26th Spring Congress of the Korean Diabetes Association
& 1st Korea-Japan Diabetes Forum
05/11/2013 (SAT)

Activation of Heat Shock Response and Insulin Resistance

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What is Heat Shock Response: HSR ?

A New Puffing Pattern Indu
Shock and DNP in

The different puffing patterns
somes of Diptera show organ-sp
stage-specificity and sometimes z
patterns can be explained in terms
some activity. It is known that
coiling of some characteristic b
which have been shown to corr
delian loci[6,7].

It has also been shown recentl
of synthetic activity and that thei
For these reasons the different p
be more precisely interpreted in t
probably due to different metab
in the various organs and devel
gated.

Some recent investigations sh
induce directed variations in th
was accomplished by KROEGER[12]
gland nuclei of *D. busckii* into egg
gaster, and by CLEVER and KARL
tions of ecdysone in *Chironomus*
The purpose of this paper is to
effect of temperature on the puff
vary glands chromosomes of *Dr*
clearly appear that temperature

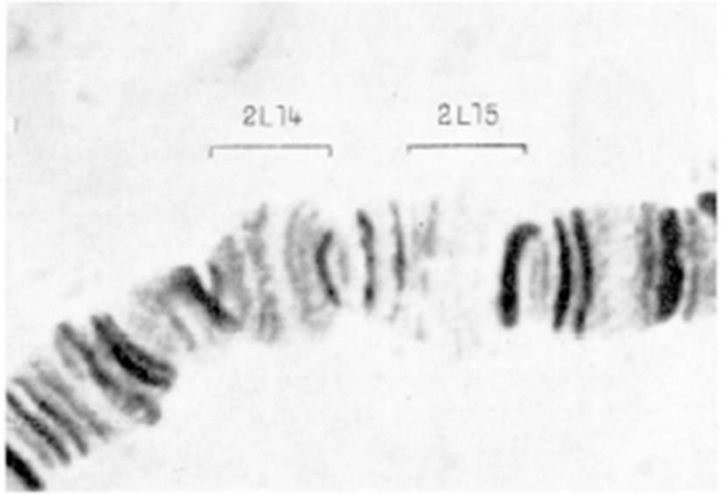


Fig. 1. The 2L 14 and 15 regions of salivary gland chromosome of *D. busckii* larvae reared at 25°C about 15 h before pupation.

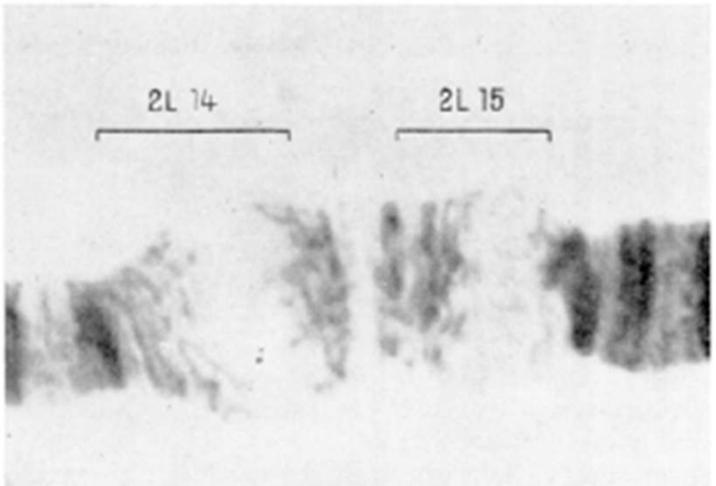


Fig. 2. The same regions as in Figure 1 after a thermal shock of 30 min at 30°C. Larvae near to pupation.

uffing patterns and that the
he same bands and involves

ited to the 2L chromosomes,
found in this region. In the

2L 14	2L 15	2L 20
– –	– –	
+	+	+

, 139 (1952).
511 (1953).
Chromosoma 7, 371 (1955).
ytology (Ed. Rudnik, Ronal Press,

. Exp. Cell Res. *6*, 195 (1954).
36 (1935).
(1938).
1957).
1959).
, 177 (1960).
al. 7, 147 (1962).
129 (1960).
xp. Cell Res. *20*, 623 (1960).

Molecular Life Sciences. 1962)

HSR and Diabetes

- Wholebody hyperthermia improves glucose homeostasis in mice and human type 2 diabetes.
  - Hooper PL., Hot-tub therapy for type 2 diabetes mellitus. *N Engl J Med.* 1999.
  - Kokura S et al., Wholebody hyperthermia improves obesity-induced insulin resistance in diabetic mice. *Int J Hyperthermia*. 2007.

- Hsp72 mRNA is decreased in insulin resistant type 2 diabetic patients.
  - Bruce CR et al., Intramuscular Hsp72 and HO-1mRNA are reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defence mechanism. *Diabetes*. 2003.
  - Kurucz I et al., Decreased expression of Hsp72 in skeletal muscle of patients with type 2 diabetes correlated with insulin resistance. *Diabetes*. 2002.

- HSP72 protein is decreased in insulin resistant type 2 diabetic patients.
- Long-term hyperthermia, muscle-specific HSP72 Tg, or BGP-15 ameliorate insulin resistance in the mouse model of type 2 diabetes.
  - Chung J et al., HSP72 protects against obesity-induced insulin resistance. *Proc Natl Acad Sci USA. 2008.*

Activation of HSR may contribute to improving metabolic abnormalities in type 2 diabetes.

Hooper PL. *N Engl J Med.* 1999 Sep 16;341(12):924-5.

Hot-Tub therapy:

- 30 min a day
- 6 days a week
- 3 weeks
- Water temp. 37.8～40.5℃
- Oral temp. 0.8℃↑

Results:

- BW 1.7 ± 2.7 kg↓ (p=0.08)
- FBS 182 ± 37 mg/dL
  → 159 ± 42 mg/dL (p=0.02)
- HbA1c 11.3 ± 3.1 %
  → 10.3 ± 2.6 % (p=0.004)

TABLE 1. CHARACTERISTICS OF THE EIGHT PATIENTS AND RESULTS OF THREE WEEKS OF EXPOSURE TO A HOT TUB.

AGE	SEX	DURATION OF DIABETES	MEDICATIONS	BODY WEIGHT (BEFORE/AFTER EXPOSURE)	FASTING PLASMA GLUCOSE (BEFORE/AFTER EXPOSURE)*	GLYCOSYLATED HEMOGLOBIN (BEFORE/AFTER EXPOSURE)†
yr		yr		kg	mg/dl	%
43	M	14	Glyburide, metformin hydrochloride	83.2/80.9	190/186	13.6/12.7
50	M	13	Glyburide, troglitazone, insulin	201.8/199.1	109/66	8.6/7.7
51	M	9	Glyburide, metformin hydrochloride, insulin	175.0/168.2	231/181	12.2/11.1
54	F	9	Metformin hydrochloride, insulin	60.9/61.8	207/156	17.4/14.8
57	F	8	Glipizide, metformin hydrochloride	64.5/64.5	197/155	11.0/11.1
57	M	3	Glyburide, troglitazone	75.0/73.6	165/162	8.6/7.6
63	M	11	Glipizide, metformin hydrochloride	91.8/91.8	158/160	9.1/8.1
68	F	9	Glyburide, metformin hydrochloride, troglitazone	85.5/84.1	197/203	9.5/8.9

*To convert the values to millimoles per liter, multiply by 0.05551.

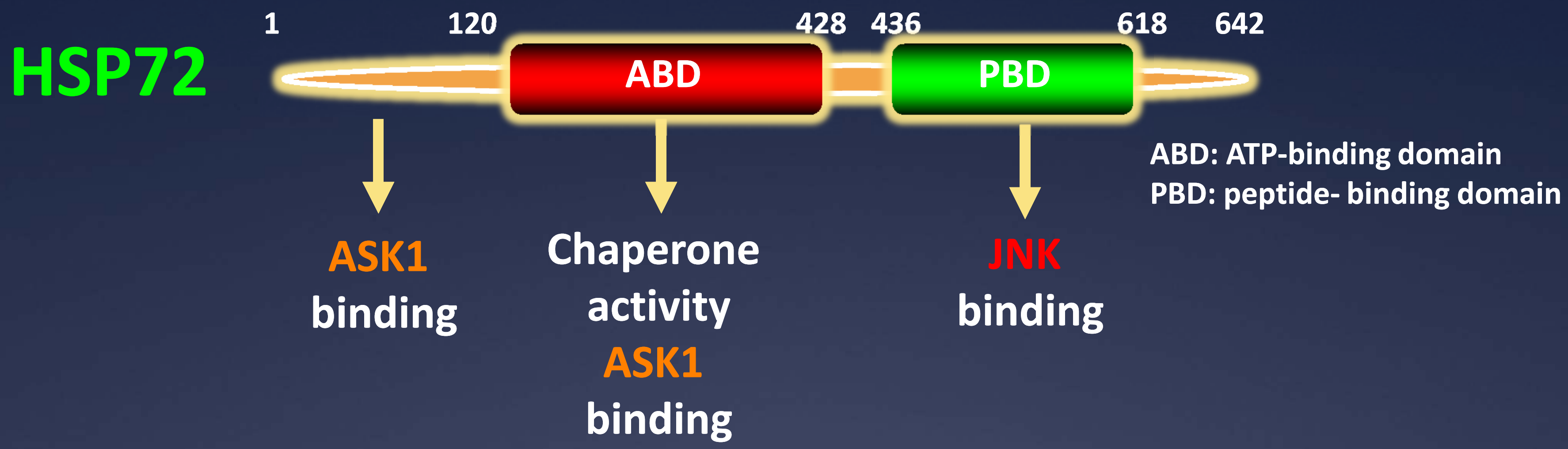
†The normal range was 4 to 8 percent.

