X86010.1 |
| Cd36 | TCATATTGTGCTTGGCAATCAGAG | TGATGATGCTTTTACCCTACAGATG | NM_001159558.1 |
| Fabp4 | TCACCGCAAGGAGCAAGAA | CACCAGCTCTGTACCATCTC | NM_024406.2 |
| Fatp4 | CATCGTCGATGGGATTTG | CAGGCTGCTGCTGCTGAATTCATC | NM_011144.6 |
| Cidea | TTCTCTGTGCTATCCCAGTG | GAGTTGTAAGGAAACTCGCTG | NM_001170.2 |
| Ppara | TGGTTGCGTCGCTCAAATTTTCG | GCAACTTCTCAATGCTCCTTATGTTT | NM_011144.6 |
| Fabp3 | ACCTGAAAGCTAGTGGGACAG | TGATGATGAGCTGCTGCTCAT | NM_010174.1 |
| Fatp1 | CGCTTTCTGCTGCTCTGCTTG | GATGGACGGATGCTGCTG | NM_011927.3 |
| Per1 | CCACGAGTGTGCTATGACAGA | CTGCTCCCGGTCTTGCTTCAG | AF543843.2 |
| Cry1 | CACCTGTTCCGAAAGAGACTC | CTGAAGACAAAAATGCGACCT | NM_0017771.3 |
| Arnt/ Bmal1 | TCAAGACGACATAGGACACCT | GGACATTGGCCTAAAACACAGTG | NM_007849.4 |
| Rplp0/ 36b4 | AGATGACGAGATCGCGGATC | GTCTTGGCACATCGACC | NM_007475.5 |
| Ppard | CAGGCGGCTACATGGAATGTC | TCCAGTGACCTGCTCTGTTA | NM_011145.3 |
| Acadm/ Mcad | TTTCGAAGACGTCAGAGTC | TGCCGAGTGGTTCGTTGTC | NM_007382.5 |

shRNA

| Gene | Sequence | Accession Number |
|------|----------|------------------|
| Cd36 | GGGAAAGTTGCTCCTTGAAGA | NM_001159558.1 |
| Ppara | CCGTATCTGAAGAATTCTGA | NM_011144.6 |