Supplemental data

Palmitate enrichment & concentration. To 200 µl of plasma, 100 µl of the internal standard heptadecanoic acid (110 mg/dl) was added and mixed. Isolation of the plasma fatty acids for the determination of palmitate concentration and isotopic enrichment was performed according to Patterson et al (1). The methylated plasma palmitate enrichment and concentration were measured on a gas chromatograph-combustion isotope ratio mass spectrometry equipped with an FID detector (GC-C-IRMS; Hewlett Packard 5890 Finnigan GC combustion III, Finnigan XL, Finnigan MAT, Bremen, Germany). The palmitate was determined by injecting 2 µl onto a 30 m capillary fused-silica column (Rtx-2330, Restex, USA), via an HP-PTV injector. The injectors vent-flow was set at 5 p.s.i., and the initial temperature was 85 °C for 1 min than with a ramp of 500 °C/min to 255 °C. After the GC-separation of fatty acids, the flow was split to the FID detector for palmitate concentration determination against a standard calibration curve, and to the combustion interface for subsequent enrichment determination on the sector magnet of the isotope ratio mass-spectrometer. The isotopic enrichment of palmitate was expressed as the $\delta$ per mil difference between $^{13}$C/$^{12}$C of the sample and a known laboratory reference standard related to PDB. The methyl derivative of palmitate contains 17 carbons of which 16 are from palmitate, thus TTR of palmitate was corrected by a factor 17/16.

Glucose & glycerol enrichment and concentration. Glucose and glycerol enrichment and concentrations were analysed simultaneously via turbulent flow chromatography (Cohesive technologies RTX1, Franklin, MA, USA) combined with the high selectivity and sensitivity of tandem mass spectrometry (Sciex API3000, Applied Biosystems, Foster City, USA). Fifty µl of plasma was accurately mixed with 50 µl internal standard solution (0.500 mM $[^{13}$C$_6$]glucose and 0.0300 mM $[^{13}$C$_3$, D$_8$]glycerol) and vortex mixed with 100 µl ethylacetate for 5 min. After
centrifugation the organic layer was discarded, and to the water layer 20 µL 1 M K₂HPO₄, 20 µL 1 M NaOH, 10 µL Benzoyl Chloride was added and mixed thoroughly. The pH was brought to ±7 with approximately 10 µL 1.4 M H₃PO₄ and extracted with 500 µL ethylacetate and vortexed for 2 – 3 min. After centrifugation 400 µl of the ethylacetate layer was transferred to a new tube and 100 µL 1 M NaOH was added. After mixing for 2 min 100 µl of the ethylacetate phase was transferred to a new Eppendorf tube. This extraction procedure was repeated and the 100 µl ethylacetate was combined with the first one after which it dried under a stream of nitrogen. The precipitate was re-dissolved in 500 µL injection buffer (10 mM NH₄AC in 75:25 acetonitrile/water) and vortexed for 1 min. The liquid was transferred to a 0.45 µm centrifugal filter (PALL life technologies, nanosep MF GHP 0.45 µm centrifugal filters, Mexico), and centrifuged for 1 min at max speed in an eppendorf centrifuge. The turbulent-flow extraction column, (Cohesive C₁₈, 50 mm × 0.5 mm, 50 µm), was used to isolate analytes from the treated human plasma with a 10 µL injection volume. The chromatographic separation was achieved on an analytical column at room temperature (Waters acquity, UPLC HSS C₁₈ 1.8 µm x 2.1 x 150 mm) with a stepwise gradient of mobile phase A (10 mM NH₄AC in water) and B (10 mM NH₄AC in 95:5 acetonitrile/water). In case of glucose, the ion pairs (precursor ion → product ion) m/z 781 → 231, m/z 720 → 233 and m/z 724 → 237 were selected for multiple reaction monitoring (MRM) of the tracee, tracer and internal standard, respectively. In case of glycerol, the ion pairs (precursor ion → product ion) m/z 422 → 283, m/z 427 → 288 and m/z 430 → 291 were selected for multiple reaction monitoring (MRM) of the tracee, tracer and internal standard, respectively.

