

Supplementary Information

Materials and methods

Plasmid constructs

Mouse active SREBP-1c (*N*-terminal, p68) and SREBP-2 cDNAs were PCR amplified from mouse (C57BL/6) liver cDNA. SREBP-1c sense (5'- C GGA TTC GAC GAG CTG GCC TTC GGT GAG -3') and anti-sense (5'- G TCT AGA CTC CAT AGA CAC ATC TGT GCC TC -3') and SREBP-2 sense (5'-TTG AAT TCG ATG AGA GCA GCG AGC TGG G-3') and anti-sense (5'-TGC AAG CTT CCC CAC TGC AGC AAG GAA GTC-3') primers were used to amplify the DNA fragment. The fragment for SREBP-1c was digested with *Bam*HI and *Xba*I, and the fragment for SREBP-2 was digested with *Eco*RI and *Hind*III and then cloned into pCMX vector containing a Flag epitope for affinity tag. Luciferase reporter plasmids containing SREBP-1c response element from RALDH1 and RALDH2 promoter was synthesized *in vitro*. For RALDH2, sense (5'- CTA GCA TCT ATC ACC CCA CCT CTA GAT CTA TCA CCC CAC C-3') and anti-sense (5'- GAT CGG TGG GGT GAT AGA TCT AGA GGT GGG GTG ATA GAT G -3') and for RALDH1, sense 5'-CTA GTC TGC CCA TCT CTA GAA GTC TGC CCA TC-3' anti-sense 5'-GAT GGG CAG ACT TCT AGA GAT GGG CAG ACT AG-3' oligonucleotides were annealed, phosphorylated by T4 polynucleotide kinase and ligated into *Bam*HI / *Nhe*I digested

pGL3-promoter vector (Promega). 1.5 kb endogenous promoters of RALDH1 and RALDH2 were amplified by PCR from mouse genomic DNA using the primers for RALDH1 sense (5'-GTG CTA GCA TCT TGG GGT GCA TTG CCT GAG-3') anti-sense (5'-CGA GAT CTG CAT GCA CTA AGG GTC ATT TGC-3') and for RALDH2 sense (5'-GTG CTA GCG TTG CTC TAG GAC GAC GTA G-3') and anti-sense (5'-ATA GAT CTT CAC CTC GCC CGC CAT GG-3') and cloned into pGL3 vector at *NheI/BglII* sites by

Primers used for RT-PCR

The primers used for the amplification of genes were as follows: for SREBP-1c (Srebf1, Accession No: NM_011480): sense: 5'-GCA GCC CAG GTG CCA ACT C-3', 184 bp downstream from the ATG codon. anti-sense: 5'-CTG GGA GGC CAA GCT TTG-3', 623 bp downstream from ATG codon and expected to amplify a PCR product of 439 bp. For SREBP-2 (Srebf2, Accession No: NM_033218): sense: 5'-ATG GAT GAG AGC AGC GAG CTG G-3' and anti-sense 5'-GCT GCA GCT GAG CTG GAG GTT G-3') to amplify 406 bp PCR product. SCAP (Scap, Accession No: NM_001001144): sense 5'-GAC CCT GAC TGA AAG GCT TGC T-3' and anti-sense 5'-CTG AGC TGT CTC TCA GCA CAT-3' to amplify 413 bp PCR fragment. RALDH1 (Aldh1a1, Accession No: NM_013467): sense 5'- ATG GCA CAA TTC

AGT GAG CGG C-3', 128 bp down-stream from the transcription initiation site (80 bp from ATG codon) and anti-sense: 5'-ACA ACC ACG GTA TTC CCA CAG C-3', 619bp down from the transcription initiation site (571 bp from ATG codon) and expected to yield a PCR product of 491 bp. RALDH2 (Aldh1a2, Accession No: NM_009022): Sense: 5'-ATG ACC ATT CCT GTA GAT GGA G-3', 476bp down-stream from the transcription initiation site and anti-sense, 5'-TGC GCC TCT TGG CCC TTT CCA C-3', 1041bp down-stream from the transcription initiation codon and which amplified a PCR product of 565 bp. LXR α (Nr1h3, Accession No: NM_013839): sense 5'-GTC CTT GTG GCT GGA GGC CTC A-3' and anti-sense 5'-CCT CGC AGC TCA GCA CGT TGT A-3') to amplify 340 bp PCR product. LXR β (Nr1h2, Accession No: NM_009473): sense 5'-CTC TGG ACA CTC CCG TGC CTG G-3' and anti-sense: 5'-GCA GCC GGC AGA GCT GGC ACT T-3' to amplify 393 bp PCR product.

