Supplementary information

Glucose uptake in skeletal muscle

Mice were anesthetized via i.p. injection of 2.5% avertin (0.02 ml/g body weight), and the extensor digitorum longus (EDL) and soleus muscles were removed for *in vitro* incubation. Isolated muscles were incubated for glucose uptake as described for the rat epitrochlearis muscle [1]. Incubation medium was prepared from a stock solution of Krebs-Henseleit bicarbonate buffer (KHB) supplemented with 5 mM Hepes and 0.1% bovine serum albumin and continuously gassed with 95% O_2: 5% CO_2. Isolated muscles were incubated in KHB in the absence or presence of insulin (0.18 or 12 nM) for 30 min. Glucose transport was assessed using 2-deoxyglucose [2]. Muscles were transferred to vials containing glucose-free KHB supplemented with 20 mM mannitol for 10 min. Thereafter, muscles were transferred to KHB containing 1 mmol/l [^{3}H]-2-deoxyglucose (3.7 GBq/ml) and 19 mmol/l [14C]-mannitol (2.59 GBq/ml) and incubated for 20 min. After incubation, muscles were immediately frozen in liquid nitrogen. The extracellular space and intracellular 2-deoxyglucose concentrations were determined [1, 2]. Glucose transport activity is expressed as µmol/ml 2-deoxyglucose per hour.

Metabonomics of lipid-soluble liver tissue extract using ^{1}H nuclear magnetic resonance (NMR) spectroscopy

Lipid-soluble liver tissue extract was prepared using a modified method of Folch et al. [3]. Briefly, pre-weighed tissue (~100-200 mg) was homogenized in 4 ml 2:1 $CHCl_3$-CH_3OH (v/v). Supernatants were extracted with 0.8 ml 50 mM NaCl and the organic phase collected and dried under a stream of nitrogen. Immediately prior to analysis, samples were reconstituted in 800 µl of (3:1) $CDCl_3$-CD_3OD containing 0.05% tetramethylsilane (an internal chemical shift reference with $\delta = 0.0$ ppm) and pipetted into 5 mm NMR tubes.

NMR spectra were recorded using a Varian UNITY INOVA 500 MHz spectrometer at 25°C. For each sample, a single pulse 1D, ^{1}H experiment was performed and spectra recorded in a data set containing 16K complex points covering a spectral width of 8000 Hz. A total of 32 transients were averaged using a 4 second recycle delay. All spectra were processed by applying cosine bell apodization before zero filling by a factor of 2, followed by Fourier transformation and DC baseline correction. Identification and quantification of peaks were made using the maximum intensity present in the spectral window assigned to lipid: lipid methylene (1.25-1.40 ppm); triglyceride: triglyceride terminal methyl (0.85-0.95 ppm); and cholesterol: cholesterol methyl (0.65-0.75 ppm) [4].

Real-Time PCR Analysis

Primer sequences were (forward 5'-3', reverse 5'-3'): glucose-6-phosphatase, catalytic (*G6pc*) (ACGTCTGTCTGTCCCGGATCTA, CCCCGGATGTGGCTGAAAGTTT), signal transducer and activator of transcription 3 (*Stat3*) (GCCCCGTACCTGAAGACCA, GACATCGGCAGGTCAATGG), CCAAT/enhancer-binding protein β (*Cebpb*) (ATCCGGATCAAACGTGGCT, CGCAGGAACATCTTTAAGGTGA), Krüppel-like factor 15 (*Klf15*) (TGCGTCGGCACACAGGCGAGAA, CCGGTGCC TTGACAACTC ATCT), glucose transporter 1 (*Glut1*, also called solute carrier family 2 (facilitated glucose transporter), member 1 (*Slc2a1*)) (GCTGTGCTGTGCTCATGACCAT, CCACGATGCTCAGATAGGACAT), very low

density lipoprotein receptor **(***Vldlr*) TGCGAGAGCCTGCCTCC, TCGCCCCAGTCTGACCA. Primer sequences for *Hprt* are from [5] and sequences for *ER*α from [6].

Western blot analysis

Liver protein extracts were prepared by homogenising and lysing tissue in 50 mM Tris-HCl pH 7.6 with 0.1% Triton X-100, supplemented with 1 x protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Total cell lysates were centrifuged at 20,000 x g for 30 min, and the cleared lysate was transferred to new tubes and centrifuged again to remove as much lipid as possible. Protein concentrations of extracts were determined using the Bradford method (Coomassie Plus, Pierce, Rockford, IL, USA). Equal amounts of protein were separated by electrophoresis through 6-12% polyacrylamide gels. Proteins were transferred to Hybond-C Super membranes (GE Healthcare, Buckinghamshire, UK). Membranes were probed using Stat3 (Thermo Scientific, Cheshire, UK), phospho-Stat3 (Tyr705, Cell Signaling Technology, Danvers, MA, USA) and glucose-6-phosphatase (C-14, Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies and protein-antibody complexes were detected using an ECL or ECL Advance chemiluminescence system (Pierce or GE Healthcare). Band densities were quantified using ImageJ [7]

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