Supporting Information

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SI Experimental Procedures

Plasma and Tissue Metabolites. Plasma metabolites and liver and muscle glycogen were determined as previously described (1). Skeletal muscle total lipids were extracted using the method described by Folch (2), and triglyceride levels were measured using colorimetric assay kit (INstruchemie).

Lipoprotein Lipase Activity. Oleic acid (400 μM) was added to cells complexed with BSA. After 24 h, the medium was aspirated, and the cells were washed twice with PBS. Lipoprotein lipase (LPL) was released from the myotubes by addition of 0.5 mL PBS containing 100 IU/mL heparin (LEO) per well. Heparin-releasable LPL was collected after incubation for 5 min. LPL activity was measured with an LPL activity assay kit (Roar Biomedical) on a Fluoroskan Ascent FL Microplate Fluorometer (Thermo Fisher Scientific). LPL activity in homogenates of mouse skeletal muscle was measured as previously described (3).

Western Blot. Protein lysates (20–30 μg protein per lane) were loaded on a denaturing gel and separated by SDS gel electrophoresis. Protein was transferred to a PDVF membrane. The primary antibody [rabbit anti–phospho-AMPKα antibody (Thr172) (40H9), rabbit anti-AMPKα antibody (Cell Signaling), or rat anti-mouse Angptl4 antibody (Kairos 142–2), AG-20A-0054-C100; Adipogen] was used at a ratio of 1:1,000 or 1:2,000 [phospho–AMP-activated protein kinase (AMPK)]. The secondary antibody (HRP-labeled goat anti-rabbit or anti-rat IgG; Sigma) was used at a ratio of 1:5,000. All incubations were performed in Tris-buffered saline, pH 7.5, with 0.1% Tween-20 and 5% dry milk, except for the final washings when dry milk was omitted.

Angiopoietin-Like 4 ELISA. Angiopoietin-like 4 (ANGPTL4) levels in cell-culture medium and in muscle homogenates were measured by ELISA as detailed previously (4). Briefly, 96-well plates were coated with anti-human ANGPTL4 polyclonal goat IgG antibody (AF3485; R&D Systems) and were incubated overnight at 4 °C. Plates were washed extensively between each step. After blocking, 100 μL of the cell medium was applied, followed by 2-h incubation at room temperature. A standard curve was prepared using recombinant human ANGPTL4 (3485-AN; R&D Systems) at 0.3–2.1 ng per well. Next, 100 μL of diluted biotinylated anti-human ANGPTL4 polyclonal goat IgG antibody (BAF3485; R&D Systems) was added for 2 h, followed by the addition of streptavidin-conjugated HRP for 20 min and tetramethylbenzidine substrate reagent for 6 min. The reaction was stopped by the addition of 50 μL of 10% H2SO4, and the absorbance was measured at 450 nm.

Microarray Processing. Total RNA (100 ng) was labeled using an Ambion WT expression kit (Life Technologies) and hybridized to human whole-genome Genechip Human Gene 1.1 ST arrays coding 19,732 genes (Affymetrix).

Microarray analysis was performed using the MADMAX pipeline for statistical analysis of microarray data (5). Quality control was performed, and all arrays met our criteria. For further analysis a custom annotation was used (6), and expression values were calculated using the robust multichip average method (7). Microarray data were filtered, and probe sets with expression values >20, located at more than five arrays, and with an interquartile range value >0.2 were stated to be expressed and were selected for further statistical analysis. Significant differences in expression were assessed using intensity-based moderated *t* statistic (8). Genes were defined as significantly changed when the *P* value was <0.01. The microarray dataset has been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database, accession number GSE41769.

Microarray analysis of human myotubes from three subjects treated with oleic acid has been described previously (9). Microarray analysis of C2C12 myotubes treated with oleic acid also has been described previously (10). The microarray datasets have been deposited in the NCBI GEO database, accession numbers GSE18589 and GSE38590.

