**Supplemental information**

**Supplemental data**

***α-MSH levels are not related to obesity***

To evaluate whether circulating α-MSH levels were different in obese compared to normal body weight subjects, we determined α-MSH concentration in 3 different mammalian species: in a well-known DIO mouse model ([Enriori et al., 2007](#_ENREF_2)), in a model of obesity in monkeys (HFD fed monkeys, S-HFD) ([McCurdy et al., 2009](#_ENREF_6)) and in obese children ([Reinehr et al., 2006](#_ENREF_8)). In all three studies, baseline α-MSH levels were not different between obese and lean groups (Figure S2A, B, C and c). In addition, we found that in both, obese children and monkeys plasma α-MSH concentrations did not correlate with homeostasis model assessment of insulin resistance (HOMA, data not shown), suggesting that α-MSH levels are not linked to obesity or insulin resistance.

**Supplemental Experimental Procedures**

*Cell culture experiments (L6-Glut myc cells)*

*α-MSH effect on glucose uptake:* L6 rat myoblast cells were maintained in low glucose DMEM media (5.5mM) enriched with 10% FBS. After confluence was reached, cells were differentiated in myotubes by reducing serum concentration (2% FBS, 7 days). Cells were serum-starved the night before the experiment. Medium was replaced in the morning by fresh no glucose non-serum medium and α-MSH (100nM) was added to the corresponding wells for 1h. After that, cells were washed and cold preincubation media was added and maintained at 37ºC (20 min). Radiolabeled 2-deoxy-D-[2,6-3H]glucose (1mmol/l, 0.5μCi/ml), 10nM insulin, 100nM α-MSH or both was added to the corresponding well and maintained for 15 min and then quickly washed with cold PBS. A similar procedure was performed to test the inhibition of PKA by pretreatment with 15µM H89 (a potent selective inhibitor of cAMP dependent protein kinase) during 30 min. PG-901 (MC5R selective agonist) effect was evaluated after incubation of 2 doses (1 and 5nM)

*Seahorse analyses:* L6 cells were cultured as above or with high glucose DMEM media (25mM) and seeded in V7 Seahorse (Seahorse Bioscience, NortBillerica, MA) plates at 25,000 cells per well. Mitochondrial function, glucose and palmitate oxidation assays were performed after four days of differentiation, as previously described ([McGee et al., 2011](#_ENREF_7))

*Ex vivo glucose metabolism:* Mice were fasted overnight and soleus muscles were dissected tendon to tendon from anaesthetised mice (2-3% isoflurane gas). Muscles were preincubated for 30 min with warmed (30°C), pre-gassed (95%O2- 5%CO2, pH 7.4), modified Krebs-Henseleit buffer supplemented with 2mmol/l sodium pyruvate, 8mmol/l mannitol, and 0.1% wt/vol BSA and were then incubated with or without 10nM insulin, 100nM α-MSH or α-MSH plus Insulin (100nM + 10nM) for 20 min. Glucose uptake was assessed for 10 min using 2-deoxy-D-[2,6-3H]glucose (1mmol/l, 0.5μCi/ml) and 1 mM D-[14C] (0.45mCi/ml) in the presence or absence of 10nM insulin. Radioactivity was measured in muscle lysates by liquid scintillation counting.

For dose-response study, we used the same procedure and the samples were incubated with different dose of α-MSH (10, 100 and 1000nM). For theophylline experiment, we used the same procedure, but soleus muscles from DIO mice were incubated with α-MSH (100nM), Theophylline (100μM) or α-MSH plus Theophylline for 20 min before glucose uptake.

*Immunoassays:* Human serum insulin and glucose concentrations were measured and homeostasis model assessment (HOMA) was calculated as previously described ([Roth et al., 2010](#_ENREF_9)). Monkey insulin was measured using an in-house assay on a Roche Diagnostics Elecsys 2010 clinical assay platform ([McCurdy et al., 2009](#_ENREF_6)). Mice insulin was measured at Monash Obesity and Diabetes Institute by an in-house Enzyme-linked immunosorbent assay (ELISA) as previously described ([Wilson et al., 2014](#_ENREF_10)).

*Immunohistochemistry:* Sections were washed in KPBS and soaked in microwave on high in 1x citra buffer for antigen retrieval protocol. Sections were then blocked in 2% donkey serum in 0.4% triton X-100/KPBS. To characterize the relative contribution of the POMC gene products, sheep anti-αMSH (#AB5087, 1:5,000; Chemicon, Temecula, CA), or rabbit anti-adrenocorticotrophic hormone (ACTH) (#20070, 1:5000, Immunostar, Hudson, WI) antibodies were used. For preabsorption studies, a ratio of 1µg of antibody: 50x excess peptide was incubated at 4°C overnight (ACTH, #001-01; αMSH, #043-01; Phoenix Pharmaceutical, Inc., Burlingame, CA) in 75µl of sterile water. Primary as well as preabsorbed antibodies were then diluted to their respective concentration in 2% donkey serum in 0.4% triton X-100/KPBS and incubated overnight. Tissues were then washed in KPBS and secondary antibodies applied for 1 hour at room temperature at dilution of 1:200 (Jackson ImmunoResearch Laboratories, Inc, West Grove, PA).

