

Figure S1. Quantitative analysis of liver fibrosis. Data shows the relative area of Sirius red staining for female and male mice as indicated in the figure. Boxplots indicate the median as the horizontal line, the top and bottom of the box show the upper and lower quartiles, maximum and minimum values are displayed as whiskers. Female significance; ^αSD + OVX vs. SD, P=0.0106. ^βHFD + OVX vs. SD + OVX, P=0.0109. Male significance; ^ωHFD vs. SD, P=0.0124. SD, standard diet; HFD, high-fat diet; OVX, ovariectomized.

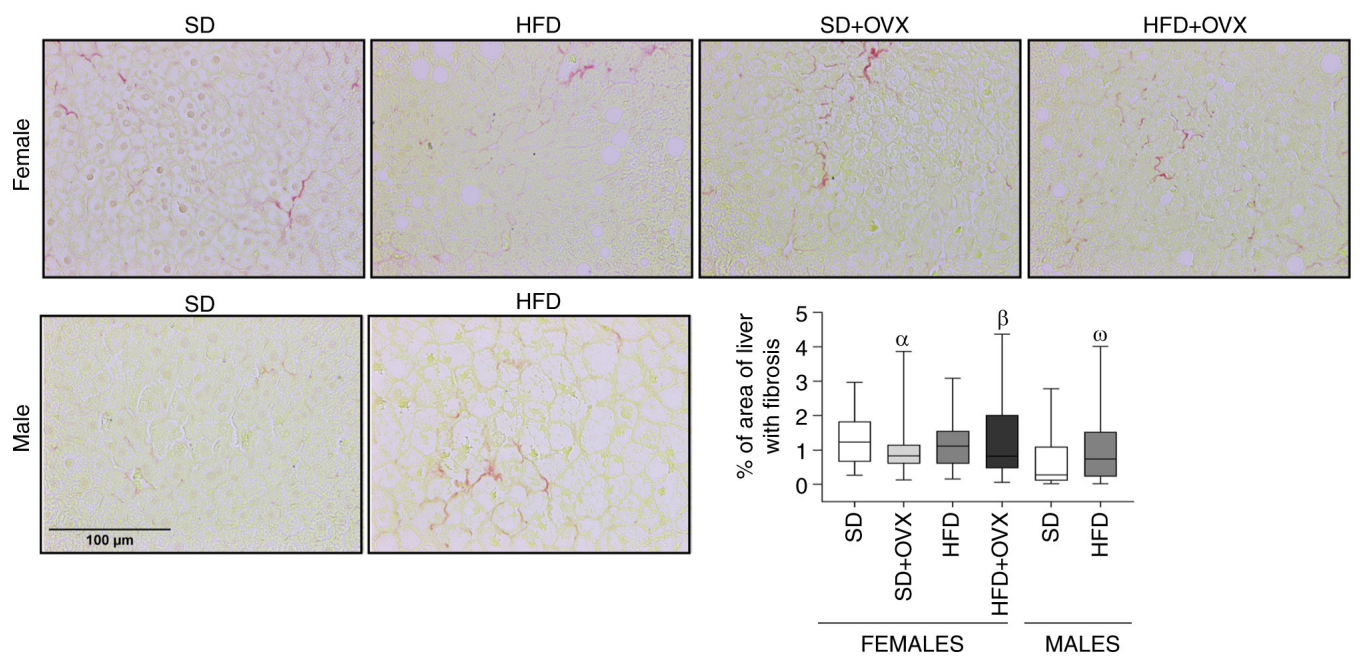
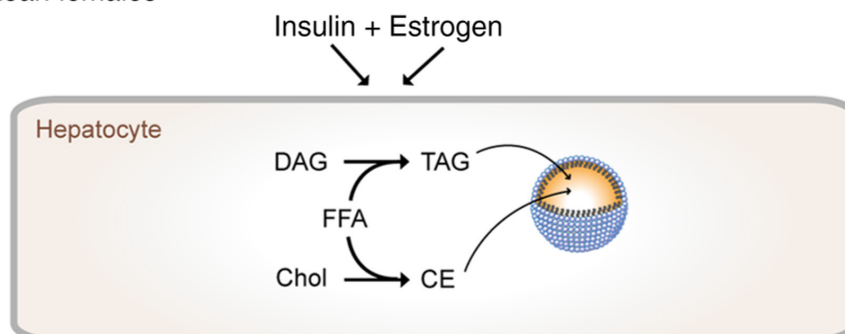
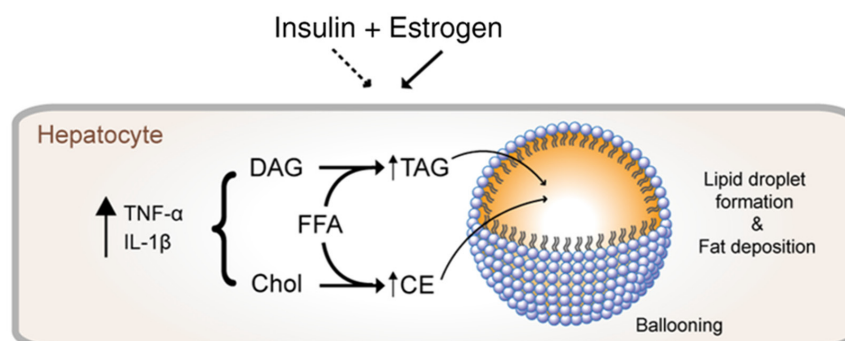


Figure S2. Effect of ovariectomy on the lipid metabolism of obese mice. (A) Lipid metabolism of hepatocytes in lean animals. DAG is esterified by the addition of a FFA to form TAG. In addition, free cholesterol is esterified to form CE. Insulin may promote the synthesis of TAG and CE, hence regulating the levels of toxic FFA and DAG in the hepatocytes to avoid lipotoxicity. TAG and CE are both incorporated into lipid droplets to form the neutral core, delimited by phosphatidylcholine and perilipins. By contrast, FFA, DAG nor free cholesterol can be incorporated into the lipid droplets. (B) Metabolic syndrome leads to insulin resistance, which appears to be ameliorated by the effect of estrogen. The excessive nutrient uptake induces the formation TAG and CE, which are incorporated into lipid droplets. The formation of lipid droplets contributes to lipid accumulation in the hepatocytes, observed as steatosis in liver tissue, but also to regulate the levels of cytotoxic FFA, DAG and free cholesterol. Increased $\text{TNF-}\alpha$ and $\text{IL-1}\beta$. (C) In obese-ovariectomized mice, the loss of estrogen as a product of the ovariectomy has been associated with higher insulin resistance. This condition undermines the formation of lipid droplets and total lipid accumulation. The impaired synthesis of TAG and CE is accompanied by higher levels of cytotoxic FFA, DAG and free cholesterol in the hepatocytes. This lipotoxic profile induced the overexpression of the pro-inflammatory $\text{NF-}\kappa\beta$ and the increase in serum transaminases indicating liver damage. DAG, diacylglycerol; FFA, free fatty acids; TAG, triacylglycerol; CE, cholesteryl esters; Chol, free cholesterol.

A Lean females



B Obese females



C Obese ovariectomized females