Heat stimulation → Blood flow↑ → Glucose uptake↑

(Baron AD. *Am J Physiol*. 1994)

Heat stimulation → HSP72↑ → JNK↓, ASK1↓

HSP72 (Heat Shock Protein 72)



- Inducible upon exposure to environmental stress that causes protein misfolding in the cytosol, such as heat shock, exposure to heavy metals and ischemia
- Strong cyto-protective effects and functions as a molecular chaperone in protein folding, transport, and degradation
- Protects against ischemic cerebro and cardio vascular disease
- Inhibits JNK by several distinct mechanisms

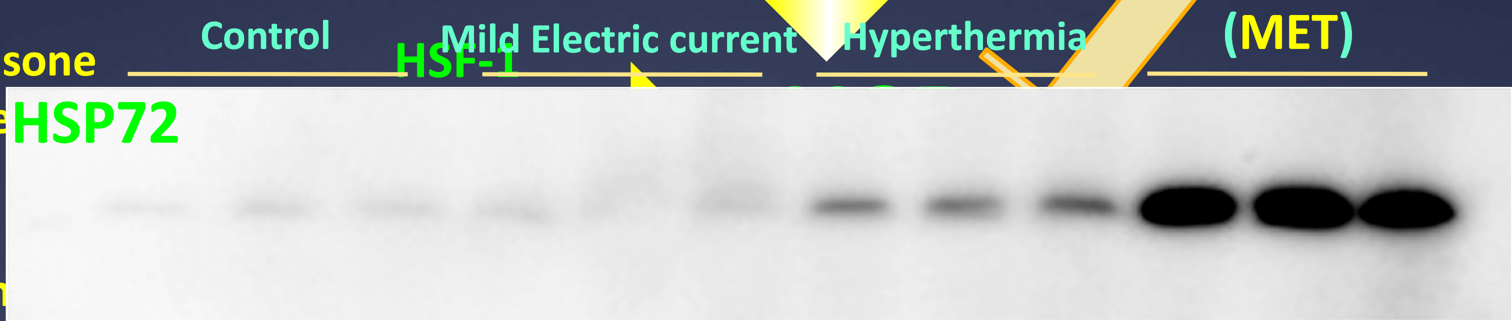
  →physical interaction, activation of MAPK phosphatase-1 and -3, inactivation of DLK-1, and suppression of MAPK kinase-1 and -7

HSR activator / suppressor

Heat shock
Cold shock (sympathetic activation)
Exercise
PPARγ agonist (PIO, TRO, CI)
GGA
BRX-220
Bimoclomol
cAMP, PKA
Dexamethasone
L-glutamate
ER stress
TNF-α
Norepineph
β-adrenergic signal (Isoproterenol)
Tyroxine
Insulin + Heat shock

Hyperthermia
+
Mild electric current

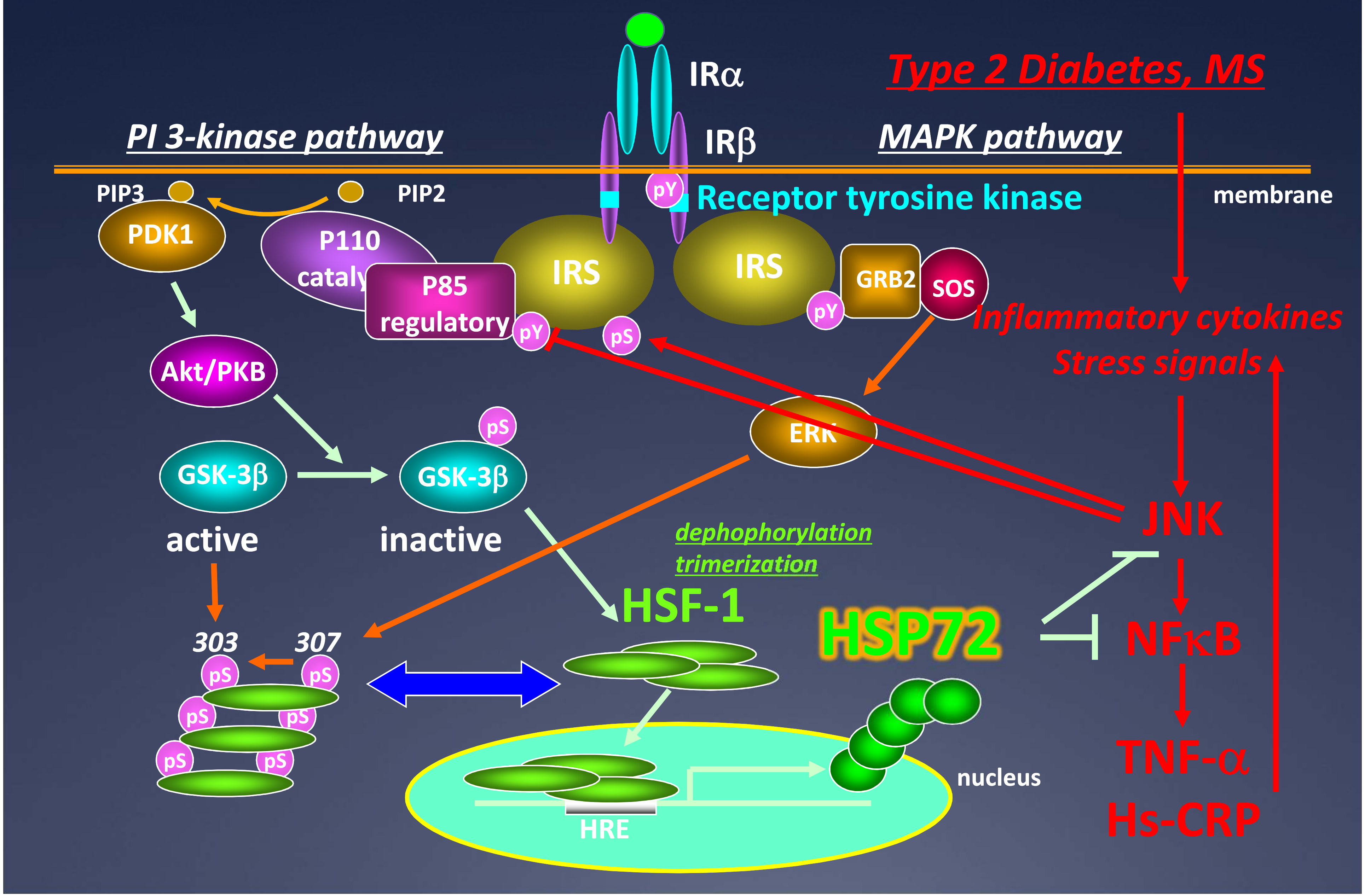
Estrogen
FAS ligand
Aging
Diabetes
(Insulin resistance)



- Cell survival
- Akt activation (PI3K dependent or independent)
- Mitochondrial biogenesis

- Apoptosis
- Apoptosis-inducing factor (AIF)
- JNK
- ASK1
- NF-κB activation

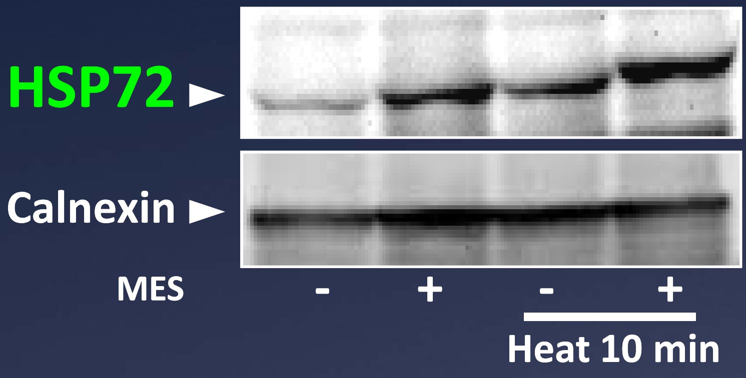
HSR attenuation and insulin resistance

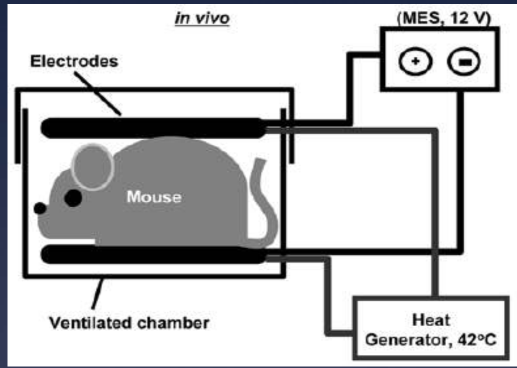


MES with hyperThermia (MET)