#### *Measurement of melanocortin receptors:* MCR mRNA expressions were assessed in soleus muscles of both control and DIO mice by RT-PCR. RNA extraction and Semiquantitative RT-PCR analysis

A Mini RNeasy kit (QIAGEN, Germantown, MD) was used to extract total RNA from soleus muscles. The total RNA was reverse transcribed using SuperScript II reverse transcriptase. PCR primers used to detect mouse MC1R mRNA were 5’-GCC ACC CTT ACT ATC CTT CT-3’ (nt 1143-1162) and 5’-ATA TCA CTG TCA CCC TCT GC-3’ (nt 1410-1391). To detect mouse MC3R mRNA we used a 5’ sense primer 5’-CAA GAT GGT CAT CGT GTG TCT-3’ (nt 923-943) and an antisense primer 5’-TAG CCC AAG TTC ATG CTG TT-3’ complementary to nt 1339-1320. MC4R primers were 5’-ATC ATT TAC TCG GAC AGC AGC-3’ (nt 985-1005) and 5’-ACA ACT CAC AGA TGC CTC CCA-3’ (nt 1417-1397) and for MC5R we used a 5’ sense primer 5’-TCA TTT GCC TCA TCT CCA TGT-3’(nt 723-743) and an antisense primer 5’-ACT GAG AGA GGA AGG CGT TT-3’ complementary to nt 1159-1140. Mouse β-actin was used as control and forward and reverse primers were respectively: 5’- TTC AAC ACC CCA GCC ATG T-3’ and 5’-TGT GGT ACG ACC AGA GGC ATA C-3’. All PCR primers were designed using the software Primer Select 6.0 (DNASTAR, Inc., Madison, WI). The PCR amplification conditions included a denaturing step at 95°C for 4 min, followed by 35 cycles of denaturing (95°C for 15 sec), annealing (at 55°C) for 1 min, and extension (72°C for 2 min).

*Measurement of gluconeogenic enzymes by quantitative RT-PCR:* RNA was extracted and reverse-transcribed as previously described ([Litwak et al., 2014](#_ENREF_5)). Real-time quantitative PCR was performed using TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA) and TaqMan primers for Glucose-6-phosphatase (G6pase, Mm00839363\_m1), phosphoenolpyruvate carboxykinase (PEPCK, Mm00440636\_m1). 18s (Hs99999901\_s1) was used as a housekeeping gene and did not vary between groups. Amplifications were performed using a Real Plex4 Mastercycler (Eppendorf, Hamburg, Germany). Thermal cycling conditions included 2 min at 36 50°C, 95°C for 10 min followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The mRNA levels were determined by the comparative ∆Ct method.

*Measurement of MC5R, and PDE4B, by Western blots: a) In basal conditions:* Four control and 4 DIO mice were fasted overnight and soleus muscles were dissected tendon to tendon from anaesthetised mice (3% of isofluorane gas). Samples were immediately homogenized in cold RIPA lysis buffer and 10µg of each sample loaded on a 10 % Tris-Glycine pre-cast gel as previously described ([Enriori et al., 2011](#_ENREF_3)). The following antibodies were used for MC5R expression: anti MC5R (1:4000, goat polyclonal antibody，Abcam, catalog no.: #ab92287 and 1:5000, anti α/β tubulin rabbit polyclonal antibody, Cell signaling, catalog no.: #2148S) Donkey anti-goat IgG horseradish peroxidase (1:10000, Santa Cruz Biotechnology, catalog no.: #sc-2020) and Goat anti-rabbit IgG horseradish peroxidase (1:50000, Sigma) were used as secondary antibody, respectively.

*For PDE4B expression*: Four control and 5 DIO mice were fasted overnight and soleus muscles were dissected and processed as above. Rabbit polyclonal anti PDE4B (1:500, Abcam, catalog no.: #ab14611) and rabbit polyclonal anti-actin antibody (1: 5000, Sigma, catalog no.: A2066) and Goat anti-rabbit IgG horseradish peroxidase (1:50000, Sigma) were used.

*cAMP measurement:* To measure cAMP concentrations in muscles during α-MSH infusion and GTT we used the same design that was described in *α-MSH infusion*; muscles were obtained after 45 min of initiating α-MSH infusion and 25 min of administering glucose injection. cAMP concentration was determined with a direct immunoassay kit (BioVision Research Products, Mountain View, Ca, USA). The kit has a cAMP polyclonal antibody onto the plate. cAMP-HRP conjugates directly compete with cAMP from sample binding to the cAMP antibody on the plate. The HRP activity at 450nm is inversely proportional to the cAMP concentration in samples.

*Glucose tolerance test during* -*MSH perfusion clamp studies:* This technique was recently validated ([Badin et al., 2013](#_ENREF_1)). Mice are catheterized at least 6 days before the experiment after being anesthetized with Isoflurane (1.5%; Air: 0.4l/min). The right jugular vein was catheterized for infusion with a silastic catheter. The free end of the catheter is tunneled under the skin to the back of the neck. Catheters are flushed daily with ~50µl of 0.9% NaCl containing 5mg/ml ampicillin and 20UI/ml heparin. Animals are individually housed after surgery and their body weight monitored daily. Animals not within 10% of presurgery weight by postsurgery day 6 were excluded from the study. Awaken animals are place in their home cage for the duration of the experiment and food-deprivated overnight. Mice will be infused for a total of 3 hours with solution of NaCl containing either 0.1%BSA or 0.1%BSA +-MSH (1µg/µl) at a rate of 1µl/min. At time=-60 min animals received a bolus of -MSH (0.5µg) through intraperitoneal injection in a total volume of 100µl of NaCl+0.1% BSA. At t=0 animals received a intraperitoneal injection of a mixture of D-glucose and (1g/kg)+ 2-deoxy-D-[1-14C]glucose (2DG) (3μCi, Perkin Elmer, Courtaboeuf, France). Blood samples (10μl) are collected from the tail at 0, 5, 10, 20, 30, 40, 60 and 120 min until the end of the experiment where mice are killed by elongation and tissues are collected.

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