

Online Appendix

Distinct effects of leptin and a melanocortin receptor agonist injected into medial hypothalamic nuclei on glucose uptake in peripheral tissues

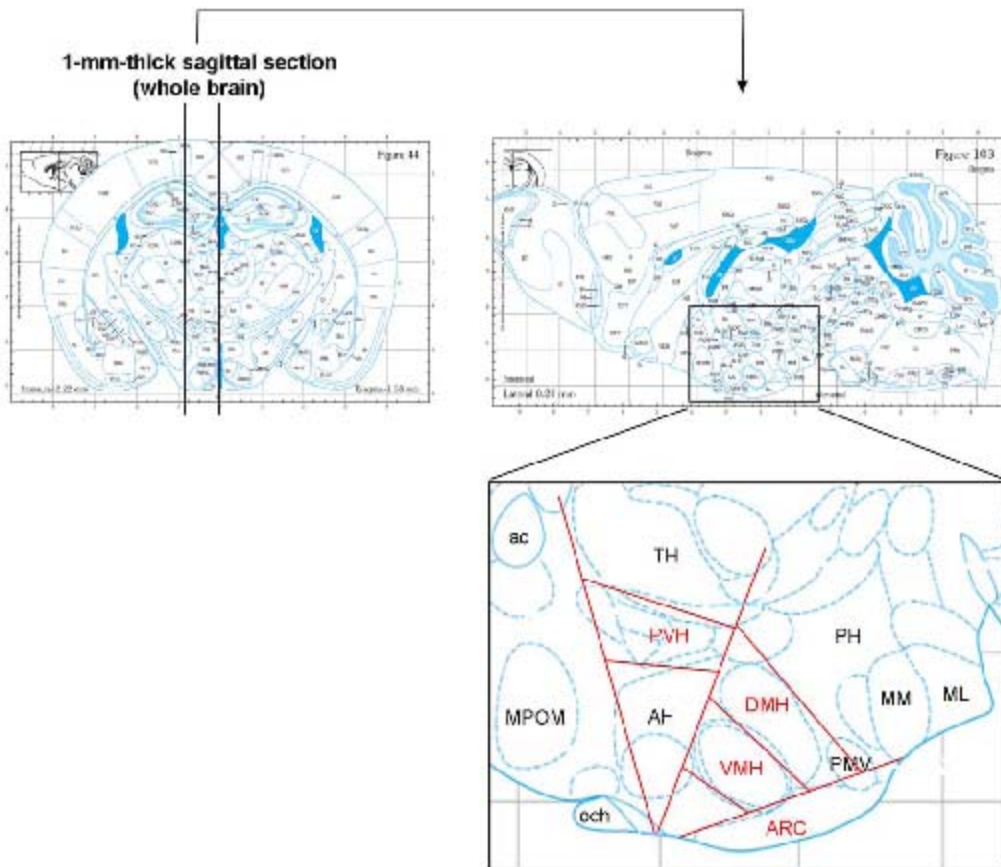
Short running title: Effects of leptin and MT-II on glucose uptake

Chitoku Toda,^{1,2} Tetsuya Shiuchi,^{1,2} Suni Lee,² Maya Yamato-Esaki,^{1,2} Yusuke Fujino,^{2,3} Atsushi Suzuki,² Shiki Okamoto,^{1,2} Yasuhiko Minokoshi^{1,2*}

¹Department of Physiological Sciences, Graduate University for Advanced Studies (Sokendai), Hayama, Kanagawa 240-0193, Japan

²Division of Endocrinology and Metabolism, Department of Developmental Physiology, National Institute for Physiological Sciences, Okazaki, Aichi 444-8585, Japan

