**SUPPLEMENTAL DATA**

# HEPATOCYTE-SPECIFIC IKK-β ACTIVATION ENHANCES VLDL-TRIGLYCERIDE PRODUCTION IN APOE*3-LEIDEN MICE

Janna A. van Diepen[1], Man-Chi Wong[2], Bruno Guigas[3], Jasper Bos[1], Rinke Stienstra[5], Leanne Hodson[6], Steven E. Shoelson[7], Jimmy F.P. Berbée[1], Patrick C.N. Rensen[1], Johannes A. Romijn[1], Louis M. Havekes[1,4,8], Peter J. Voshol[1,*]

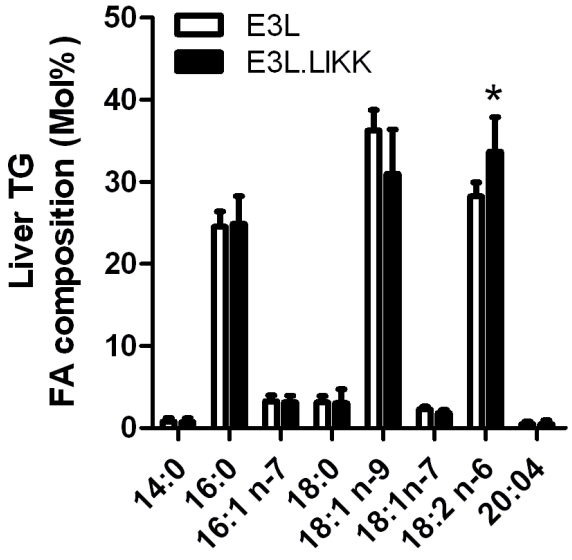
Depts. of [1]General Internal Medicine, Endocrinology and Metabolic Diseases, [2]Pulmonology, [3]Molecular Cell Biology and [4]Cardiology, Leiden University Medical Center, Leiden, The Netherlands; [5]Dept. of General Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands; [6]Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford, UK; [7]Joslin Diabetes Center and the Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA; [8]Netherlands Organization for Applied Scientific Research – Biosciences, Gaubius Laboratory, Leiden, The Netherlands. *Present address: Metabolic Research Laboratories, Institute of Metabolic Science, University of Cambridge, Cambridge, UK.

| Gene | Forward primer | Reverse primer |
|---|---|---|
| *Abcg5* | TGTCCTACAGCGTCAGCAACC | GGCCACTCTCGATGTACAAGG |
| *Abcg8* | GACAGTTCACAGCCCACAA | GCCTGAAGATGTCAGAGCGA |
| *Acox1* | TATGGGATCAGCCAGAAAGG | ACAGAGCCAAGGGTCACATC |
| *Apob* | GCCCATTGTGGACAAGTTGATC | CCAGGACTTGGAGGTCTTGGA |
| *Cidea* | CTCGGCTGTCTCAATGTCAA | CCGCATAGACCAGGAACTGT |
| *Cidec* | CTGGAGGAAGATGGCACAAT | GGGCCACATCGATCTTCTTA |
| *Cpt1a* | GAGACTTCCAACGCATGACA | ATGGGTTGGGGTGATGTAGA |
| *Cyclo* | CAAATGCTGGACCAAACACAA | GCCATCCAGCCATTCAGTCT |
| *Cyp27a1* | TCTGGCTACCTGCACTTCCT | CTGGATCTCTGGGCTCTTTG |
| *Cyp7a1* | CAGGGAGATGCTCTGTGTTCA | AGGCATACATCCCTTCCGTGA |
| *Cyp8b1* | GGACAGCCTATCCTTGGTGA | CGGAACTTCCTGAACAGCTC |
| *Dgat1* | TCCGTCCAGGGTGGTAGTG | TGAACAAAGAATCTTGCAGACGA |
| *Fasn* | TCCTGGGAGGAATGTAAACAGC | CACAAATTCATTCACTGCAGCC |
| *Gapdh* | TGCACCACCAACTGCTTAGC | GGCATGGACTGTGGTCATGAG |
| *Hmgcr* | CCGGCAACAACAAGATCTGTG | ATGTACAGGATGGCGATGCA |
| *Mttp* | CTCTTGGCAGTGCTTTTTCTCT | GAGCTTGTATAGCCGCTCATT |
| *Nr1h3* | CTGCACGCCTACGTCTCCAT | AAGTACGGAGGCTCACCAGCT |
| *Nr1h4* | GGCCTCTGGGTACCACTACA | ACATCCCCATCTCTTTGCAC |
| *Pklr* | GCAGAACGAGTCACAGCAAT | GTGGAGGCTTCCTTCAAGTG |
| *Plin2* | CAGGATGGAGGAAAGACTGC | CTTATCCACCACCCCTGAGA |
| *Plin5* | TGTCCAGTGCTTACAACTCGG | CAGGGCACAGGTAGTCACAC |
| *Ppara* | ATGCCAGTACTGCCGTTTTC | GGCCTTGACCTTGTTCATGT |
| *Ppargc1b* | TTGTAGAGTGCCAGGTGCTG | CCTCCATAGCTCAGGTGGAA |
| *Srebp-1c* | GGAGCCATGGATTGCACATT | CCTGTCTCACCCCCAGCATA |