Primers used for in vivo ChIP assay

For amplification of SRE site on RALDH2 promoter two sets of primer were used.

Forward: 5'-GCT GGA AGG AGA CCA TCA AGA C-3', starting from -624 bp, and reversed: 5'-GAG CCT TAG CGC TGG TGG GCC G-3' starting from -267 bp and expected to amplify 357 bp PCR product covering SRE site (TATCACCCAC)

between -442 bp and -431 bp. In the second set, forward: 5'-CAG CAG TTG GGC TGA GAA GCC-3' at -526 bp and anti-sense at -382 bp 5'-GAG CTT TCG CTC AGC CTT CCG-3' to amplify a PCR fragment of 144 bp. For SRE on RALDH1 promoter, forward primer: 5'-CTG GCT TCC AGT GTC TGG AGC A-3' at -285 bp, and reversed: 5'-GCA TGC ACT AAG GGT CAT TTG-3' at -32 bp were used to amplify a PCR fragment of 253 bp covering the two cluster of SRE sites TCATGCCCT (A) (and TCTGCCCAT (A) located between -98 bp and -90 bp and -81 bp and -73 bp upstream of transcription start site.

Affinity capture of RAR ligands and HPLC analysis

His-tagged RAR-LBD (250 µg) purified protein from bacteria was incubated with 25 µL of liver isopropanol extract (500 µg) for 6 hour at 4°C in 2.5 mL of 50 mM HEPES (pH 8.0) binding buffer containing 150 mM NaCl, 10% glycerol and 0.1% NP-40. The receptor bound ligand was extracted by 100% isopropanol and run over HPLC. Then the receptor was affinity bound to the Ni-agarose beads (Qiagen). The unbound ligands were washed three times with the same binding buffer, and the bound ligand was eluted from the receptor. In controls, the beads plus extract without receptors, and the receptors bound to beads without the extract were set and processed in parallel to the experimental sample. The eluted extracts were run by HPLC

(Beckman) over C18 (4.6 mm id) column under UV detection at 254 nm with a gradient flow of solvent B (100% acetonitrile plus 0.1% TFA) for 45 minutes from 0% (100% solvent A: 5% acetonitrile in H₂O plus 0.1% TFA) to 100% for 30 minutes and 100% solvent B for 15 minute.

Hydrodynamic based siRNA delivery and knock-down of SREBP-1c

To knock-down SREBP-1c in mouse liver, the siRNA sequence (5'-GCAAGGCCAUCGACUACAU-3') targeting N-terminus established by screening of individual siGENOME duplex was placed into the synthetic oligonucleotide, sense: 5'-TCG ACG CAA GGC CAT CGA CTA CAT GGA ATT CCA TGT AGT AGT CGA TGG CCT TGC GTT TTT-3' and anti-sense: 5'-CTA GAA AAA CGC AAG GCC ATC GAC TAC ATG GAA TTC CAT GTA GTC GAT GGC CTT GCG-3', the oligonucleotides were self annealed and cloned into IMX-800 vector (IMGENEX) containing U6 promoter to generate shRNA. The plasmids was purified from *E. coli* using endotoxin free plasmid isolation kits (Qiagen) and dissolved in 0.9% normal saline. The plasmid was delivered once to the mice (100 µg/mice/1.6 mL in 5 seconds) by hydrodynamics-based delivery following the established protocols (Kobayashi *et al*, 2004, Liu *et al*, 1999). The mice were sacrificed after 3 days to collect liver tissue for extraction of RNA and protein.

Supplementary Figure legends

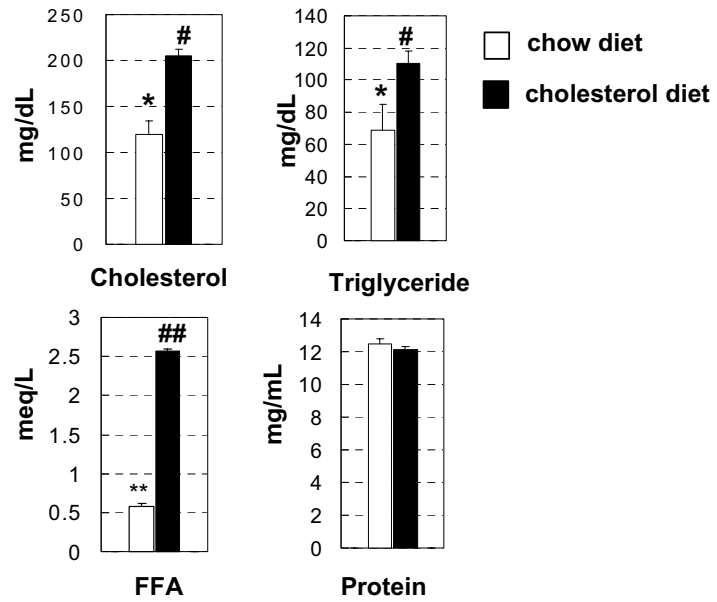
Figure S1 Effects of cholesterol and cholesterol-lowering agent on blood profiles. **(A)**