1. Sanderson LM, Boekschoten MV, Desvergne B, Müller M, Kersten S (2010) Transcriptional profiling reveals divergent roles of PPARalpha and PPARbeta/delta in regulation of gene expression in mouse liver. *Physiol Genomics* 41(1):42–52.
2. Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226(1):497–509.
3. Ruge T, Wu G, Olivecrona T, Olivecrona G (2004) Nutritional regulation of lipoprotein lipase in mice. *Int J Biochem Cell Biol* 36(2):320–329.
4. Kersten S, et al. (2009) Caloric restriction and exercise increase plasma ANGPTL4 levels in humans via elevated free fatty acids. *Arterioscler Thromb Vasc Biol* 29(6): 969–974.
5. Lin K, et al. (2011) MADMAX - Management and analysis database for multiple ~omics experiments. *J Integr Bioinform* 8(2):160.
6. Dai M, et al. (2005) Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. *Nucleic Acids Res* 33(20):e175.
7. Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* 19(2):185–193.
8. Sartor MA, et al. (2006) Intensity-based hierarchical Bayes method improves testing for differentially expressed genes in microarray experiments. *BMC Bioinformatics* 7:538.
9. Hessvik NP, et al. (2010) Metabolic switching of human myotubes is improved by n-3 fatty acids. *J Lipid Res* 51(8):2090–2104.
10. Bosma M, et al. (2012) Perilipin 2 improves insulin sensitivity in skeletal muscle despite elevated intramuscular lipid levels. *Diabetes* 61(11):2679–2690.

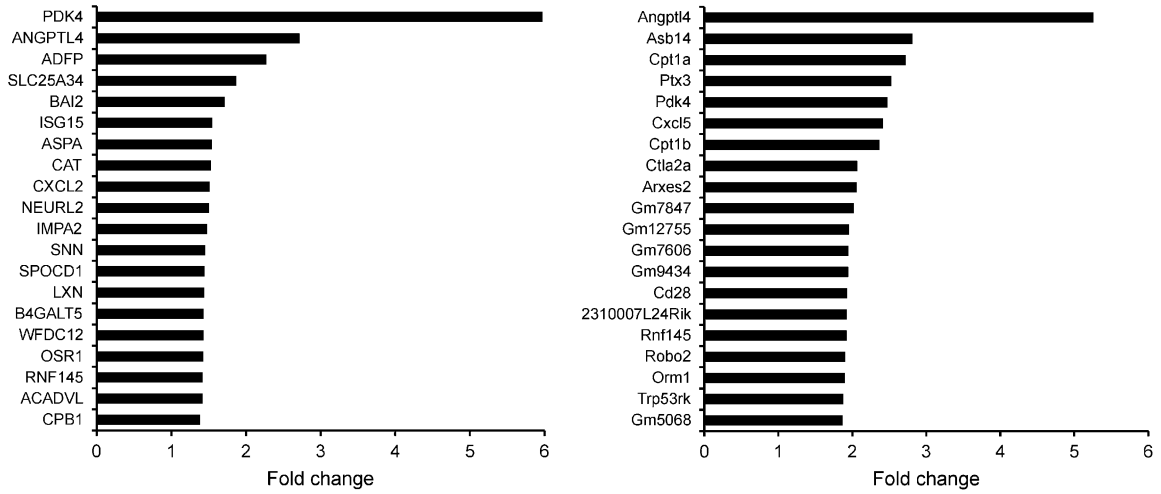

Fig. S1. The top 20 genes most highly induced by oleic acid in primary human myotubes (*Left*) or mouse C2C12 myotubes (*Right*) ranked according to fold-change, as determined by Affymetrix microarray.

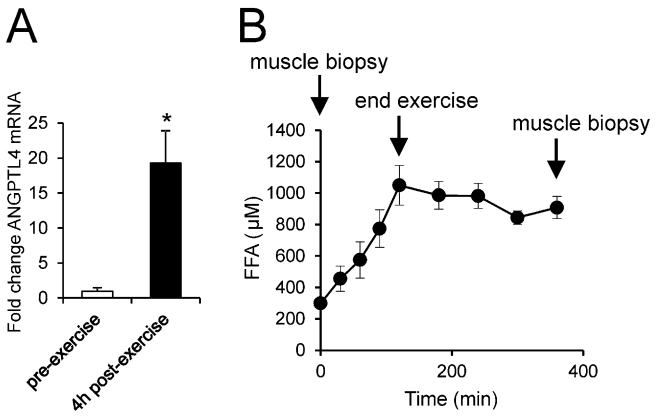

Fig. S2. (*A*) *ANGPTL4* mRNA in human vastus lateralis muscle collected before a 2-h cycling exercise bout and after 4 h of postexercise recovery, all in the fasted state. (*B*) Plasma free fatty acid (FFA) concentrations during the entire exercise trial (1). Error bars represent SEM.

1. Schrauwen P, et al. (2002) Effect of acute exercise on uncoupling protein 3 is a fat metabolism-mediated effect. *Am J Physiol Endocrinol Metab* 282(1):E11–E17.

