Supplementary Materials

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Supplementary materials and methods

Computed tomography (CT)-based measurement of visceral fat depots in mice

Abdominal visceral fat depots were quantified using based on the abdominal computed tomography (CT) images using micro-X-ray CT (CosmoScan FX; Rigaku Corporation, Tokyo, Japan) as described previously with brief modification [1]. Semi-quantification was performed using the CT images of abdominal region between the L1 and L5. Images were acquired with a tube voltage of 90 kV, tube current of 88 μA, 120 μm slice thickness, and total scan time of 2 min [2,3]. Following scanning, the visceral and subcutaneous fat mass were calculated using 3-dimensional image analysis software Slice-O-Matic (Tomovision, Montreal, Canada). The higher tissue density of abdominal muscular wall was used as the demarcation line for visceral fat and subcutaneous adipose tissue [4]. These analyses were performed at six weeks after the start of treatment.

Measurement of plasma leptin level

The plasma leptin concentrations in the mice were measured using a Mouse Leptin ELISA Kit (Abcam) according to the manufacturer's protocol.

Cell viability assay

C2C12 myocytes were seeded on uncoated plastic tissue culture dishes at a density of 1.5×10^4 cells/mL, and then cells were treated with different concentrations of DMSO or palmitic acid (PA) (0, 0.25, 0.5, 0.75 and 1 mM) for 24 hr. To assess the effect of inhibition of semaglutide, C2C12 cells were pre-treated with semaglutide (0.3, 0.6 and 0.9 μM). The Premix WST-1 Cell

Proliferation Assay system (Takara Bio, Kusatsu, Japan) was used to evaluate cell viability according to the manufacturer's protocol. Three independent experiments were performed, and the mean results were calculated from five replicates of each experiment. The proliferative rate was indicated as the ratio to the value of non-treatment group at the start of each experiment.

References

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Supplementary Figure 1. Effect of semaglutide on lipid metabolism in DDC-fed KK-Ay mice. (A) Visceral fat volume. Abdominal fat mass was measured by CT scan analysis at 6 weeks of treatment. Visceral fat was evaluated in abdominal regions between the L1 and L5. (B) Plasma leptin level at 6 weeks of treatment. Data are the mean ± SD (n=10). **P<0.01, significant difference between groups. N.S, not significant; ND, normal diet-fed group; DDC, diethoxycarbonyl-1,4-dihydrocollidine diet-fed group; Veh, vehicle-treated group; Sem, semagutide-treated group.

Supplementary Figure 2. Dose-dependent effects of semaglutide on the cell viability and UPS markers in C2C12 myocytes. (A) Dose-dependent effects of palmitic acid (PA) (0-1 mM) on cell viability in C2C12 myocytes. Cells were treated with PA for 24 hr. (B) Relative mRNA levels of *Fbxo32*, *Trim63* and *Mstn*. *Gapdh* was used as an internal control for qRT-PCR. (C) Cell viability by treatment with semaglutide. C2C12 cells were pre-treated with semaglutide (Sem) (0.3−0.9 μM) before stimulation with PA (0.75 mM) (B and C). Quantitative values are indicated as fold changes to the values of non-treatment group. Data are the mean ± SD (n=8). **P<0.01, significant difference between groups.

Supplementary Table 1. List of primers used in q-PCR

gene	Sense (5'-3')	Antisense (5'-3')
Mouse		
Fbxo32	GCAAACACTGCCACATTCTCTC	CTTGAGGGGAAAGTGAGACG
Trim63	TGACCACAGAGGGTAAAG	TGTCTCACTCATCTCCTTCTTC
Mstn	GGCCATGATCTTGCTGTAAC	TTGGGTGCGATAATCCAGTC
MyoD	CTTCTATCGCCGCCACTC	AAGTCGTCTGCTGTCTCAA
MyoG	CCAACCCAGGAGATCATTTG	ACGATGGACGTAAGGGAGTG
Tnfa	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
Il6	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
Il1b	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG
Ppargc1a	GAATCAAGCCACTACAGACACCG	CATCCCTCTTGAGCCTTTCGTG
Tfam	GAGGCAAAGGATGATTCGGCTC	CGAATCCTATCATCTTTAGCAAGC
Sirt1	CGGCTACCGAGGTCCATATAC	CAGCTCAGGTGGAGGAATTGT
Sesn2	GCGCTTTCATTCCAGTGGAAGAG	CAGAAGCTGCTAAGGTAGTCCG
Hmox1	CACTCTGGAGATGACACCTGAG	GTGTTCCTCTGTCAGCATCACC
Nqo1	GCCGAACACAAGAAGCTGGAAG	GGCAAATCCTGCTACGAGCACT
Gstm3	AGAGCAATGCCATCCTGCGCTA	GGTTCTCCAAAGTATCCACACGG
Gapdh	GACCCCTTCATTGACCTCAAC	GATGACCTTGCCCACAGCCTT