Supporting Information

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SI Materials and Methods

Human Subjects. Healthy, lean young men (body mass index, BMI = 21.8 ± 0.4 kg/m^2; age = 26.7 ± 2.8 y, n = 10) participated in the study. The medical ethics committee of Maastricht University approved the study, and subjects gave their written informed consent. Each subject underwent two treatments in randomized order, as described previously (1). In short, every treatment lasted for 5 d. To create a situation in which fat oxidative capacity was maximally used, subjects were provided with high-fat diets for consumption at home during 3 consecutive days. On the evening of the third day subjects entered the respiration chamber for a 36-h stay (2000 h–0800 h) to allow the continuous determination of fatty acid (FA) oxidation. During the stay in the respiration chamber, subjects again consumed a high-fat diet and they were given oral dosages of either etomoxir (day 3, 75 mg in the evening; day 4, 150 mg in the morning and afternoon and 75 mg in the evening; and day 5, 150 mg in the morning) or placebo (same time schedule with the same number of capsules, but not containing any drug) under supervision in randomized order. These multiple doses resulted in a total etomoxir dose of 600 mg over the 36 h in the respiration chamber. A fasting blood sample was taken on the morning of day 5. On day 5 at 0800 h, subjects left the respiration chamber and a muscle biopsy was taken. Thereafter subjects engaged in a 2-h cycling protocol at 50% of their predetermined maximal workload, and blood was collected before exercise and at time points 90, 100, 110, and 120 min during exercise.

Western Blotting. Tibialis anterior muscle samples of mice were homogenized as described previously (2) and muscle lysates of the mice and human primary myotubes were processed for standard SDS/PAGE and Western blotting. Protein concentration was assessed, and equal amounts of protein were loaded per lane. Actin was used as a loading control. Membranes were incubated with antibodies against perilipin 5 (PLIN5) (GP31; Progen), perilipin 2 (PLIN2) (GP40; Progen), oxidative phosphorylation (OXPHOS) (MS601; MitoSciences), Akt and pAkt (Ser473) (9271 and 9272, respectively; Cell Signaling Technology, Bioké), and AMP-activated protein kinase (AMPK) and phosphorylated (p)AMPK (Thr172) (2532 and 2531, respectively; Cell Signaling).

Blots were incubated with the appropriate secondary antibodies and bands at a molecular weight corresponding to the control samples were quantified using the Odyssey infrared imaging system (Licor Biosciences).

For pIRS1, PKCθ, and GLUT4 detection equal amounts of muscle membrane protein fractions were loaded on SDS/PAGE. Caveolin-3 was used as a loading control. After Western blotting, membranes were incubated with antibodies against pIRS1 (Ser307) (2381S; Cell Signaling), PKCθ (2059; Cell Signaling), and GLUT4 (sc-1608; Santa Cruz, Bio-Connect). After incubation with the appropriate secondary antibody, the specific IRS1 and GLUT4 proteins were detected and analyzed using the Odyssey near-infrared scanner (Licor).

1. Schrauwen P, et al. (2002) Etomoxir-induced increase in UCP3 supports a role of uncoupling protein 3 as a mitochondrial fatty acid anion exporter. *FASEB J* 16:1688–1690.

2. Schrauwen P, et al. (2003) Uncoupling protein 3 as a mitochondrial fatty acid anion exporter. *FASEB J* 17:2272–2274.

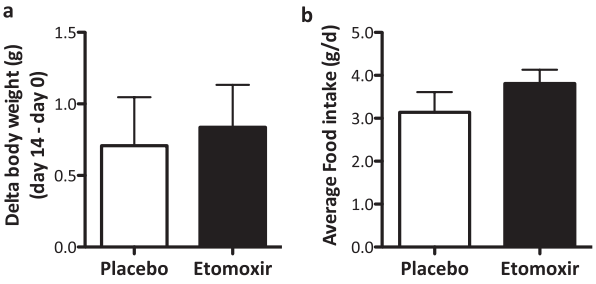

Fig. S1. Body weight gain and food intake were not affected by etomoxir treatment in C57BL6 mice. (*A*) Delta body weight [day (d) 14 – d0] and (*B*) average food intake. Data are expressed as mean + SEM (placebo n = 16, etomoxir n = 15).

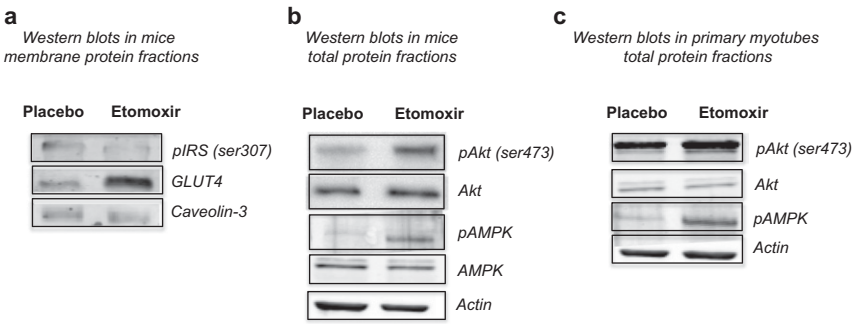

Fig. S2. Representative Western blots of the quantified bars included in Fig. 4. (*A*) Representative Western blots of pIRS (Ser307), GLUT4, and Caveolin-3 (loading control) in membrane fractions of tibialis anterior muscle of etomoxir- vs. placebo-treated mice. (*B*) Representative Western blots of pAkt (Ser473), Akt, pAMPK, AMPK, and actin (loading control) in total protein fractions of tibialis anterior muscle of etomoxir- vs. placebo-treated mice. (*C*) Representative Western blots of pAkt (Ser473), Akt, pAMPK, and actin (loading control) in total protein fractions of primary myotubes treated with etomoxir vs. placebo.