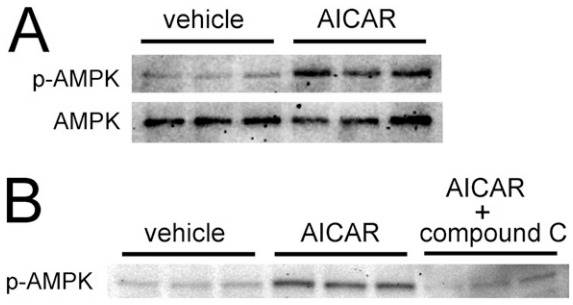

Fig. S3. (*A*) Immunoblot for AMPK and phospho-AMPK in C2C12 myotubes treated with 5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide (AICAR) (1 mM) for 12 h. (*B*) Immunoblot for phospho-AMPK in C2C12 myotubes treated with AICAR (0.5 mM) and compound C (20 μM) for 12 h.

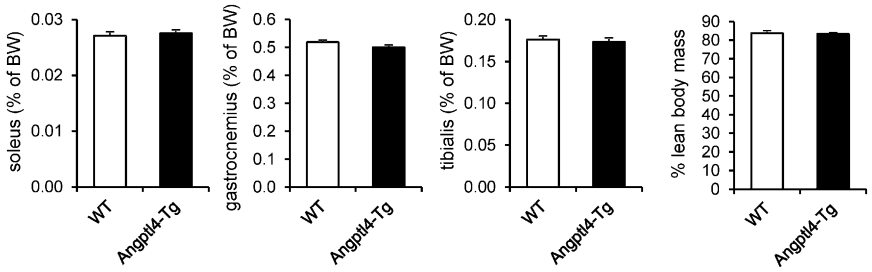

Fig. S4. Weight of soleus, gastrocnemius, tibialis anterior, and lean body mass shown as percent body weight (BW) in WT and *Angptl4*-transgenic (*Angptl4*-Tg) mice.

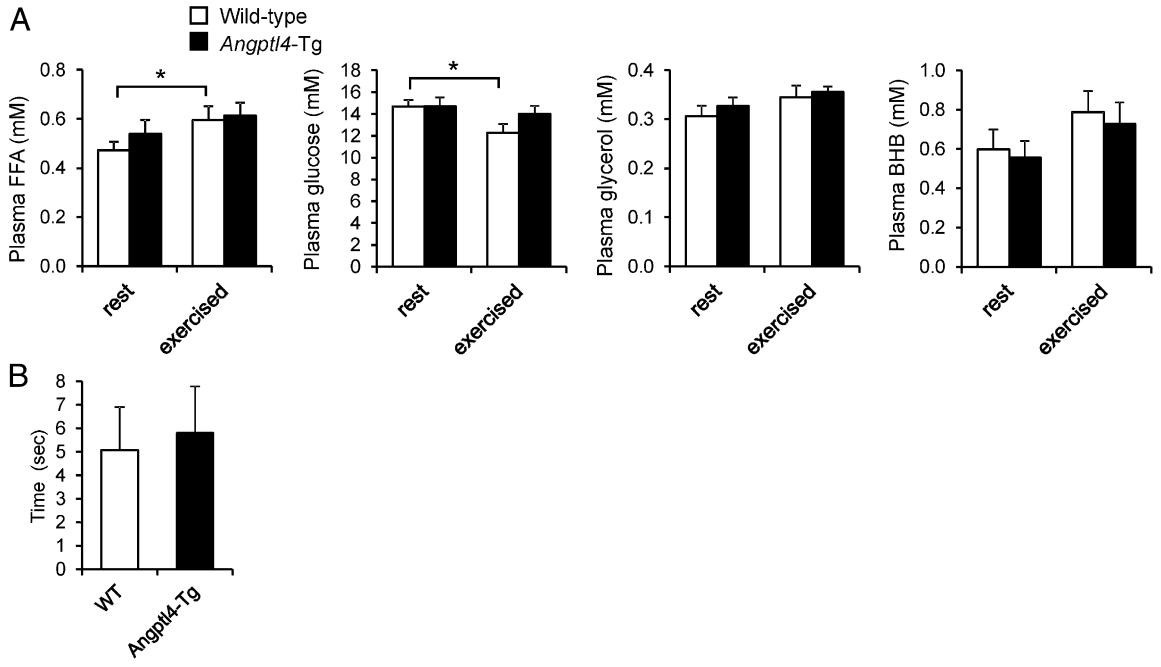

Fig. S5. (*A*) Plasma metabolic parameters in WT and *Angptl4*-Tg mice at rest or after 90 min of moderate running exercise (12 m/min). (*B*) Horizontal wire test: average time until mice grasped wire with both hind legs. *Significantly different according to Student *t* test ($P < 0.05$). Error bars represent SEM.