Figure legends

Figure 1. **Leptin induces the elongation of cardiac myocytes.** Neonatal rat ventricular cardiac myocytes were isolated, treated with saline or leptin (50 ng/mL) for 48 h, and subjected to immunocytochemistry. The primary antibody to β-MHC was stained with a secondary antibody conjugated with peroxidase (*brown signals*). *A*, representative photomicrographs. *B*, Myocardial cell surface area, and short- and long-axis lengths were measured as described under "Materials and Methods." Values are means ± S.E. The data are from 40 cells in each group. *C*, mRNA expression levels of atrial natriuretic factor (ANF) and GAPDH were measured by real-time PCR after leptin treatment. Mean ANF/GAPDH value was set at 1.0. Values are means ± S.E.

Figure 2. **Analysis of the expression of Ob-Ra and -Rb and the phosphorylation state of STAT3, ERK1/2, p38 and JNKs.** mRNA was prepared from neonatal rat cardiac myocytes and RT-PCR analysis was performed by the standard method. Western blotting analysis was performed using anti-phosphospecific STAT3, ERK1/2, p38 and JNK antibody. To normalize for protein loading after immunoprecipitation, blots were stripped and probed with an antibody that recognizes both phosphorylated and non-phosphorylated forms of ERK1/2, p38, JNK, and STAT3. *A*, Expression of the Ob-Ra and -Rb receptors is shown as 495bp and 436bp bands, respectively. *B*, Activation (phosphorylation) of ERK1/2, p38 and JNK in cardiac myocytes was examined at 10 min after leptin stimulation. *C*, Activation of STAT3 was examined in cardiac myocytes stimulated with leptin (50 ng/mL). STAT3 signals were quantified by densitometry using NIH Image 1.62, and the levels of phosphorylated STAT3 relative to the level of total STAT3 were determined. The relative level in cardiac myocytes without treatment was set at 1.0 in each experiment. Values are the means ± S.E. from three independent experiments.

Figure 3. **Leptin induces the nuclear translocation of STAT3 in cardiomyocytes.** Immunocytochemical staining for STAT3 was performed using the indirect immunofluorescence method. The cells were incubated with anti-STAT3 monoclonal antibody and STAT3 signals were detected with anti-mouse FITC-conjugated secondary antibody. β-MHC staining was performed using rabbit anti β-MHC polyclonal antibody and the signals were detected with anti-rabbit rhodamine-conjugated secondary antibody. DAPI staining indicates the nucleus of the cells.

Figure 4. **AG490 suppresses the leptin-induced increase in the DNA-binding activity of STAT3.** Nuclear extracts were prepared from neonatal cardiac myocytes that had been stimulated with leptin in the absence or presence of AG490 (1 μM) or treated with saline as a control. These extracts were probed with a radiolabeled double-stranded oligonucleotide containing the consensus STAT3 site (*A*) or the SP1 site (*B*). The *arrows* indicate the complex corresponding to the interaction between the STAT3 probe and STAT3(*A*) or the interaction between the SP1-probe and SP1 (*B*). *A*, competition study in which unlabeled competitor DNAs were present as indicated: *lane 4*, wild-type (*wt*) STAT3; *lane 5*, STAT3 with a mutation (*mut*). *C*, the amount of STAT3/DNA binding in *A* was quantified by densitometry using NIH Image 1.62, and the relative amount of DNA binding (STAT3/SP1) was determined in each sample. The amount in saline (*SS*)-treated cardiac myocytes was set at 1.0 in each experiment. Values are the means ± S.E. of three independent experiments.

Figure 5. **AG490 suppresses the leptin-induced phosphorylation of STAT3 and inhibits cardiac myocyte hypertrophy.** *A*, Total cell lysates were prepared from neonatal cardiac myocytes that had been stimulated with leptin in the absence or presence of AG490 (1 μM) or treated with saline as a control. Western blotting analysis with anti-phosphospecific and total STAT3 was performed and their signals were quantified as described in the legend for Figure 2. The relative level in cardiac myocytes without treatment was set at 1.0 in each experiment. Values are the means ± S.E. from three independent experiments. *B*, Cell surface area was measured as described in the legend for Figure 1. Values are means ± S.E. (in μm^2 of myocardial cell surface area). The data are from 40 cells in each group.

Figure 6. **Plasma leptin concentration and physiological data of mice with MI or sham operation under chronic infusion of PBS or leptin.** Twelve-week-old mice were subjected to MI or sham operation. Each mouse was subcutaneously fitted with an osmotic pump that delivered either PBS or 400 ng/hr of leptin. *A*, Plasma concentration of leptin at 4 weeks after sham or MI operation was measured by a mouse leptin ELISA kit. Values are means ± S.E. *B*, Average food intake for 4 weeks and body weight at 4 weeks after sham or MI operation. Values are means ± S.E. *C*. Heart weight / body weight ratio at 4 weeks after sham or MI operation. mRNA expression levels of atrial natriuretic factor (ANF) and GAPDH at 4 weeks after sham or MI operation were measured by real-time PCR. The mean ANF/GAPDH value was set at 1.0. Values are means ± S.E.