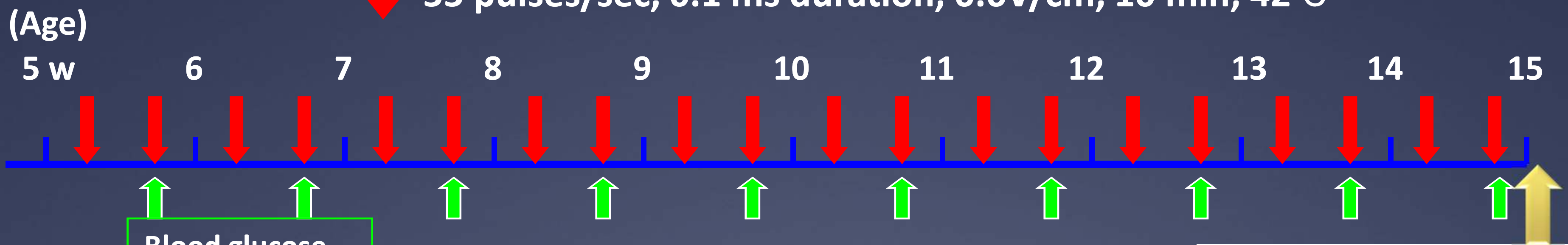
in vivo (muscle)

MET





MET-treatment, twice a week (Tue, Fri)
55 pulses/sec, 0.1 ms duration, 0.6V/cm, 10 min, 42℃



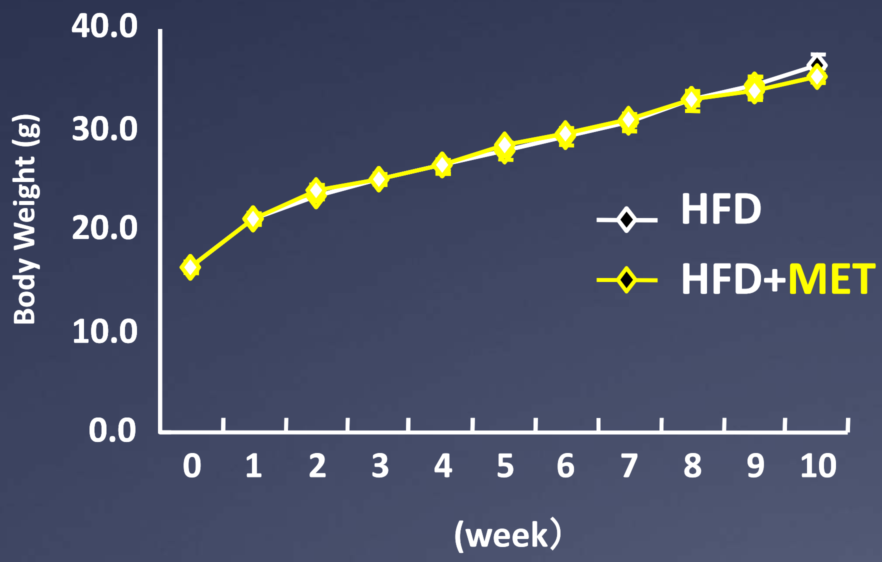
Blood glucose
Body weight
Food intake

i.p. GTT, i.p. ITT
Biochemical markers
Inflammatory markers
Insulin signal etc

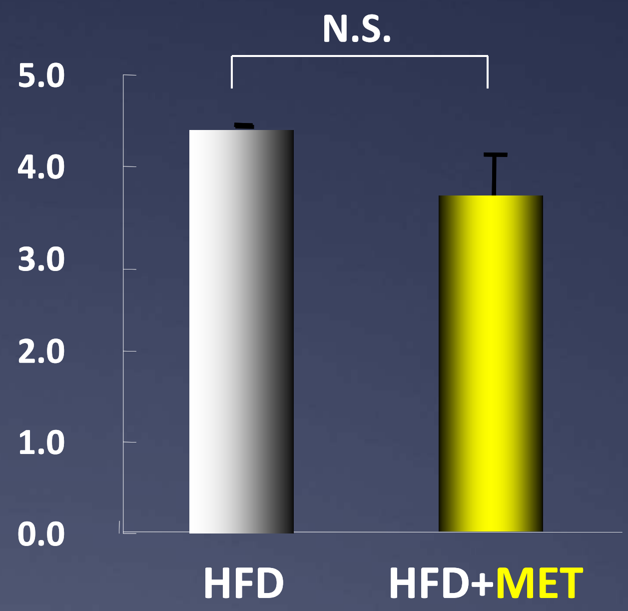
Morino S, Kondo T et al. *PLoS ONE* 2008.

Body Weight and Food Intake in HFD mice

Body Weight



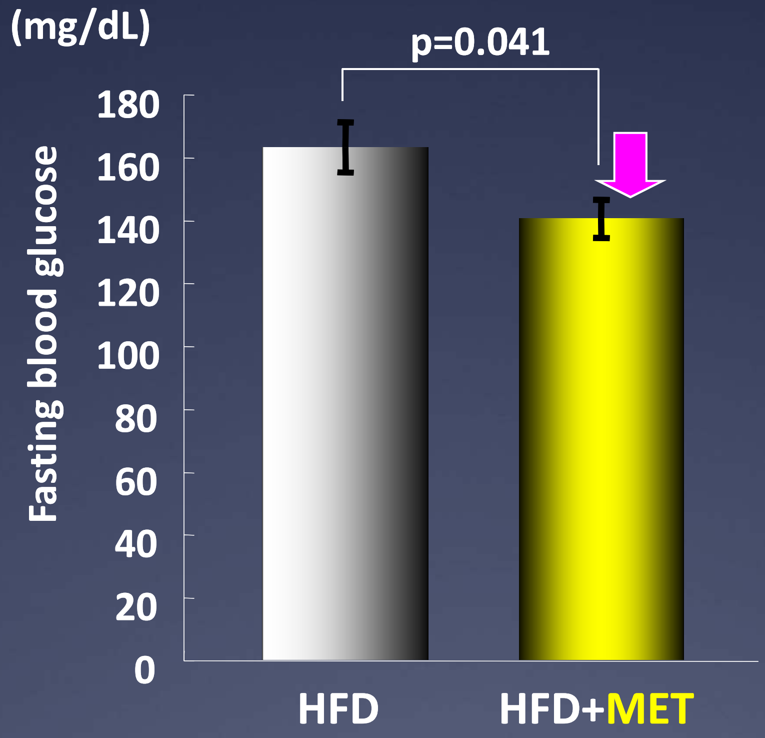
Food Intake



Morino S, Kondo T et al. *PLoS ONE* 2008.

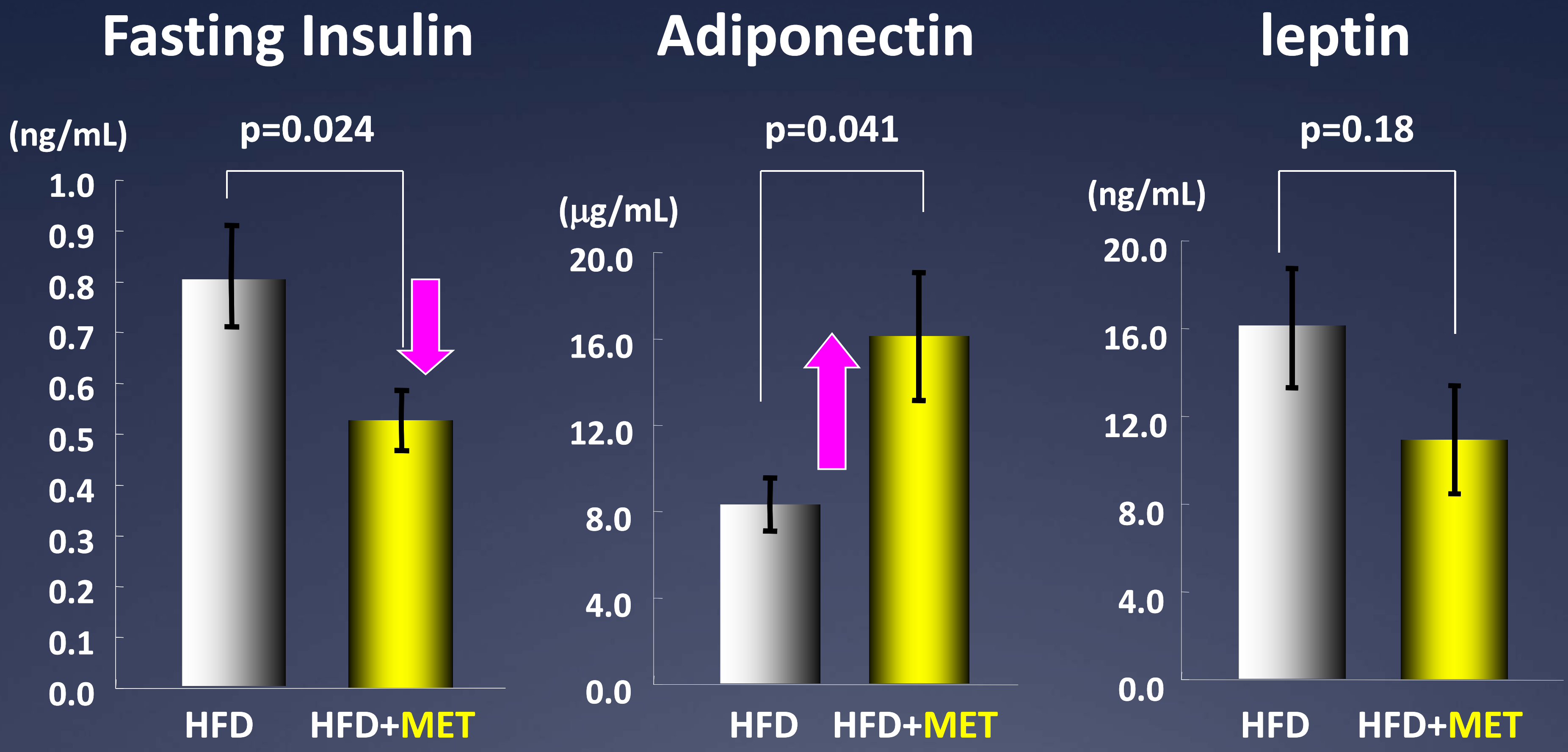
Blood Glucose Levels in HFD mice

Fasting Blood Glucose (10w)