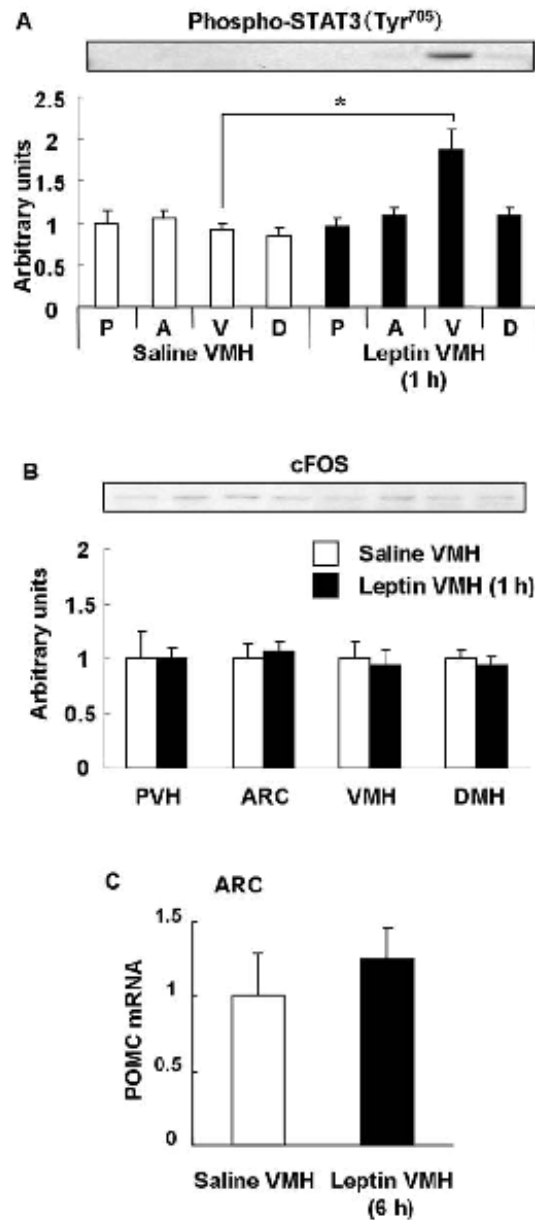
³Department of Internal Medicine 1, Faculty of Medicine, Oita University, Hasama, Oita 879-5593, Japan



Supplemental figure 1. Sampling of medial hypothalamic nuclei.

The hypothalamus present in 1-mm-thick sagittal sections of the entire mouse brain was dissected into PVH, ARC, VMH, and DMH along the indicated red lines. PVH was collected as the square area with an anterior margin of the border with the posterior region of the anterior commissure, a dorsal margin of the border with the thalamus, a ventral margin 1 mm ventral to the border with the thalamus, and a posterior margin of the white matter separating PVH from VMH-DMH. ARC was isolated as the ventral portion of the medial hypothalamus with a dorsal margin of the border with the ventral part of VMH and DMH. VMH and DMH were collected from the triangular area with an anterior-dorsal margin of the white matter separating PVH and the anterior hypothalamus from VMH-DMH, a ventral margin of the border with ARC, and a posterior margin of the border with the mammillary body.

ac, anterior commissure; MPOM, medial preoptic nucleus, medial part; TH, thalamus; AH, anterior hypothalamic area; ooh, optic chiasm; PH, posterior hypothalamic nucleus; PMV, pre-mammillary nucleus, ventral part; MM, medial mammillary nucleus, medial part; ML, medial mammillary nucleus, lateral part. Figures are modified with permission from (26).

**Supplemental figure 2.**

- (A) Immunoblot analysis of the phosphorylation of STAT3 on Tyr⁷⁰⁵ in the medial hypothalamic nuclei at 1 h after leptin injection into VMH. Data are means \pm SEM for 6 mice.
- (B) Immunoblot analysis of c-FOS expression in the medial hypothalamic nuclei at 1 h after leptin injection into VMH. Data are means \pm SEM for 6 mice.
- (C) The mRNA levels of POMC in ARC at 6 h after leptin injection into VMH were quantified by real-time PCR. Values are normalized to the level of eEF2 mRNA. Data are means \pm SEM for 4 mice. * $P < 0.05$ versus the corresponding value for saline-injected controls.