Figure 7. **Effect of chronic leptin infusion on cardiac structure and function following MI.** *A*, Heart rate and blood pressure data at 4 weeks after sham or MI operation. SBP and DBP indicate systolic and diastolic blood pressure. *B*, Echocardiography data at 4 weeks after sham or MI operation. LVEDD indicates left ventricular chamber diameter in end-diastole; LVESD, left ventricular chamber diameter in end-systole; FS, fractional shortening. *C*, mRNA expression levels of mouse Ob-Ra, Ob-Rb and GAPDH in the heart were measured by real-time PCR after MI. The mean ANF/GAPDH value was set at 1.0. Values are means ± S.E.

Fig.1

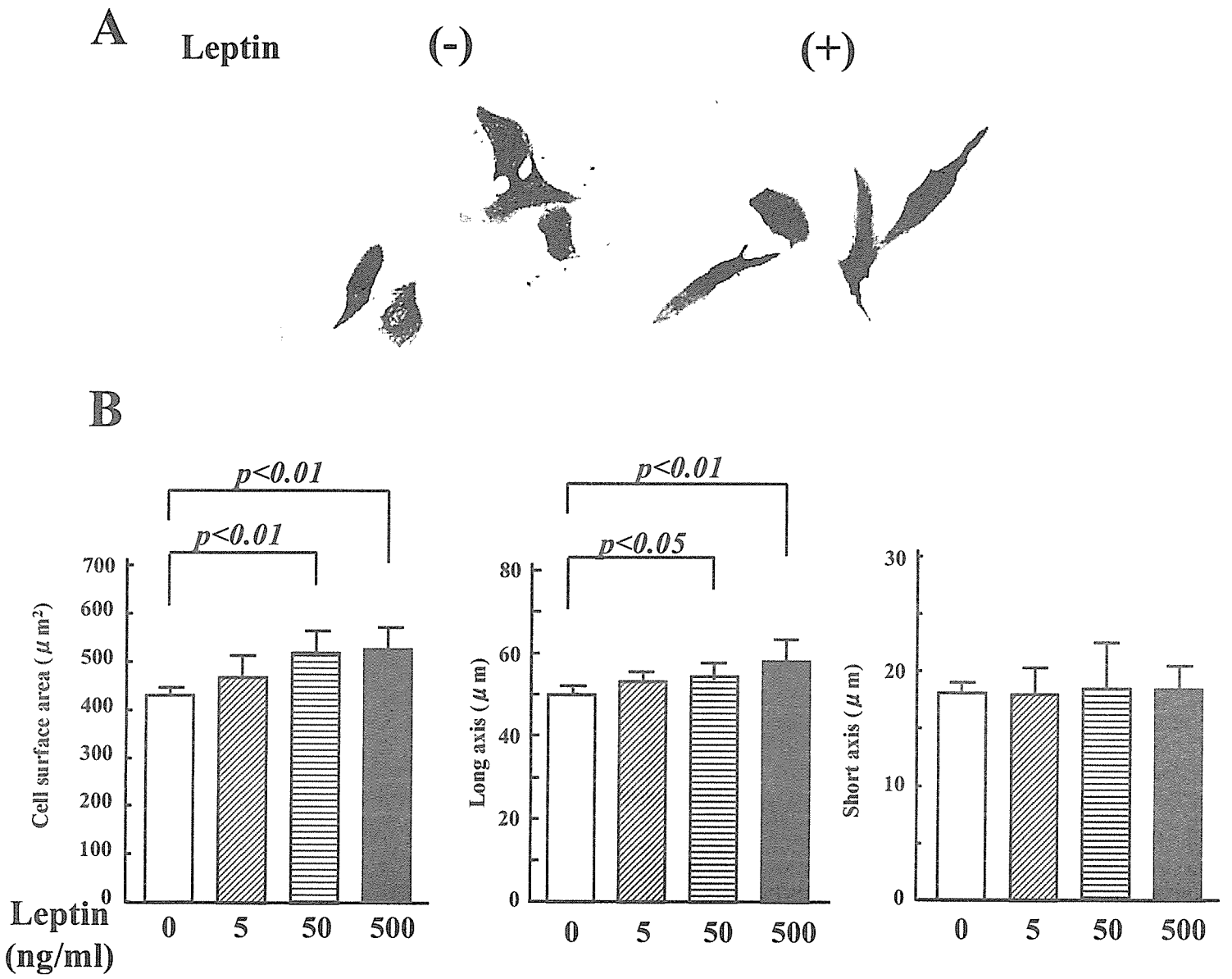
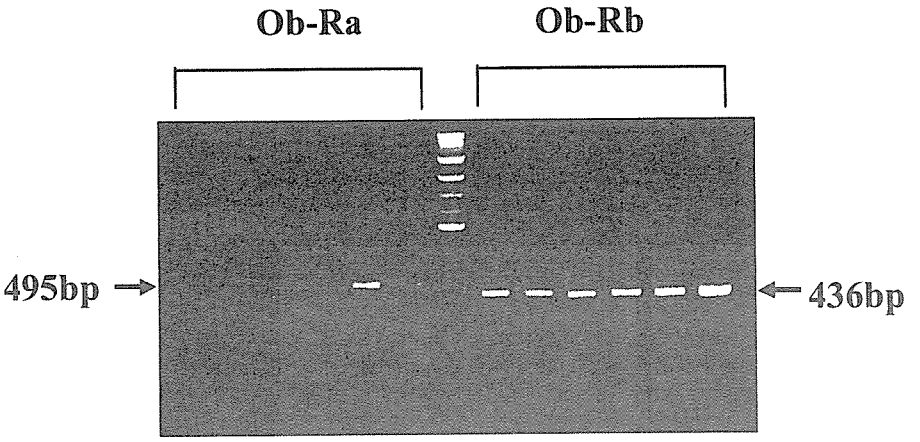
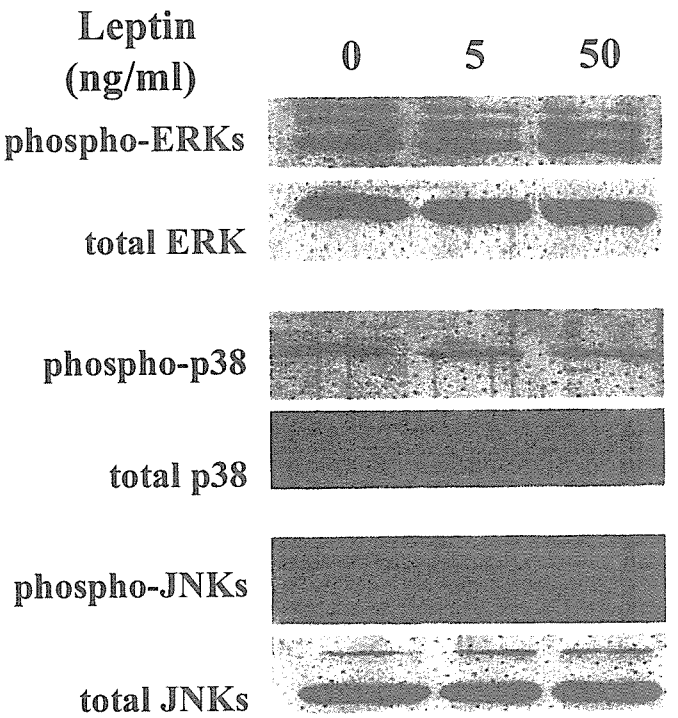