**Supplemental Table S1.** Primers used for quantatitive real-time PCR analysis.

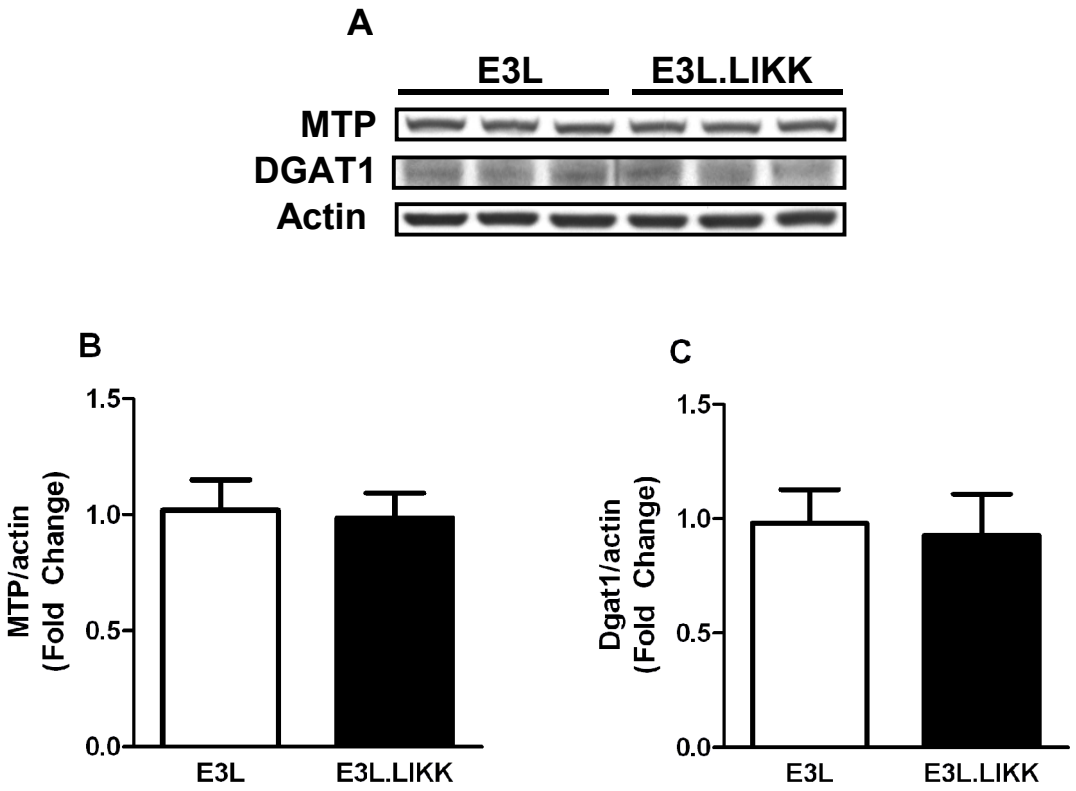
*Abcg5*, ATP-binding cassette sub-family G member 5 ; *Abcg8*, ATP-binding cassette sub-family G member 8; *Acox1*, acyl-Coenzyme A oxidase 1; *Apob*, apolipoprotein B; *Cidea*, cell death activator CIDE-A; *Cidec*, fat-specific protein FSP27; *Cpt1a*, carnitine palmitoyltransferase 1a; *Cyp27a1*, cholesterol 27 hydroxylase; *Cyp7a1*, cholesterol 7 alpha hydroxylase; *Cyp8b1*, sterol 12 alpha-hydrolase; *Dgat1*, diglyceride acyltransferase 1; *Fasn*, fatty acid synthase; *Hmgcr*, HMG-CoA reductase; *Mttp*, microsomal triglyceride transfer protein; Nr1h3, liver X receptor alpha; *Nr1h4*, farnesoid X activated receptor; *Pklr,* liver-type pyruvate kinase*; Plin2*, perilipin 2;

*Plin5*, perilipin 5; *Ppara*, peroxisome proliferator activated receptor alpha; *Ppargc1b*, PPAR-gamma coactivator 1-beta; *Srebp-1c*, sterol-regulatory element binding protein.




**Supplemental Figure S1.** Effect of LIKK on fatty acid composition of hepatic triglycerides in E3L mice.

Livers were obtained from overnight fasted E3L and E3L.LIKK mice and lipids were extracted. TG were isolated by thin-layer chromatography followed by fatty acid separation and quantification using gas chromatography. Fatty acid composition was then determined (in Mol%). Values are means ± SD (n=5-7).




**Supplemental Figure S2.** LIKK does not affect hepatic MTP and DGAT1 protein levels.
E3L and E3L.LIKK mice were fed a chow diet and sacrificed at the age of 14 weeks after an overnight fast. MTP and DGAT1 levels were measured in liver tissue by Western blots and Actin was used as an internal control. Representative Western blots are shown for 3 mice per group (A). Ratios of MTP (B) and DGAT1 (C) proteins over Actin levels were quantified. Values are means ± SD (n=5-7).