SUPPLEMENTAL DATA

S1 – Medication used by participants

All participants were treated with insulin at an average dose of 80±8.1 IU/day. Insulin treatment comprised the following: Insulatard (n=1), Levemir (n=1), Lantus (n=1), Novomix (n=1), Insulatard and Novomix (n=2), Insulatard and NovoRapid (n=1), Insulatard and Actrapid (n=1), Mixtard and NovoRapid (n=1). Eight patients also received oral anti-diabetic treatment in the form of metformin (n=7) and sulfonylurea (n=1). Additional medication comprised the following: statins (n=7), acetylsalicylic acid (n=7), antihypertensive treatment (n=6), NSAID other than acetylsalicylic acid (n=3), antacids (n=2), gemfibrozil (n=1), anti-rheumatic treatment (methotrexate, infliximab and folic acid, n=1), and antidepressants (fluanxol, n=1).

S2 - Blood analysis and Indirect Calorimetry

Plasma glucose was measured immediately (Beckman Instruments, Palo Alto, CA, USA). Serum free fatty acids (FFAs) were analyzed with a commercially available kit (NEFA-HR 2; Wako Chemicals, Richmond, VA, USA). Insulin, GH and IGF-1 was analyzed by time-resolved fluoroimmunoassay (AutoDELFIA; PerkinElmer, Turku, Finland) and C-peptide and Cortisol was measured by ELISA (DakoCytomation, Cambridgeshire, UK). Glucagon was analyzed using a validated in-house radioimmunoassay (RIA) and adrenaline and noradrenaline was analyzed by time-resolved HPLC.

The respiratory exchange ratio (RER) and resting energy expenditure were estimated by indirect calorimetry (Deltatrac monitor; Dantes Instrumentarium, Helsinki, Finland). The measurements were performed at t=210-240 min and the mean values of the last 25 min were used for calculations. Lipid and glucose oxidation were estimated after correction for protein oxidation, which were calculated on the basis of urea nitrogen excretion. All calculations were performed essentially as described by Ferrannini (1).
S3 – Adipose tissue homogenization and western blotting

AT biopsies were homogenized using a Teflon/glass Potter-Elvehjem homogenizer in a hypotonic buffer containing 20 mM HEPES pH 7.4, 10 mM NaF, 1 mM Na3VO4, 1 mM EDTA, 5% SDS, 50 µg/ml Soybean Trypsin Inhibitor, 4 µg/ml Leupeptin, 0.1 mM Benzamidine, 2 µg/ml Antipain and 1 µg/ml Pepstatin. Crude homogenates were incubated in a Thermomixer (Eppendorf, Hamburg, Germany) at 37°C and 1000 rpm for 1 hour followed by centrifugation at 14.000g for 20 min. The lipid-free infranatant was aspirated and used for western blot analysis.

Equal volumes of protein samples were resolved by SDS-PAGE (4-12% Bis-Tris gels, Criterion XT system; Bio-Rad, Hercules, CA, USA) and transferred to PVDF membranes, according to manufacturer instructions. After blocking (5% BSA and 0.01% Tween-20 in TBS) the membranes were incubated with primary antibodies. The following primary antibodies were used: anti-ATGL, anti-CGI-58, anti-β-actin (Abcam, Cambridge, UK), anti-HSL, anti-phospho-HSL (Ser552), anti-phospho-HSL (Ser554), anti-phospho-HSL (Ser650), anti-Perilipin A, anti-PPAR-γ, anti-phospho-Akt (Ser473), anti-phospho-AMPKα (Thr172), anti-Akt (Cell Signaling, Beverly, MA, USA), anti-CIDEA (Novus Biologicals, Cambridge, UK), anti-G0S2 (Santa Cruz Biotechnology; Santa Cruz, CA, USA), anti-AMPK α-pan (Millipore, Billerica, MA, USA). Polyclonal anti-GLUT4 antibody was produced as previously described (2). In order to determine relative phosphorylation levels, phospho-specific blots were stripped and reprobed with primary antibody for the detection of the total amounts of the protein.

Stripping was performed by incubation of the membranes in stripping buffer (62.5 mM Tris, pH 6.8 and 2% SDS) at 55°C for 30 min in a shaking water bath. Stripping efficiency was assessed by reprobing with secondary antibody. If necessary, the stripping procedure was repeated until no remaining primary antibody could be detected. Horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham, GE-Healthcare, Pittsburgh, PA, USA) and goat anti-mouse HRP-IgG (Abcam, Cambridge, UK) was used as secondary antibodies. Proteins were visualized by enhanced chemiluminescence (Pierce Supersignal West Dura; Thermo Scientific, Rockford, IL, USA), and quantified with UVP BioImaging System (UVP, Upland, CA, USA). Protein levels were determined relative to β-actin, and phosphorylations were determined relative to the total amount of the protein of interest.
**S4 – Isolation of mRNA and Real-time PCR Analysis**

Total RNA was isolated from the adipose tissue fragments using TriZol reagent (Gibco BRL, Life Technologies, Roskilde, Denmark); RNA was quantified by measuring absorbance at 260 and 280 nm with a ratio $\geq$1.8. Finally, the integrity of the RNA was checked by visual inspection of the two ribosomal RNAs, 18S and 28S, on an agarose gel.

For real-time reverse transcriptase PCR, complementary DNA was constructed using random hexamer primers as described by the manufacturer (Verso cDNA kit from VWR, Herlev, Denmark). PCR-master mix, containing the specific primers, were added and real-time quantification of genes was performed by SYBR-green real-time reverse transcription PCR assay (KAPA SYBR Fast Universal kit from Ken-En-Tec, Taastrup, Denmark) in 384 well format in a LightCycler from Roche (RocheApplied Science, Mannheim, Germany). Real-time PCR for target genes were performed with mRNA levels of $\beta_2$-microglobulin as internal control, and this expression did not change during intervention. The following primer pairs were used:

- **ATGL:** TGCACCATTGTGTATGTG and CCAGGAGTGCGACGCT
- **G0S2:** CGAGAGCCAGAGCCAGATG and AGCACCAGCCGAAGAG
- **CGI-58:** GGCTGCTGTTACTCGCTGAA and GCTCCCAAGGCTCTGATCCAA
- **Plin:** GGAGCGAGGATGGCATGCAAC and TCTGGAAGCATTCGCAGGT
- **CIDEA:** CGGCTGCCCTTAACGTGAA and AGATGAGAAACTGTCCCGTCA
- **HSL:** GAAGGCGGCACGGACGCC and GCTGGTGCGGCGGGACAC
- **B2 micro:** AATGTCGGATGGATGAAACC and TCTCTCTTTCTGGCCTGGAG

All primers were from DNA Technology (Risskov, Denmark). cDNA with specific primers was amplified in separate tubes, and the increase in fluorescence was measured in real time. The threshold cycle was calculated, and the relative gene expression was calculated essentially as described in the User Bulletin No- 2, 1997 from Perkin Elmer (Perkin Elmer Cetus, Norwalk, CT) using the formula: $k \cdot 2^{-\Delta\Delta CT}$, were $k$ is a constant, set to 1. All samples were amplified in duplicate. A similar set-up was used for negative controls, except that the reverse transcriptase was omitted and no PCR products were detected under these conditions.