A
Leptin
(-)
(+)
B
p<0.01
p<0.01
p<0.01
p<0.05
Cell surface area (μm²)
Long axis (μm)
Short axis (μm)
Leptin (ng/ml)
0
5
50
500
C
p<0.01
p<0.01
Relative expression level (ANF/GAPDH)

Fig.2

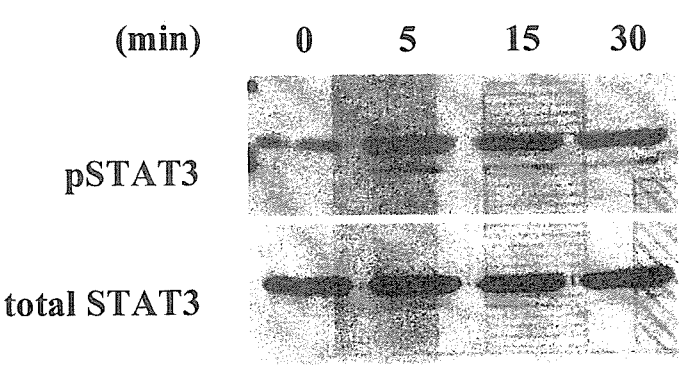
A


Ob-Ra
Ob-Rb
495bp
436bp

B


Leptin
(ng/ml)
0
5
50
phospho-ERKs
total ERK
phospho-p38
total p38
phospho-JNKs
total JNKs