**Breath CO₂ enrichment.** Ten ml of expired air was collected in a vacutainer. The ¹³C/¹²C ratio was determined by split injection (ratio 1:4) of 20 µl of the expired air onto a poraplot Q column (Chrompack, The Netherlands), injector and column at 30 °C. The ¹³C- to ¹²C-ratio was determined
by split injection (ratio 1:50) of 10 µl of the headspace on the GC-IRMS (Hewlett Packard 5890 - Finnigan GC combustion III, Finnigan XL, Finnigan MAT, Bremen, Germany).

The isotopic enrichment of breath CO₂ was expressed as the δ per mil difference between ¹³C/¹²C of the sample and a known laboratory reference standard related to PDB.

**Analytical methods.** Blood was drawn into pre-chilled tubes containing EDTA and immediately spun at 3500 g for 10 minutes at 4°C before they were frozen at –80°C until analyses.

Blood from all three catheters was collected into syringes with heparin (80 IU) (PICO50, Radiometer, Copenhagen, Denmark) and analysed immediately for glucose (ABL 615, Radiometer, Copenhagen, Denmark). Adrenaline and glucagon were measured in duplicate using commercially available RIA kits (Labor Diagnostika Nord, Nordhorn, Germany, and Linco Research, MI, USA, respectively). leptin was measured in duplicate using commercially available ELISA kits (R&D systems, MD, USA). Insulin and cortisol were measured in duplicate using commercially available ELISA kits (DakoCytomation, Cambridgeshire, UK and R&D systems, MD, USA, respectively).

T3 and T4 were measured using automated routine methods at the Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark.

**Western Blotting.** Human muscle samples were homogenized in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 50 mM NaF, 5 mM NaP, pH 7.4,) using a Tissuelyser (Qiagen, CA, USA). Phosphatase inhibitor cocktail 1 and 2 (Sigma Aldrich, MO, USA), 3 mM NaV, 1 mM DTT, 0.2% lpegal-CA-630 (Sigma Aldrich, MO, USA) and protease inhibitor complete mini (Roche, Mannheim, Germany) was added to the buffer immediately before homogenization.

Following homogenization, protein lysates were centrifuged at 13000 g for 15 min at 4°C and the pellet was discarded. Protein concentration was measured using a colorimetric protein assay (Bio-Rad, Copenhagen, Denmark). Samples were mixed with 5 x Laemmli buffer and boiled for 5 min before subsequent loading of 15 µg onto a 4%-12% gradient bis-Tris NuPage gel (Invitrogen, CA,
USA). The gel was run for 1h and 30 minutes at 125V and protein was transferred onto a PVDF membrane using a semi-dry blotting system for 1 h at 20V (Invitrogen, CA, USA). The membrane was blocked for 1 h at room temperature in 5% skimmed milk. Incubation with primary antibody took place overnight at 4ºC. Antibodies were STAT3, p-STAT3, p-ACC, AMPK, and p-AMPK (all from Cell Signaling Technology, MA, USA). Blots were washed in TBS-T and incubated with an anti-rabbit or anti-mouse IgG HRP conjugated antibody (1:5000, Dako, Copenhagen, Denmark) for 1 h at room temperature. Specific signal was detected using Supersignal West Femto Luminal/Enhancer Solution (Thermo Scientific, MA, USA) and subsequent exposure in a charge-coupled device camera (Bio-Rad, Copenhagen, Denmark). Following exposure, blots were briefly rinsed in TBS-T and then incubated in 0.5% Reactive Brown (Sigma Aldrich, MO, USA) for 15 min. Blots were analyzed and quantified using ImageQuant software, with the reactive brown image as a control for equal loading and transfer.

Reference List