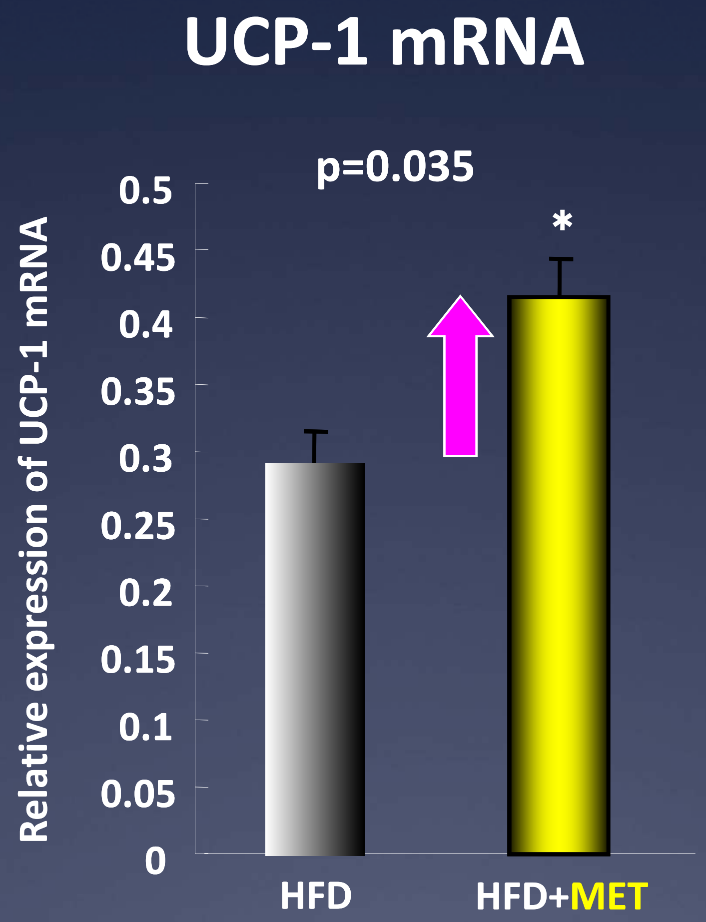
Morino S, Kondo T et al. *PLoS ONE* 2008.

Metabolic Parameters in HFD mice



Morino S, Kondo T et al. *PLoS ONE* 2008.

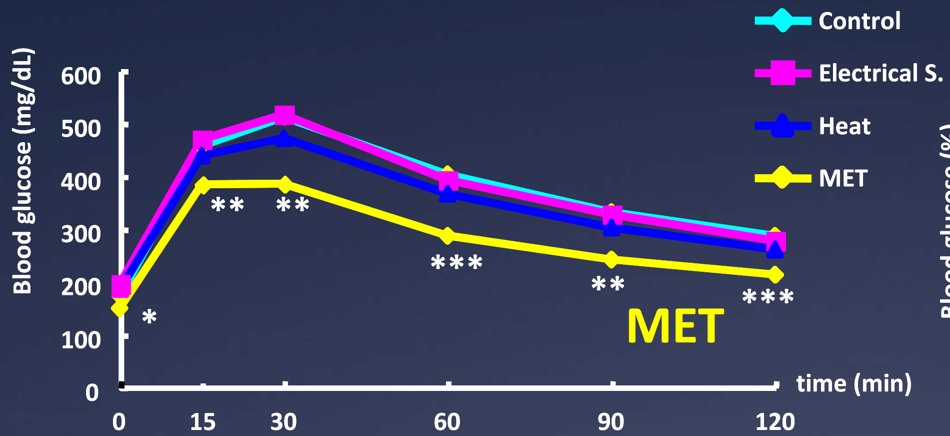
UCP-1 mRNA Expression in BAT of HFD mice



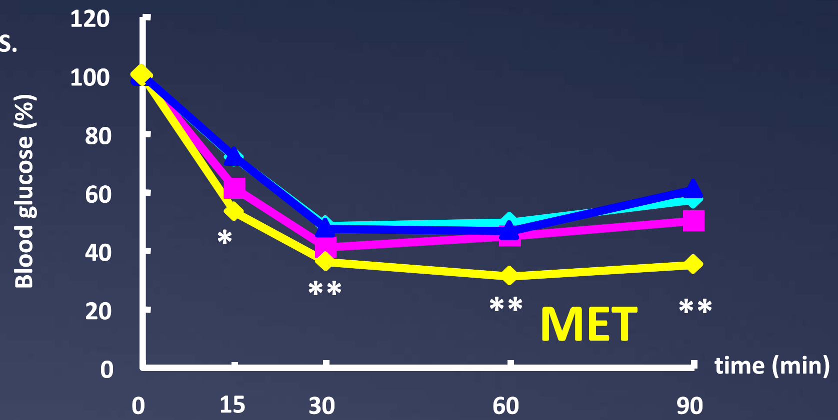
Morino S, Kondo T et al. *PLoS ONE* 2008.

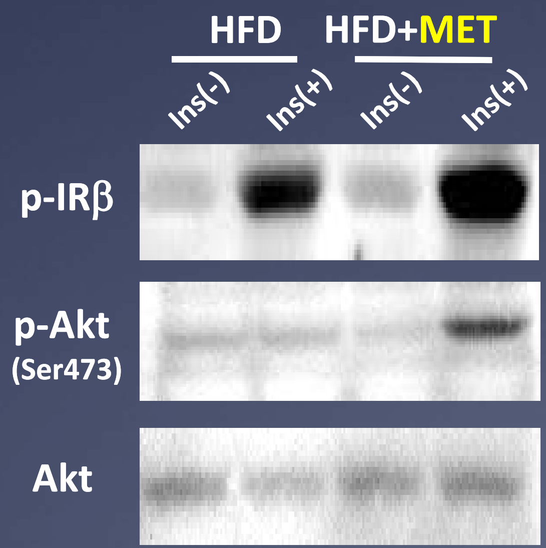
Glucose Tolerance and Insulin Resistance in HFD mice

