Supplemental Material

Methods

Animal experiments

All experiments were performed on male mice housed in individually ventilated cages in a temperature controlled room (23 ± 2°C) with a light/dark cycle of 12/12 hours. DEHP concentrations in the different diets were adjusted to expose mice to the amounts indicated in the figures according to food intake as described in (Feige et al. 2008). PPARα wild-type and null mice were on a pure SV129 background (Lee et al. 1995) and PPARβ wild-type and null mice were on a mixed SV129/C57Bl6J background (Nadra et al. 2006). The PPARα-humanized mice were generated by inserting a phage artificial chromosome containing the entire human PPARα gene and its regulatory sequences in the PPARα-null SV129 background as previously described (Yang et al. 2007).

DEHP treatment on CD was started at 4 weeks of age and the HFD treatments were started at 7 weeks of age. All treatments were performed over 13 weeks except for the high-fat diet protocol in WT mice which lasted 22 weeks because of metabolic phenotyping. Food intake and body mass were recorded weekly. Animal experimentations were approved by the relevant commission of the canton of Vaud (Switzerland) and of the National Cancer Institute Animal Care and Use Committee.

Biochemical assays

Plasmatic levels were determined from blood sampled retro-orbitally right before sacrifice. Insulin and adiponectin were measured by ELISA kits from Mercodia (Uppsala, Sweden) and Linco-Millipore (Billerica, MA, USA), respectively. Total ketone bodies were measured manually by colorimetric assay (Wako Diagnostics, Richmond, VA, USA). All other plasmatic parameters were measured on a Hitachi 902 robot using assays from Roche diagnostics (Basel, Switzerland). 3-Hydroxyacyl-CoA dehydrogenase (HAD) activity was measured as reported previously (Bedu...
et al. 2007). Fecal lipid content was measured as described in (Argmann et al. 2006), using kits from Wako following Folch extraction.

**Quantitative RT-PCR**

Reverse transcription was performed with random hexamers on 1µg of total RNA using the superscript first-strand synthesis system (Invitrogen, Basel, Switzerland) and the reaction was diluted 100 times for amplification. PCR reactions were performed in triplicate in 384-well plates on an Applied Biosystems 7900HT cycler using commercial Taqman probes (Applied Biosystems, Foster City, CA, USA). Results were normalized to 3 house-keeping genes and quantified using qBase (Hellemans et al. 2007).

**Primary hepatocytes and adenoviral infections**

Liver cells were prepared by the collagenase two-step method (Berry and Friend 1969) from PPARα wild-type or null male mice (25–30 g) after anesthesia with ketamin/xylazin (8/1 mg per 100 g body weight). Hepatocytes were seeded for 4h on 6-well plates in a ‘Hepatocyte plating medium’: M199 + Glutamax (Invitrogen) supplemented with 10% fetal calf serum, 7,5% BSA, insulin 10 nM, 1 mM T3 hormone, 500 nM dexamethasone (Sigma Aldrich) and 50 U/ml penicillin/50 g/ml streptomycin. Hepatocytes were infected overnight with 20 MOI of adenovirus encoding mPPARα or green fluorescence protein (GFP) in a serum-free medium supplemented with 7,5% BSA, 100 nM dexamethasone and 50 U/ml penicillin/50 g/ml streptomycin. 12h after infection and serum deprivation, cells were exposed to DMSO or 100 mM of MEHP for 12h in serum-free medium.
References


Figure legends

Supplemetal Material, Figure 1: DEHP exposure under chow diet does not affect glucose tolerance.

C57Bl6J WT male mice fed for 11 weeks with chow diet supplemented with vehicle (10ml/kg oil) alone or in combination with doses of DEHP leading to an exposure of 100 and 1000 mg / kg body mass / day (n=10 / group) were subjected to an intra-peritoneal glucose tolerance test (IP-GTT) with 2 g glucose per kg body mass after 4 hours of fasting.

Supplemetal Material, Figure 2: Exercise test protocol.
Supplemental Material - Figure 1

IP-GTT in chow fed mice

Glycemia (mM) vs. Time after glucose injection (min)

- DEHP 1000mg/kg
- DEHP 100mg/kg
- Vehicle