Cholesterol feed in mice (n=5) an average of 20 mg/day/mice for two weeks elevated total plasma cholesterol and serum triglyceride level by 2-fold (# vs *, $P < 0.01$, n=5, Student's t-test) and free fatty acid by 5-fold (### vs **, $P < 0.005$, n=5, Student's t-test) compared to control mice on standard chow diet. No change in plasma protein level due to cholesterol feeding. **(B)** Intra-peritoneal administration of HMG-CoA reductase inhibitor (pravastatin sodium, a cholesterol-lowering agent) in mice (n=5) at a dose of 15 mg/kg body-weight once daily for two weeks reduced plasma total cholesterol and serum triglyceride level by 2-fold (# vs *, $P < 0.01$, Student's t-test) compared to the control mice. No significant change was shown in the circulatory free fatty acids level.

Figure S2 Activation of SRE on RALDH1 by SREBP-1c. **(A)** EMSA assay on SRE

site on RALDH1 promoter. SREBP-1c could bind to SRE on RALDH1. **(B)** Reporter assay containing the SRE site on RALDH1 promoter. SREBP-1c dose-dependently activated SRE reporter in COS-1 cells, but relatively weaker than SRE site on RALDH2 promoter. * vs control bars 1 and 3 ($P < 0.005$), # not significantly different from control bar 3 ($P < 0.05$).

A Blood profile for cholesterol rich diet



B Blood profile for cholesterol lowering agent (pravastatin)

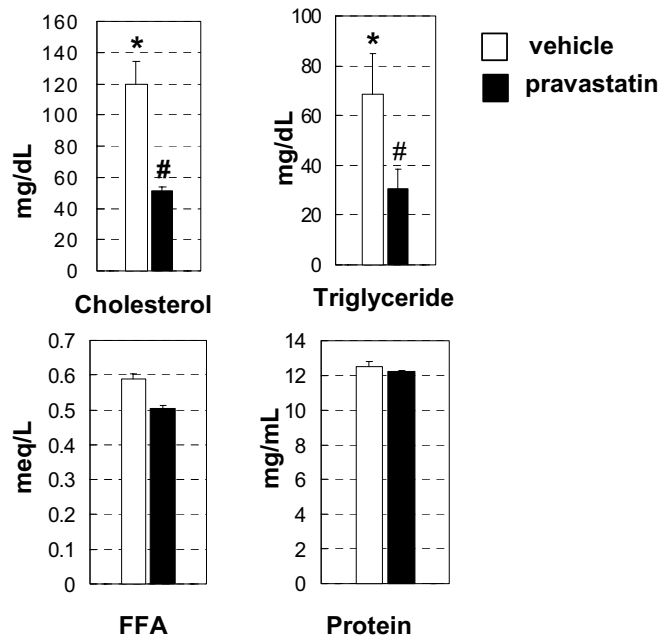
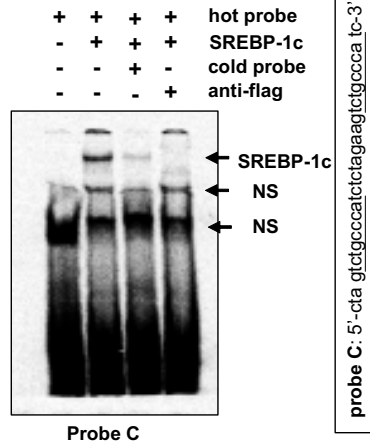


Figure S1 (Huq et al.)

A EMSA on RALDH1 promoter



B SRE on RALDH1 activation by SREBP-1c

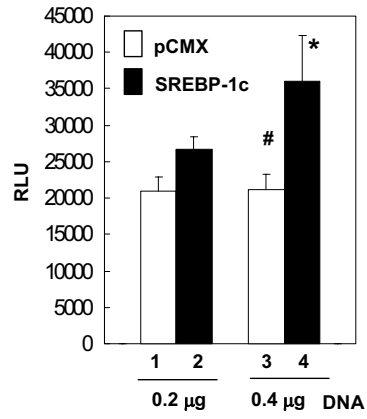


Figure S2 (Huq et al.)