Glucose tolerance test

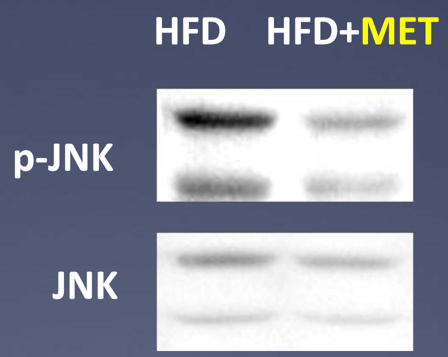


Insulin tolerance test





JNK inactivation

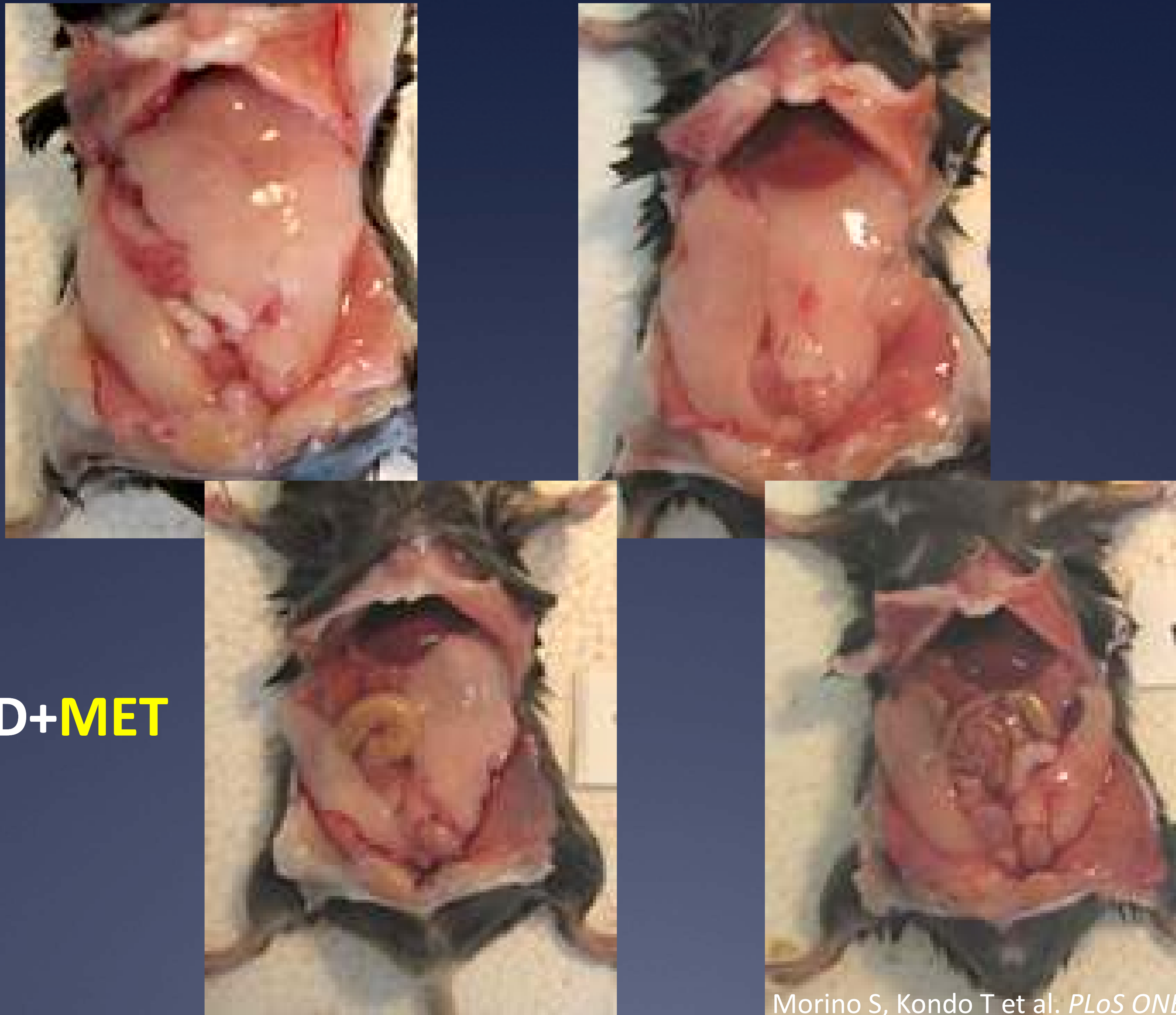


Morino S, Kondo T et al. *PLoS ONE* 2008.

Intra-Abdominal Adiposity in HFD

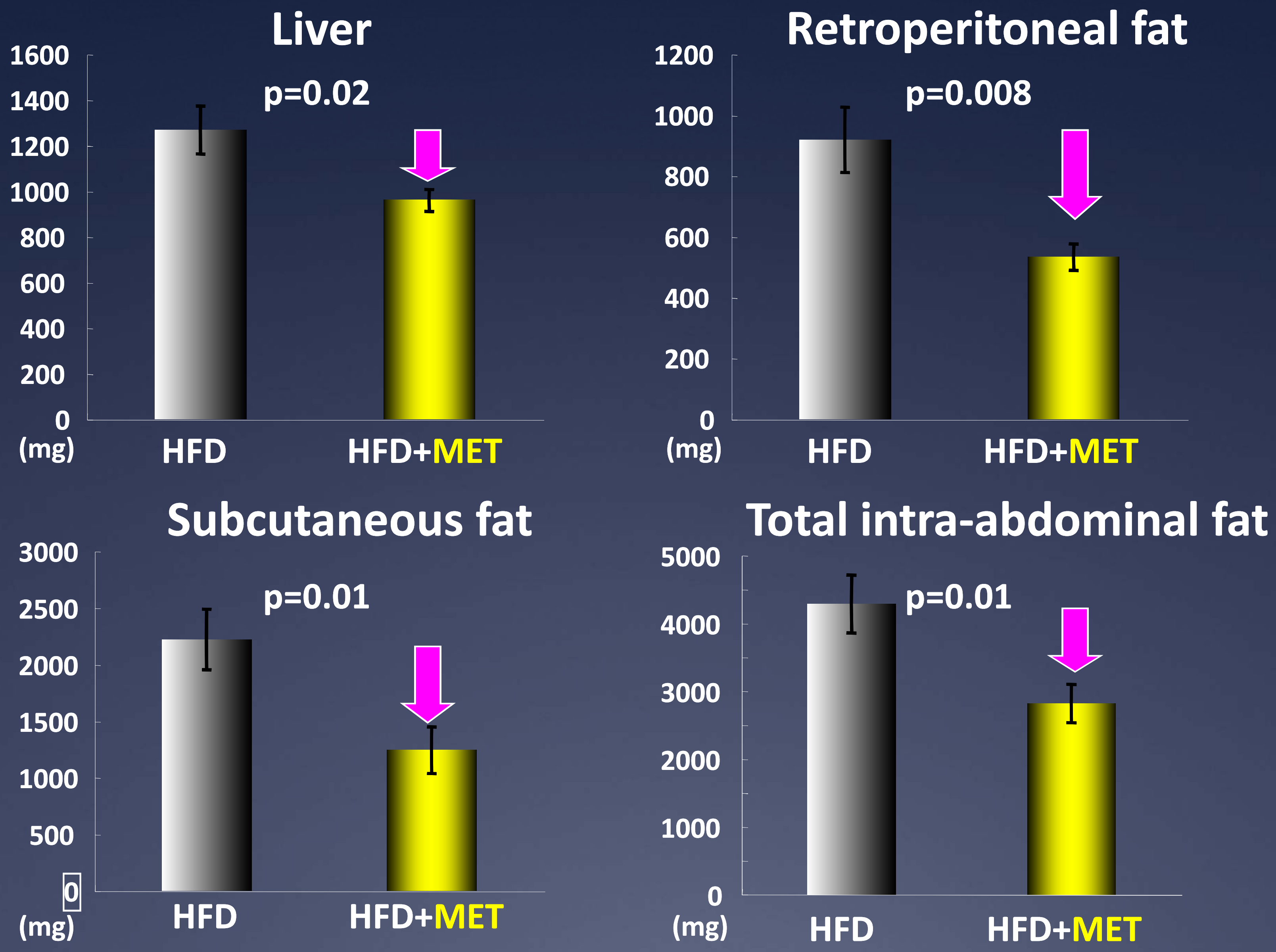
HFD

HFD+MET



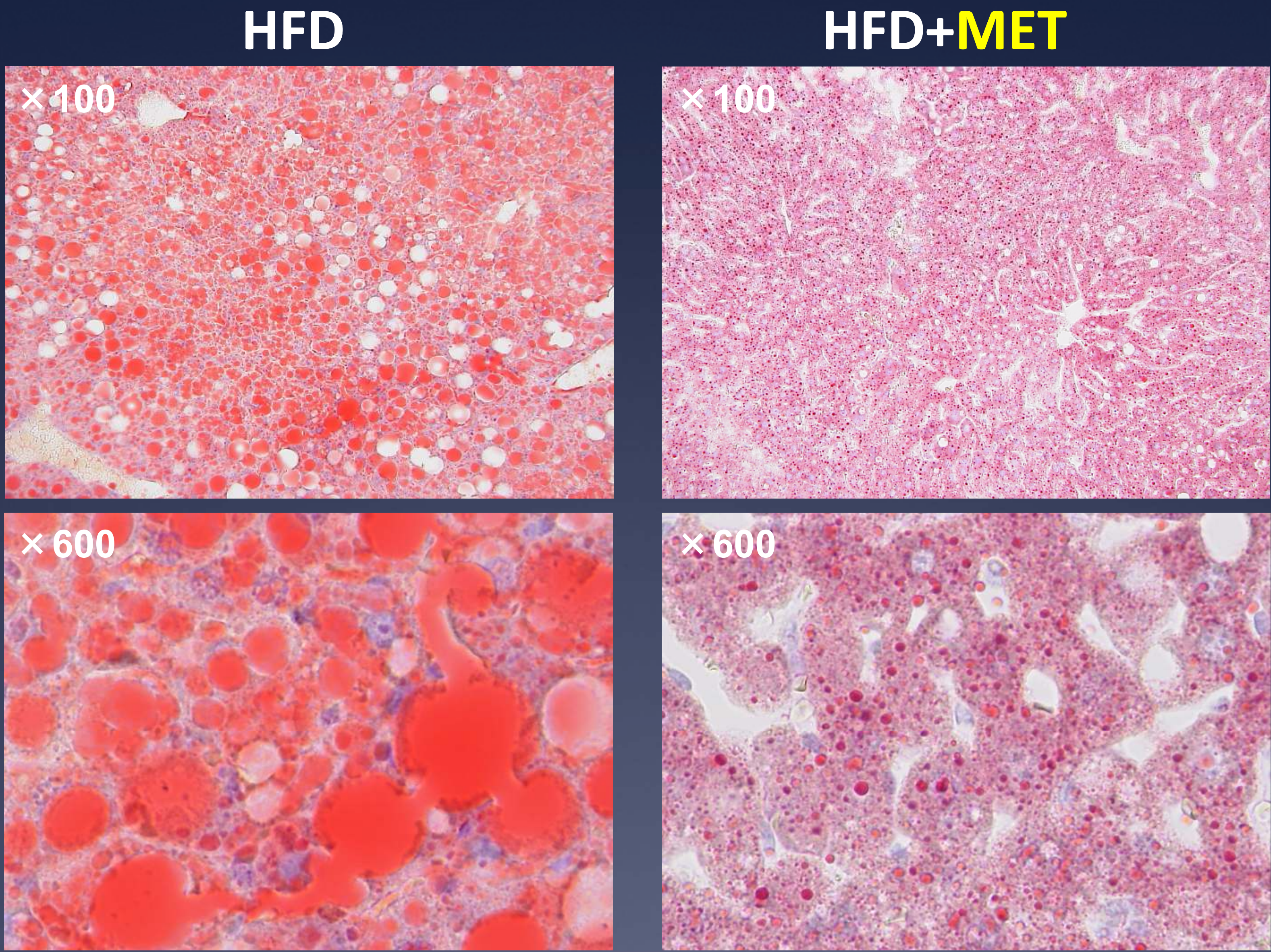
Morino S, Kondo T et al. *PLoS ONE* 2008.

