Supporting Information

Galbo et al. 10.1073/pnas.1311176110

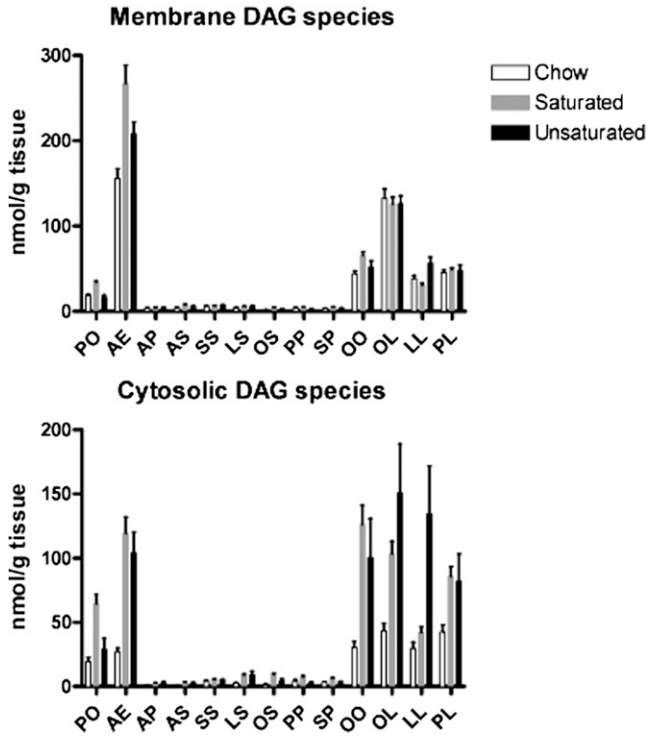

Fig. S1. Specific hepatic diacylglycerol (DAG) species in the membrane and cytosolic fractions following saturated or unsaturated fat feeding in rats. Related to Fig. 1. Hepatic DAG concentrations in the membrane and cytosolic fractions as assessed by liquid chromatography (LC)/MS/MS. n is 9–11 per group. A, arachidonic acid; E, eicosapentaenoic acid; L, linoleate; O, oleate; P, palmitate; S, stearate.

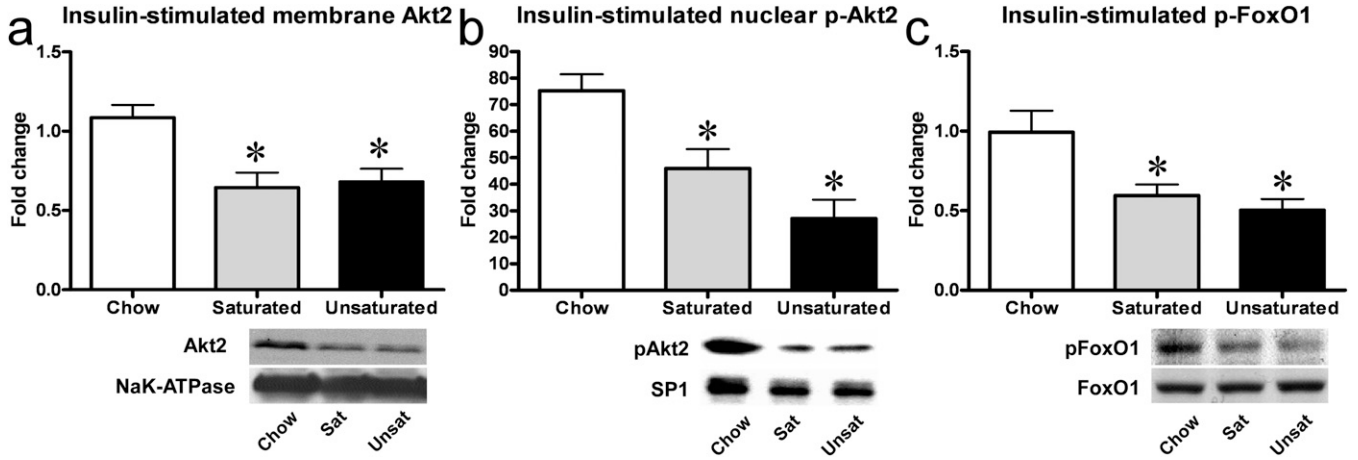

Fig. S2. Fat-feeding impairs translocation of Akt2 to the plasma membrane and nucleus, and phosphorylation of the nuclear substrate FoxO1 in rat livers. Related to Fig. 1. Feeding of either saturated or unsaturated fat resulted in a ~30–40% decrease in insulin-stimulated Akt2 recruitment to the plasma membrane. (*A*) A ~50–60% decrease in phosphorylated, nuclear Akt2 (*B*) and ~40–50% decrease in FoxO1 phosphorylation (*C*). $n = 5–6$ per group. * $P < 0.05$.

