SUPPLEMENTARY DATA

Analyses and calculations:
Blood chemistry. Blood glucose and lactate levels were measured on whole blood using ABL 615 (Radiometer Medical, Brønshøj, Denmark). Plasma long-chain fatty acid (LCFA) concentrations were measured using NEFA C kit (Wako Chemicals GmbH). Plasma concentrations of insulin were measured by enzyme-linked immunosorbent assay (ELISA, DakoCytomation K6219). Plasma epinephrine and norepinephrine were determined by radioimmunoassay (2-CAT RIA ^{125}I RIA kit, Labor Diagnostika Nord GmbH & Co. KG).

Respiratory exchange ratio (RER). Expired air was collected in Douglas bags and the volumes of the air expired were measured with a chain-suspended Collins spirometer and analyzed for O_2 (Servomex S-3A; Servp,ex. Crowborough, UK) and CO_2 (Beckman LB2; Beckman, Irvine, CA, USA).

Body composition. Lean bodymass (LBM) and lean leg mass (LLM) were determined by dual-energy x-ray absorptiometry (DPX-IQ Lunar, Lunar Corporation Madison WI, USA) and by hydrostatic weighing (1).

Stable isotopes. Stable isotope enrichments were measured using liquid chromatography-mass spectrometry (Finnegan aQa) as previously described (2).

Muscle tissue processing. Muscle biopsies were freeze-dried and dissected free of any visible fat, blood and connective tissue. Dissected tissue was then homogenized in ice-cold buffer [10% glycerol, 20 mM sodium pyrophosphate, 1% NP-40, 2 mM PMSF, 150 mM sodium chloride, 50 mM HEPES, 20 mM β-glycerophosphate, 10 mM sodium fluoride, 1 mM EDTA, 1 mM EGTA, 10µg·ml^{-1} aprotinin, 3 mM benzamidine, 10µg·ml^{-1} leupeptin, 2 mM sodium orthovanadate (pH 7.4)]. Homogenates were rotated end over end for 1 h at 4 °C and subsequently centrifuged at 17,500 *g* for 20 min. Supernatants were collected and protein concentrations of the lysates were determined using the bicinchoninic acid method (Pierce, Rockford, IL, USA).

Glycogen. Muscle glycogen concentration was determined as glycosyl units after acid hydrolysis of freeze-dried and dissected muscle tissue by the fluorometric method (3)
IMTG content. IMTG content was determined by biochemical methods on freeze-dried and dissected tissue as described previously (4).

SDS-PAGE and western blot analyses. Muscle lysate proteins were separated by SDS-PAGE and transferred (semi-dry) to PVDF membranes (Immobilon Transfer Membranes; Millipore, Bagsvaerd, Denmark). Membranes were blocked for 1 h at room temperature (RT) in TBST + 2% skim milk powder (wt/vol, pH 7.4). Blocked membranes were probed with primary antibodies (see *Antibodies*) overnight at 4°C and subsequently incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody for 1 h at RT. Thereupon, membranes were probed with ECL (Amersham Biosciences, Piscataway, NJ), and immune-complexes were visualized using a Kodak Image Station 2000MM. Signals were quantified (Kodak 1D 3.6) and expressed as arbitrary units. Membranes used for detection of phosphorylated Akt, TBC1D1 or TBC1D4 were stripped [100 mM 2-mercaptoethanol, 2% SDS, and 62.5 mM Tris-HCl] and re-probed with the corresponding total antibody.

Antibodies. The following antibodies were used to determine the phosphorylation status of the corresponding proteins: AMPK T172 (Cell Signaling Technologies, Danvers, USA), Akt T308 (Upstate Biotechnologies, Waltham, USA), Akt S473 (Cell Signaling Technologies, Danvers, USA), phospho-specific antibodies against TBC1D4 (S318, S341, S588, T642 and S751) have previously been described

(5) as have phospho-specific antibodies against TBC1D1 S237 and T596 (6) as well as PDH site 1 and site 2 (7). Determination of TBC1D4 protein expression as well as IP of TBC1D4 was performed using an antibody specific for human TBC1D4 (Abcam, Cambridge, UK) and antibody against total PDH has been described previously (7). TBC1D1 total antibody has previously been described (8) as has the α2AMPK antibody (9).

14-3-3 overlay assay. 14-3-3 proteins (*Saccharomyces cerevisiae* 14-3-3 isoforms BMH1/2) were expressed in *E. coli* DH5*α* and purified as previously described (10). Purified 14-3-3 proteins were labeled with digoxygenin-3-*O*-methylcarbonyl-*ε*-aminocaproic-acid-*N*-hydroxysuccinimide (DIG) according to manufacturer's recommendation (Roche, Basel, Switzerland). PVDF membranes containing TBC1D4 protein after immuno-precipitation (from 150 µg muscle lysate) and SDS-PAGE were blocked (TBS-T + 1% skim milk) and incubated in DIG–14-3-3 protein (TBS-T + 1% skim milk) overnight (4◦C). Next, membranes were incubated in HRP-conjugated anti-DIG antibody (Roche, Basel, Switzerland) allowing for detection and quantification. Membrane was subsequently stripped (as described above) and incubated with total TBC1D4 antibody (as described above).

Glycogen Synthase (GS) activity. Muscle GS activity in the presence of 0 mM, 0.17 mM or 8 mM glucose 6-phosphate~~n~~ (G6P) was measured in triplicate using a 96-well plate assay (Unifilter 350 plates; Whatman, Cambridge, UK) (19).

Calculations. GS activities are presented as the percent of G6P-independent GS activity (%I form) [100 x activity in the presence of 0 mM G6P divided by the activity at 8 mM G6P (saturated)] or as the percent of fractional velocity (%FV) (100 x activity in the presence of 0.17 mM G6P divided by the activity at 8 mM G6P).

Hepatic Glucose Production (HGP) was calculated pre-clamp and during the last 40 min of the clamp (end of clamp) by subtracting the rate of glucose appearance, calculated using a single pool non-steady-state model modified after the Steele equation procedure, from the exogenous GIR as previously described (35).

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