C


(min)
0
5
15
30
pSTAT3
total STAT3

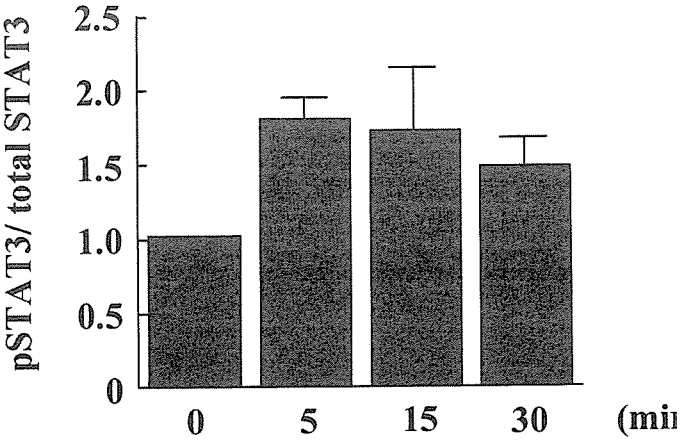

pSTAT3/ total STAT3
2.5
2.0
1.5
1.0
0.5
0
0
5
15
30
(mi

Fig.3

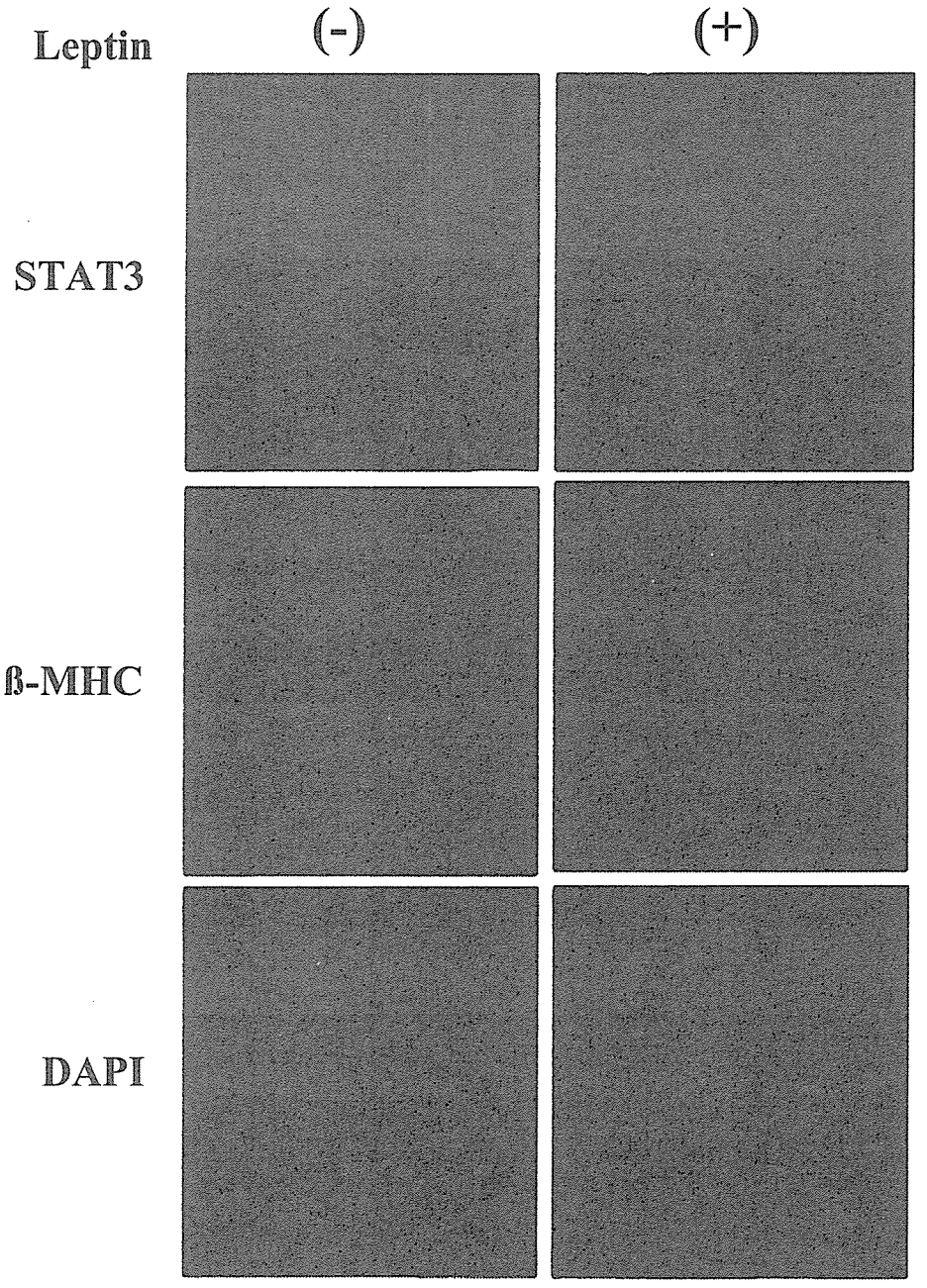


Fig.4

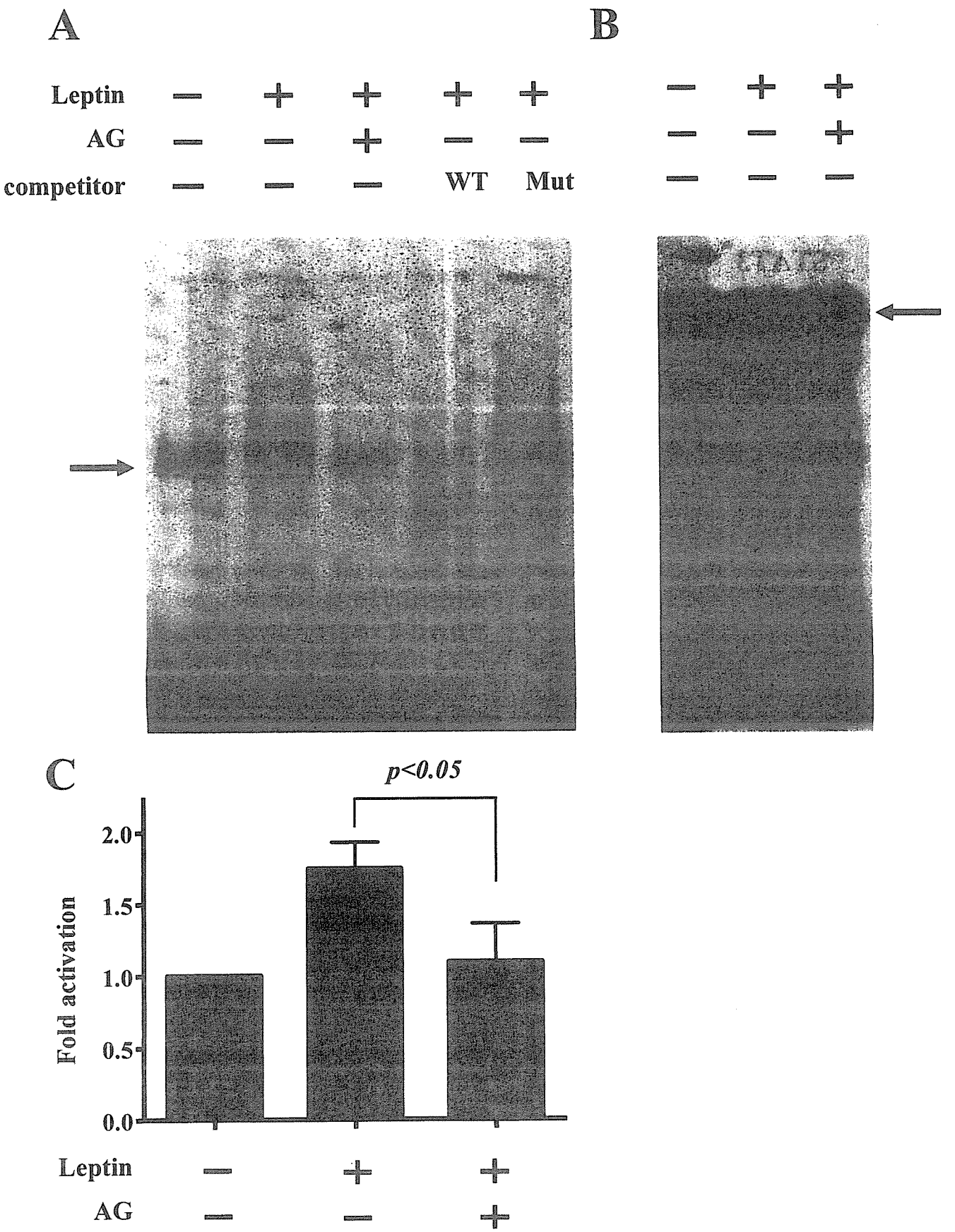
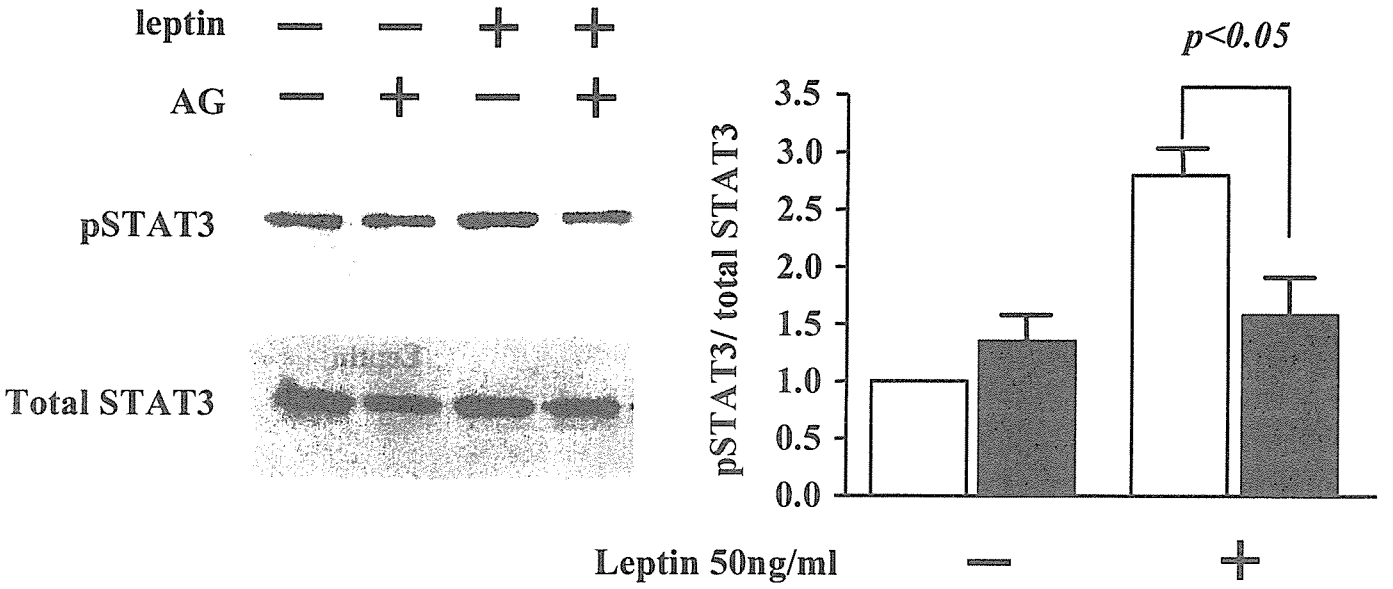