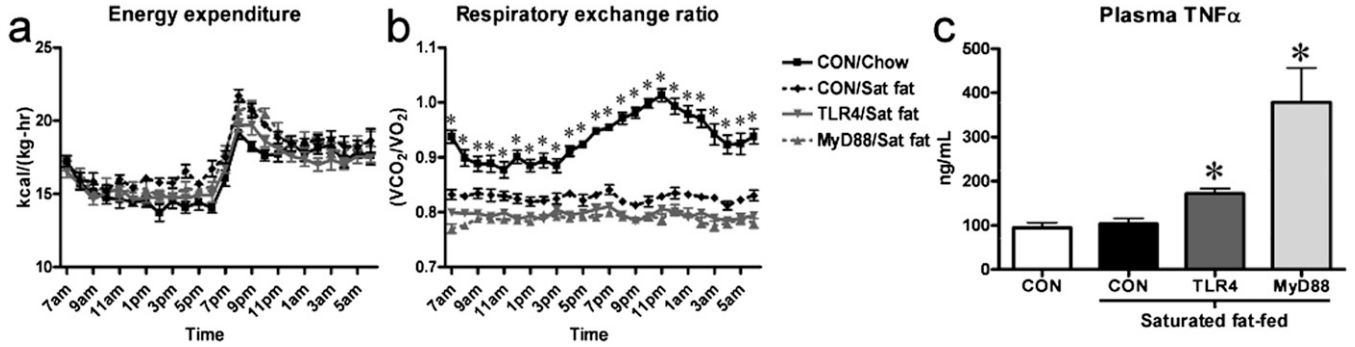

Fig. S3. Knockdown of TLR-4/MyD88 in mice did not affect energy expenditure or respiratory exchange ration, but was associated with increased levels of plasma TNF-α. Related to Fig. 2. Metabolic cage studies showed that antisense oligonucleotide knockdown of TLR-4 or MyD88 did not affect energy expenditure (*A*) or respiratory exchange ratio (*B*). Knockdown was, however, associated with increased levels of the anorexic cytokine TNF-α (*C*). $n = 5–6$ per group. * $P < 0.05$. Con, gavaged control.

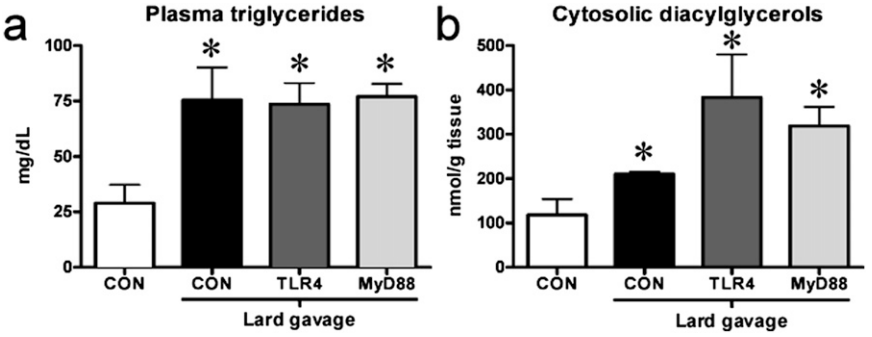

Fig. S4. Lard gavage in mice with knockdown of TLR-4/Myd88 results in increased plasma triglycerides and hepatic cytosolic diacylglycerols. Related to Fig. 2. All mice exposed to lipid gavage with lard displayed an approximately threefold increase in plasma triglycerides (*A*) and a twofold increase in hepatic cytosolic diacylglycerols (*B*). $n = 5–10$ per group. *$P < 0.05$.

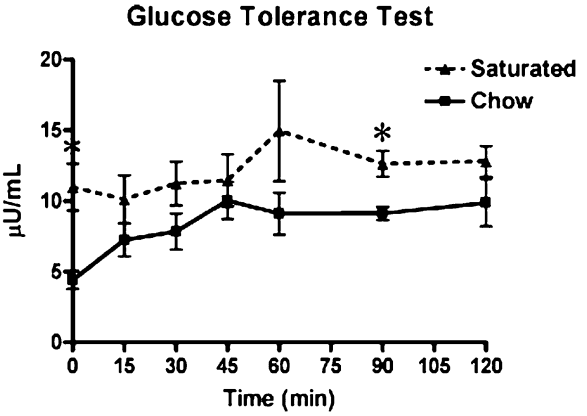

Fig. S5. Plasma insulin concentrations in TLR-4–deficient mice fed either saturated fat or chow during i.p. glucose tolerance tests (IPGTT). $n = 7–10$ per group. *$P < 0.05$.

Table S1. Hepatic mRNA expression of de novo ceramide synthesis enzymes is not increased by saturated or unsaturated fat-feeding in rats

	Chow	Saturated	Unsaturated
CerS1	1.023 ± 0.234	1.192 ± 0.309	0.844 ± 0.230
CerS2	1.037 ± 0.302	0.760 ± 0.205	0.614 ± 0.0581
Des1	1.020 ± 0.204	1.034 ± 0.0404	0.968 ± 0.210
Des2	1.100 ± 0.584	1.314 ± 0.796	0.476 ± 0.214
SPT1	1.005 ± 0.109	0.910 ± 0.154	0.884 ± 0.126

The basal hepatic expression of the key enzymes CerS1, CerS2, Des1, Des2, and serine palmitoyltransferase in the de novo ceramide synthesis pathway was not increased by fat feeding. n = 6–8/group. *$P < 0.05$.