Supplemental Table 1. Primer sets for RT-PCR and real-time PCR.

for RT-PCR	
CRF	5' -GCTAACTTTTTCCGCGTGTT-3' (forward) 5' -GGTGGAAGGTGAGATCCAGA-3' (reverse)
POMC	5' -ACCACGGAGAGCAACCTGCT-3' (forward) 5' -CATGGAGTAGGAGCGCTTGC-3' (reverse)
NPY	5' -CTAGGTAACAAGCGAATGGG-3' (forward) 5' -AATCAGTGTCTCAGGGCT-3' (reverse)
SF1	5' -GCCAGGAGTTCGTCTGTCTC-3' (forward) 5' -ACCTCCACCAGGCACAATAG-3' (reverse)
eEF2	5' -TTCATGATGTGACCCTGCAT-3' (forward) 5' -CACTTGCTCAGGACACTGGA-3' (reverse)

for real-time PCR	
GLUT4	5' -ACTCTTGCCACACAGGGTCT-3' (forward) 5' -AATGGAGACTGATGCGCTCT-3' (reverse)
HKII	5' -GGGTAGCCACGGAGTACAAA-3' (forward) 5' -TGGATTGAAAGCCAACCTCC-3' (reverse)
UCP1	5' -GGGCCCTTGTAACAACAAA-3' (forward) 5' -GTCGGTCCTTCCTTGGTGTA-3' (reverse)
POMC	5' -CTCCTGCTTCAGACCTCCAT-3' (forward) 5' -CAGCGAGAGGTGAGTTTG-3' (reverse)
eEF2	5' -GCATCTACGGTGTCTGTAAC-3' (forward) 5' -AGCGGTGAAGCCAAAGGAC-3' (reverse)

Supplemental Table 2. Effects of leptin, MT-II, and SHU9119 on plasma glucose and insulin concentrations.

	Glucose (mg/dl)	Insulin (ng/ml)
Fig. 1		
Saline VMH (6 h)	141 ± 9.0	0.76 ± 0.23
Leptin VMH (3 h)	174 ± 14.8	0.80 ± 0.19
Leptin VMH (6 h)	147 ± 8.8	0.52 ± 0.13
Fig. 2		
Saline ARC	135 ± 4.0	0.86 ± 0.30
Leptin ARC	140 ± 4.2	0.73 ± 0.21
Leptin DMH	137 ± 19.9	0.72 ± 0.22
Leptin PVH	156 ± 6.0	0.83 ± 0.19
Fig. 4		
Saline VMH	136 ± 5.2	0.79 ± 0.22
SHU9119 i.c.v.	145 ± 7.7	0.90 ± 0.42
Leptin VMH	147 ± 8.8	0.52 ± 0.13
Leptin VMH + SHU9119 i.c.v.	131 ± 11.7	0.61 ± 0.19
Fig. 5		
Saline i.c.v. (6h)	148 ± 7.3	0.87 ± 0.29
MT-II i.c.v. (3h)	184 ± 12.8 [*]	0.88 ± 0.22
MT-II i.c.v. (6h)	183 ± 7.0 [*]	0.70 ± 0.12
Fig. 6		
Saline VMH	150 ± 8.7	0.88 ± 0.29
MT-II VMH	168 ± 8.7	0.67 ± 0.24
MT-II PVH	150 ± 12.7	0.85 ± 0.20
MT-II DMH	152 ± 8.5	0.73 ± 0.21
MT-II ARC	139 ± 11.2	0.70 ± 0.24

Plasma glucose and insulin concentrations were measured at 3 or 6 hours after injection of the compounds by using glucose CII test (Wako pure chemical, Osaka, Japan) and mouse insulin ELISA KIT (U-type) (Shibayagi, Gunma, Japan), respectively. Results are the means ± SEM for 5 to 7 mice. *Significantly different from saline injection at P<0.05.