A
B
Leptin
AG
competitor
WT
Mut
C
p<0.05
Fold activation
2.0
1.5
1.0
0.5
0.0
Leptin
AG

Fig.5

A


leptin
AG
pSTAT3
Total STAT3
p<0.05
pSTAT3/ total STAT3
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Leptin 50ng/ml

B

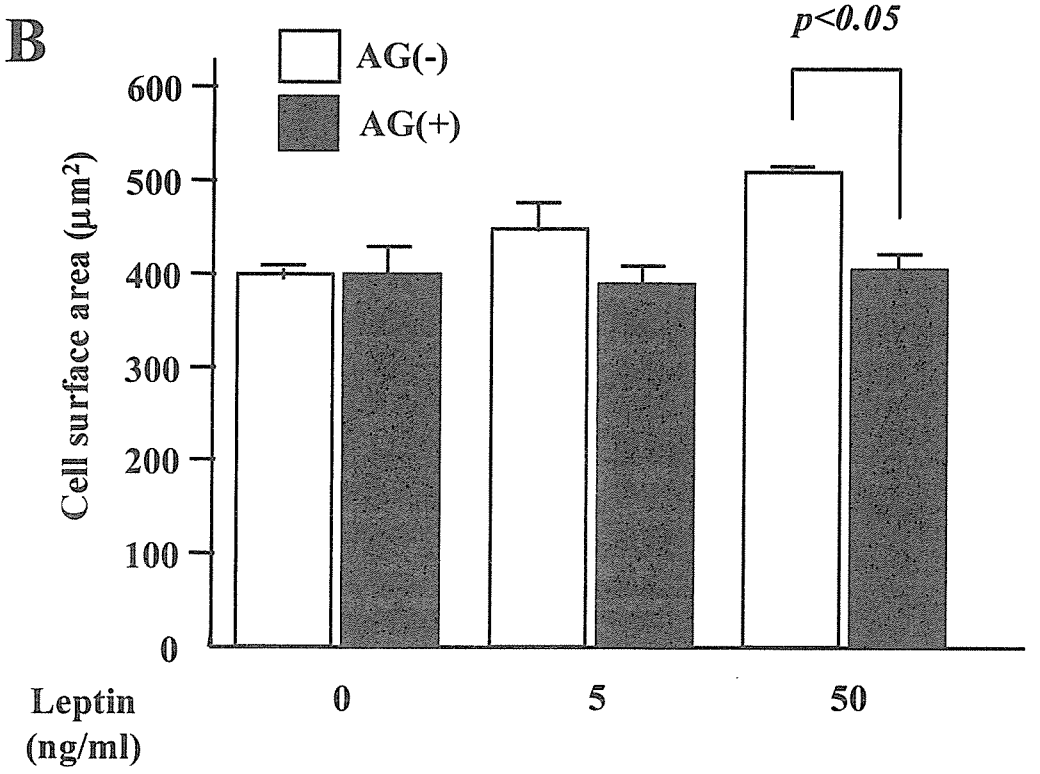

p<0.05
AG(-)
AG(+)
Cell surface area (μm²)
600
500
400
300
200
100
0
Leptin
(ng/ml)
0
5
50