Reduction of liver and adipose tissue weight



Morino S, Kondo T et al. *PLoS ONE* 2008.

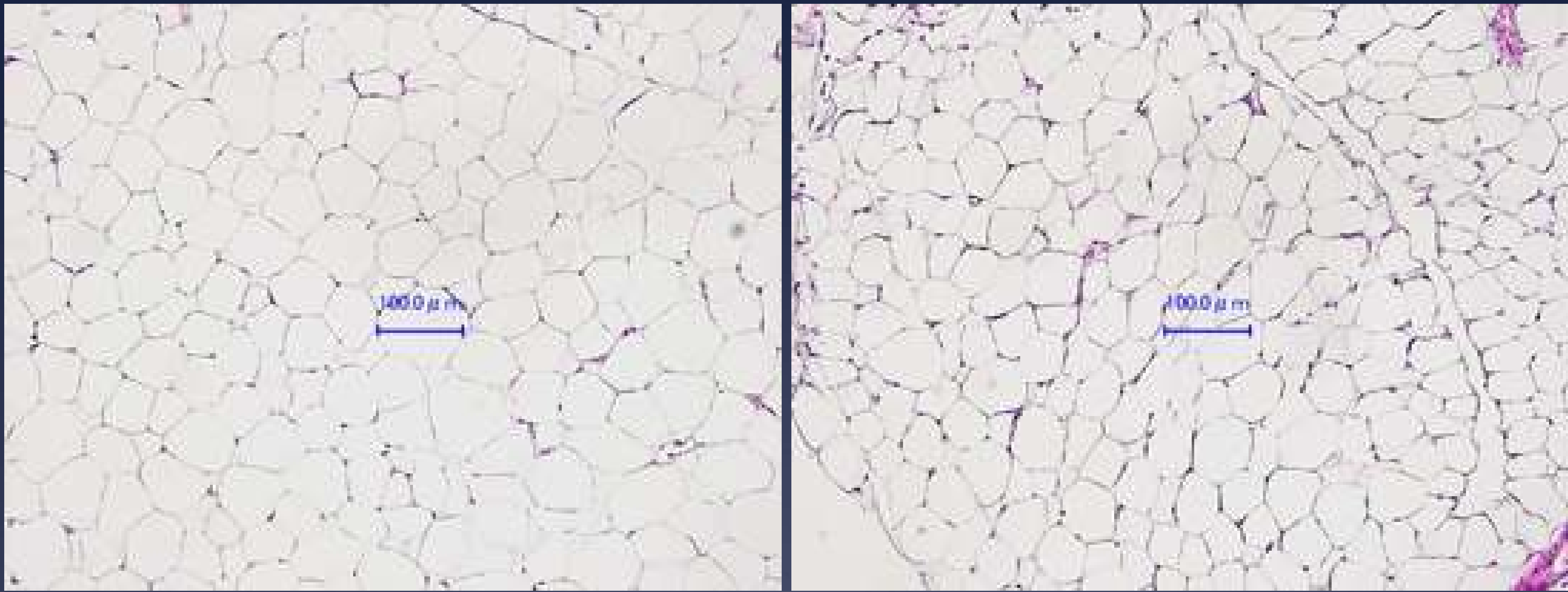
Lipid Accumulation in Liver of HFD mice

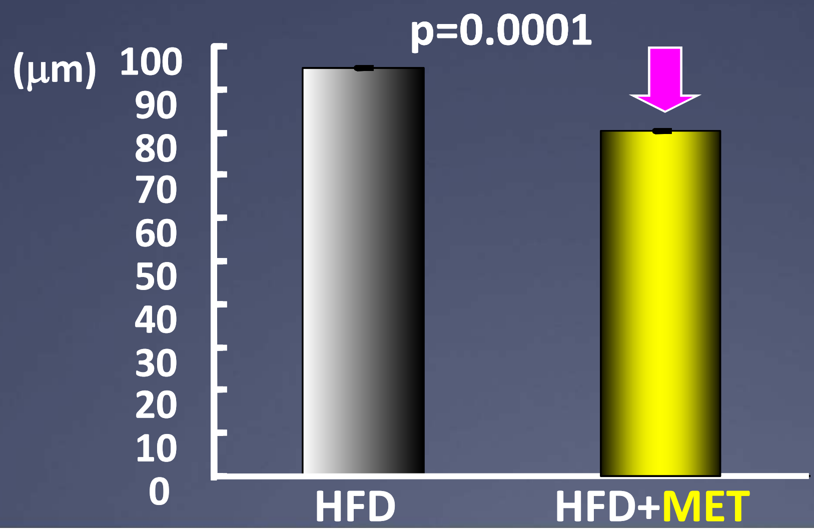


Morino S, Kondo T et al. *PLoS ONE* 2008.

Adipocyte Size in Mescenteric Fat of HFD mice

HFD HFD+MET

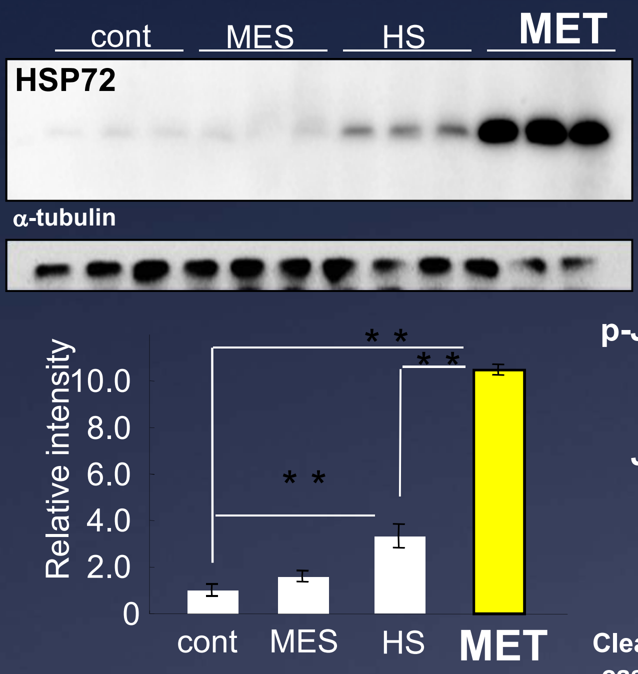




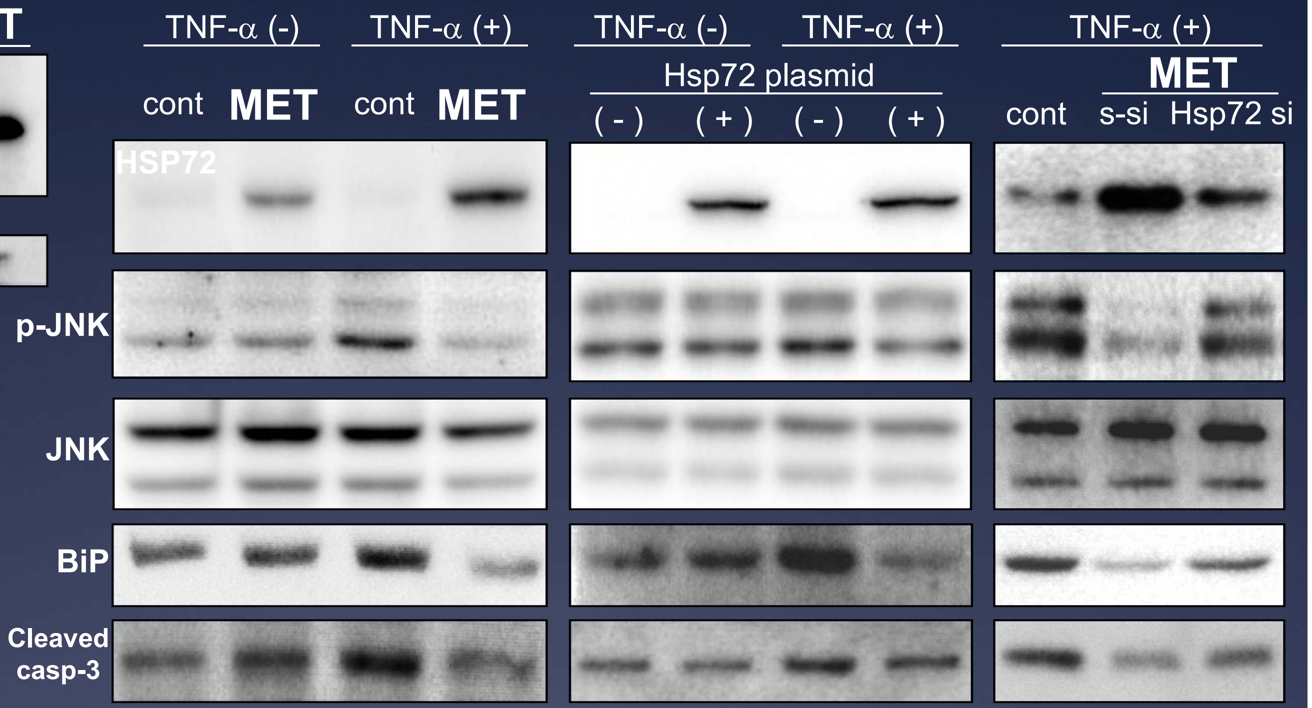
Morino S, Kondo T et al. *PLoS ONE* 2008.

Activation of HSR by MET in MIN6 cells

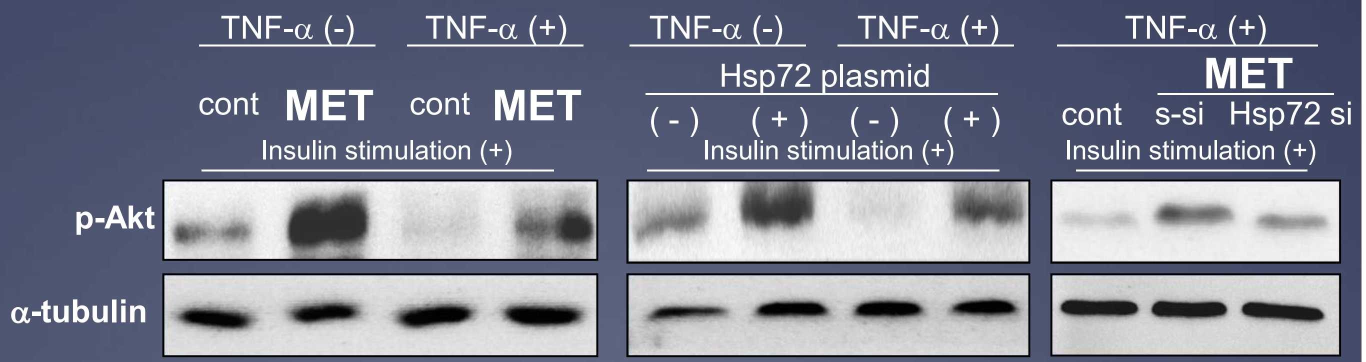
HSP72 protein expression



MET or HSP72 regulates stress and apoptotic signal

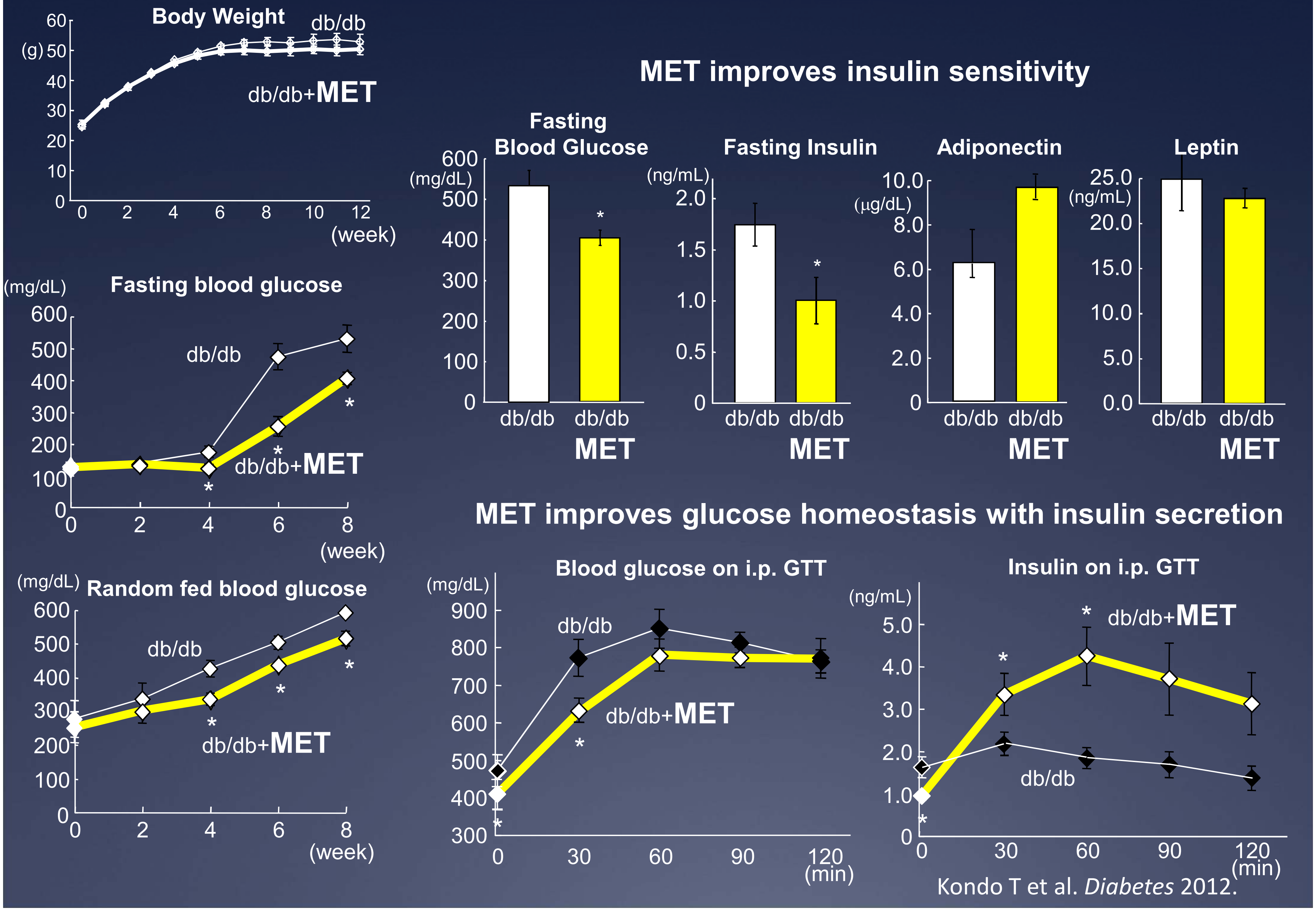


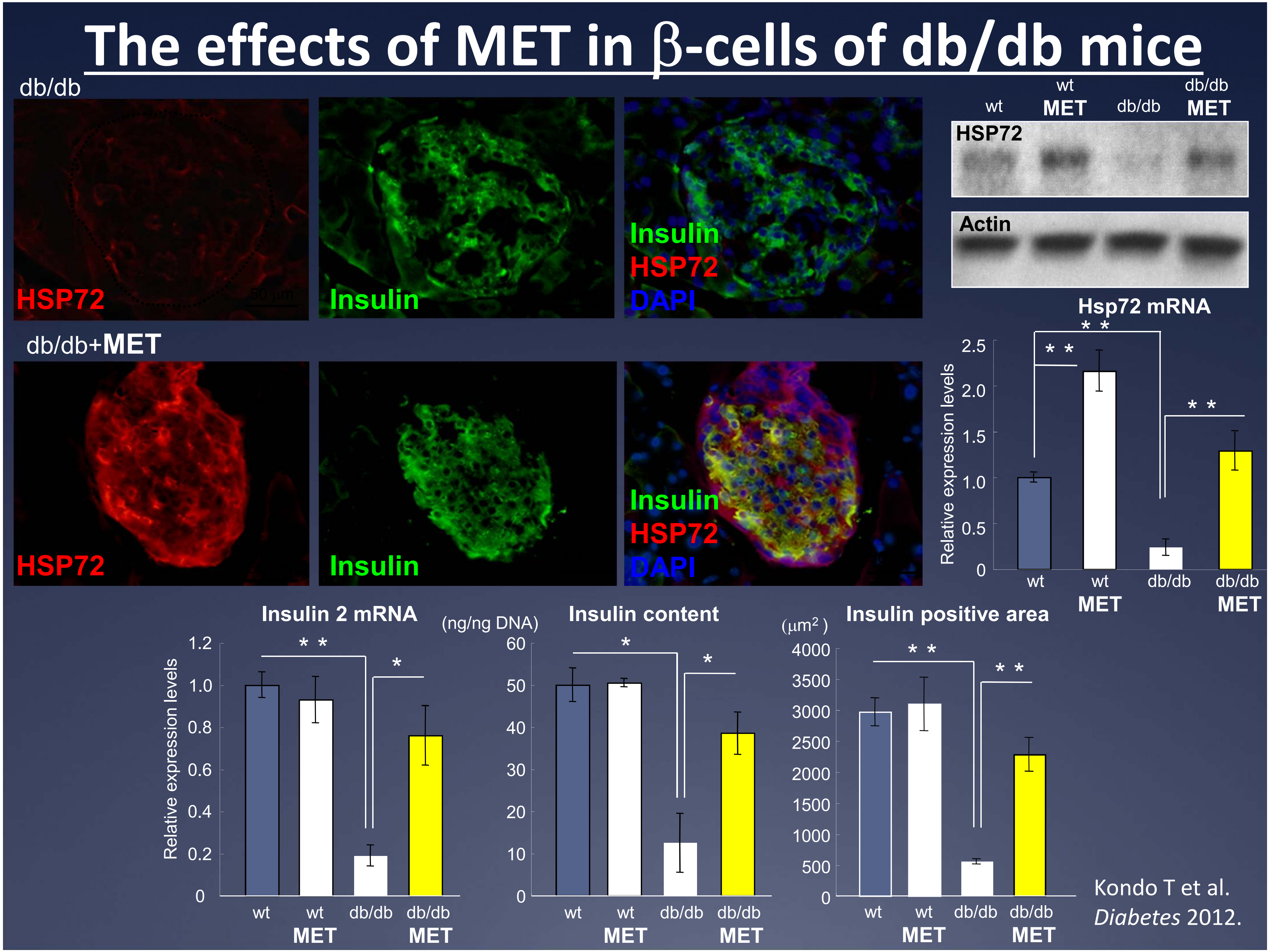
MET or HSP72 activates Akt upon insulin stimultaion