Fig.6

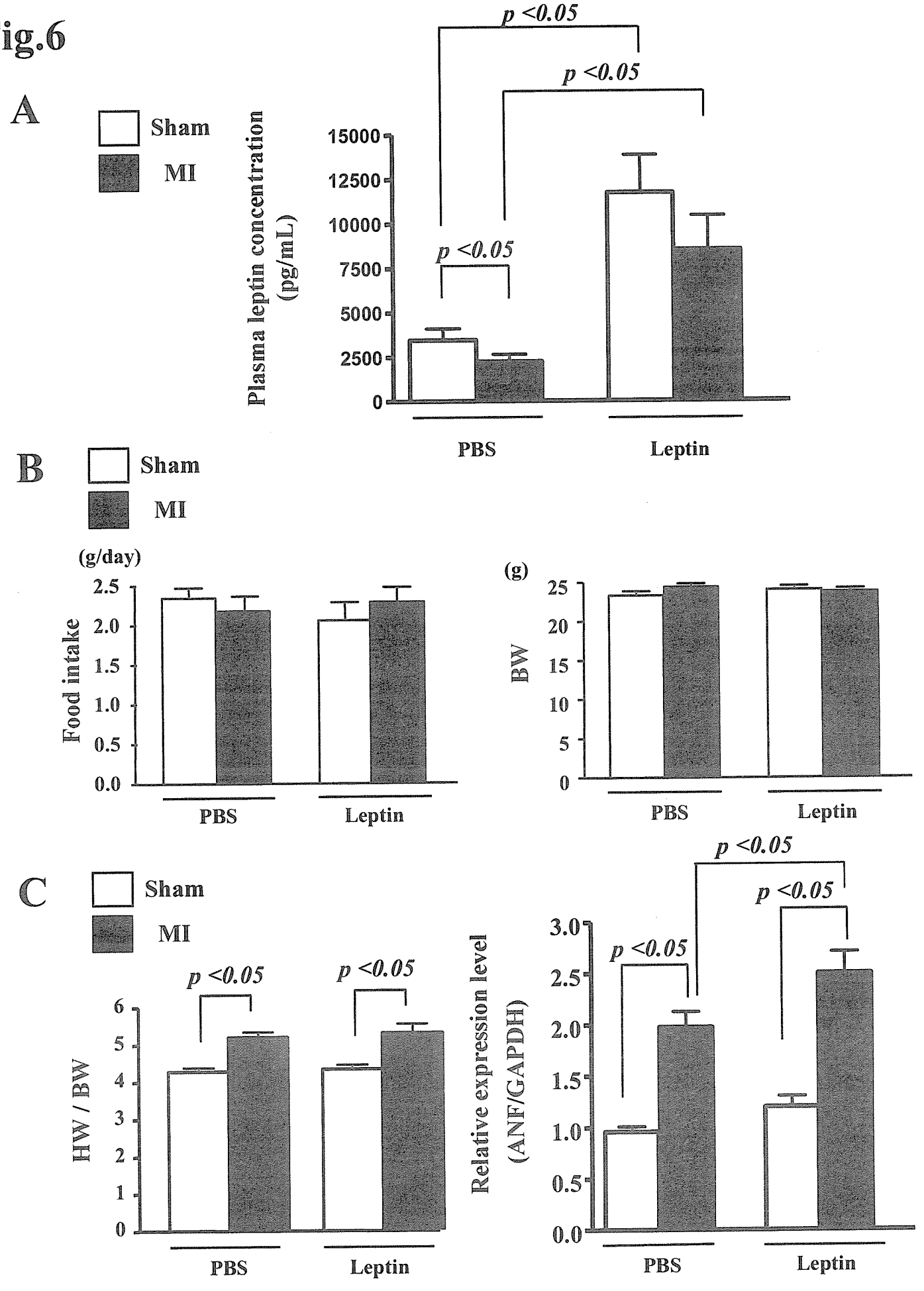
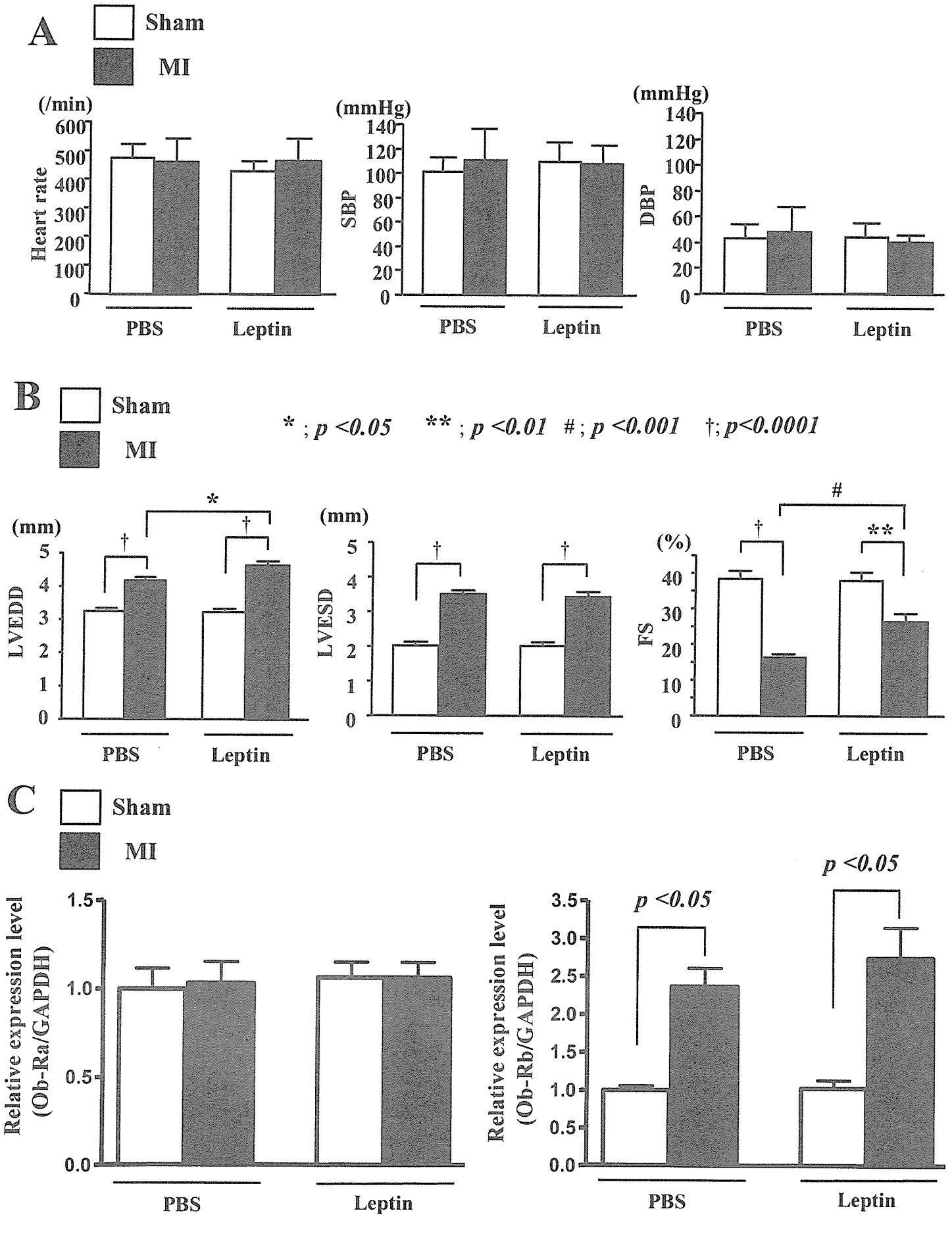

A
Sham
MI
Plasma leptin concentration (pg/mL)
15000
12500
10000
7500
5000
2500
0
p <0.05
p <0.05
p <0.05
PBS
Leptin
B
Sham
MI
(g/day)
Food intake
2.5
2.0
1.5
1.0
0.5
0.0
PBS
Leptin
(g)
BW
25
20
15
10
5
0
PBS
Leptin
C
Sham
MI
HW / BW
6
5
4
3
2
1
0
p <0.05
p <0.05
PBS
Leptin
Relative expression level (ANF/GAPDH)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
p <0.05
p <0.05
p <0.05
PBS
Leptin

Fig.7


A
Sham
MI
(/min)
Heart rate
600
500
400
300
200
100
0
PBS
Leptin
(mmHg)
SBP
140
120
100
80
60
40
20
0
PBS
Leptin
(mmHg)
DBP
140
120
100
80
60
40
20
0
PBS
Leptin
B
Sham
MI
* ; p <0.05
** ; p <0.01
# ; p <0.001
†; p<0.0001
(mm)
LVEDD
5
4
3
2
1
0
*
†
†
PBS
Leptin
(mm)
LVESD
5
4
3
2
1
0
†
†
PBS
Leptin
(%)
FS
40
30
20
10
0
#
†
**
PBS
Leptin
C
Sham
MI
Relative expression level
(Ob-Ra/GAPDH)
1.5
1.0
0.5
0.0
PBS
Leptin
Relative expression level
(Ob-Rb/GAPDH)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
p <0.05
p <0.05
PBS
Leptin