Kondo T et al. *Diabetes* 2012.

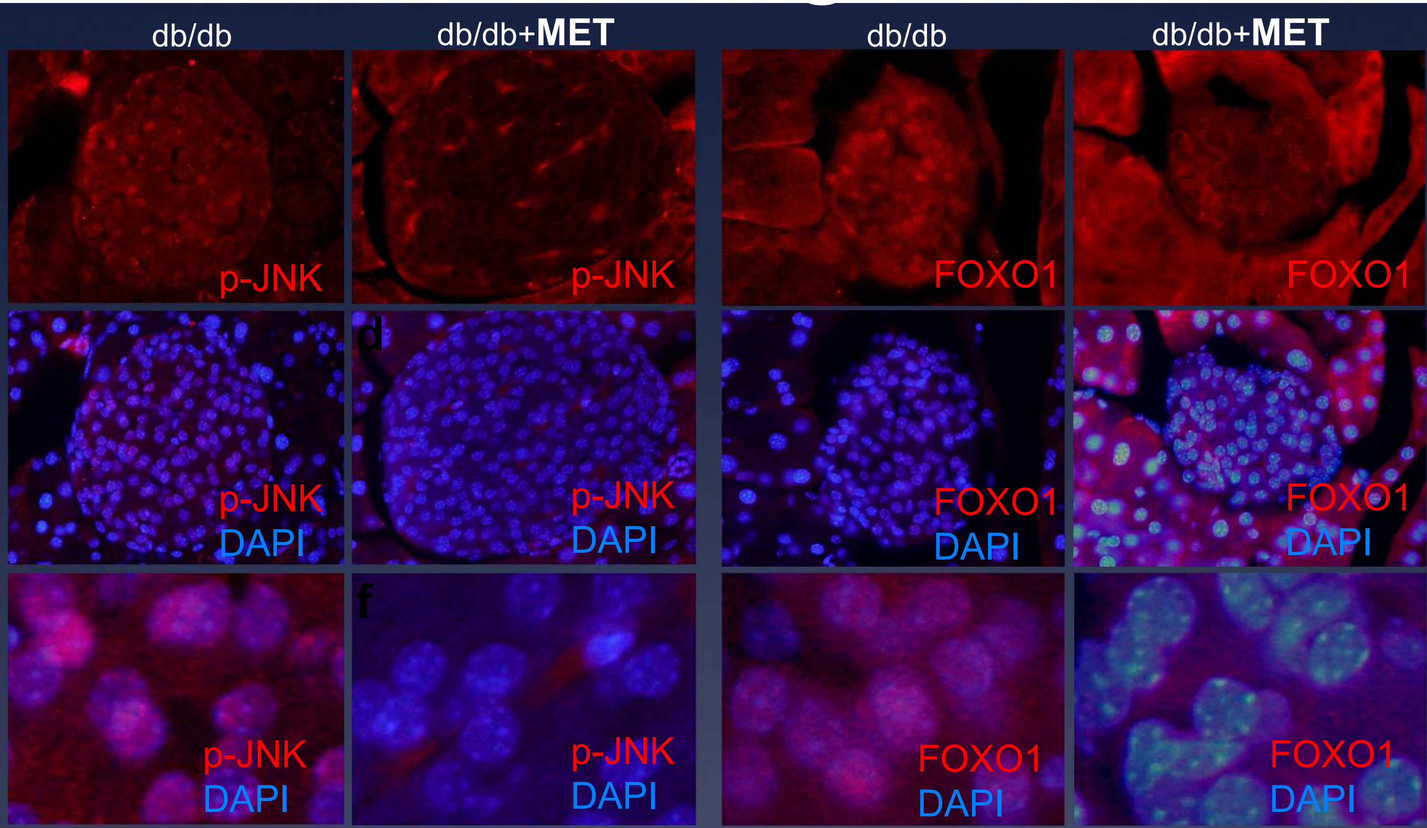
Metabolic impacts of MET in db/db mice

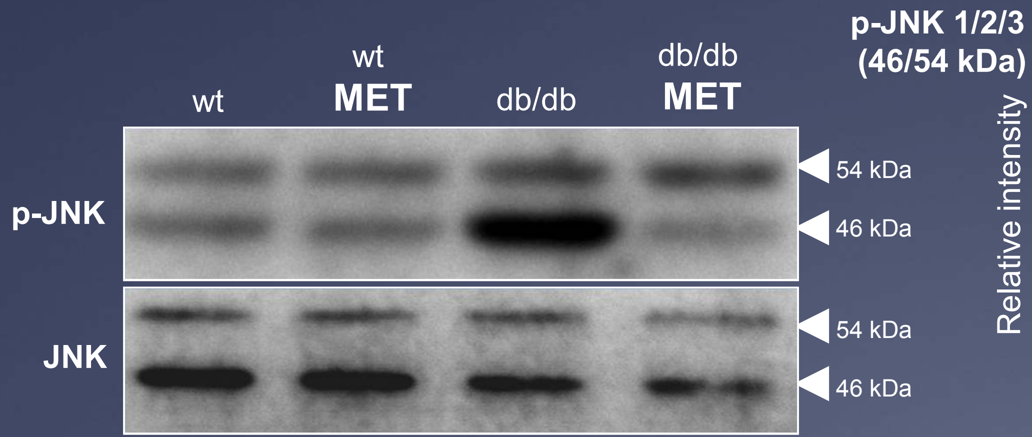


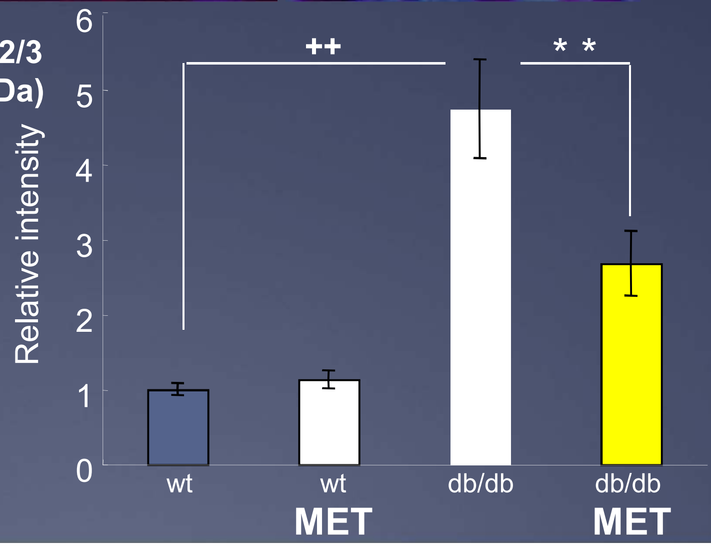


The effects of MET in β-cells of db/db mice
db/db
HSP72
Insulin
Insulin
HSP72
DAPI
db/db+MET
HSP72
Insulin
Insulin
HSP72
DAPI
wt
wt MET
db/db
db/db MET
HSP72
Actin
Hsp72 mRNA
Relative expression levels
wt
wt MET
db/db
db/db MET
Insulin 2 mRNA
Relative expression levels
wt
wt MET
db/db
db/db MET
(ng/ng DNA)
Insulin content
wt
wt MET
db/db
db/db MET
(μm²)
Insulin positive area
wt
wt MET
db/db
db/db MET
Kondo T et al.
Diabetes 2012.

MET attenuates JNK signal in db/db mice

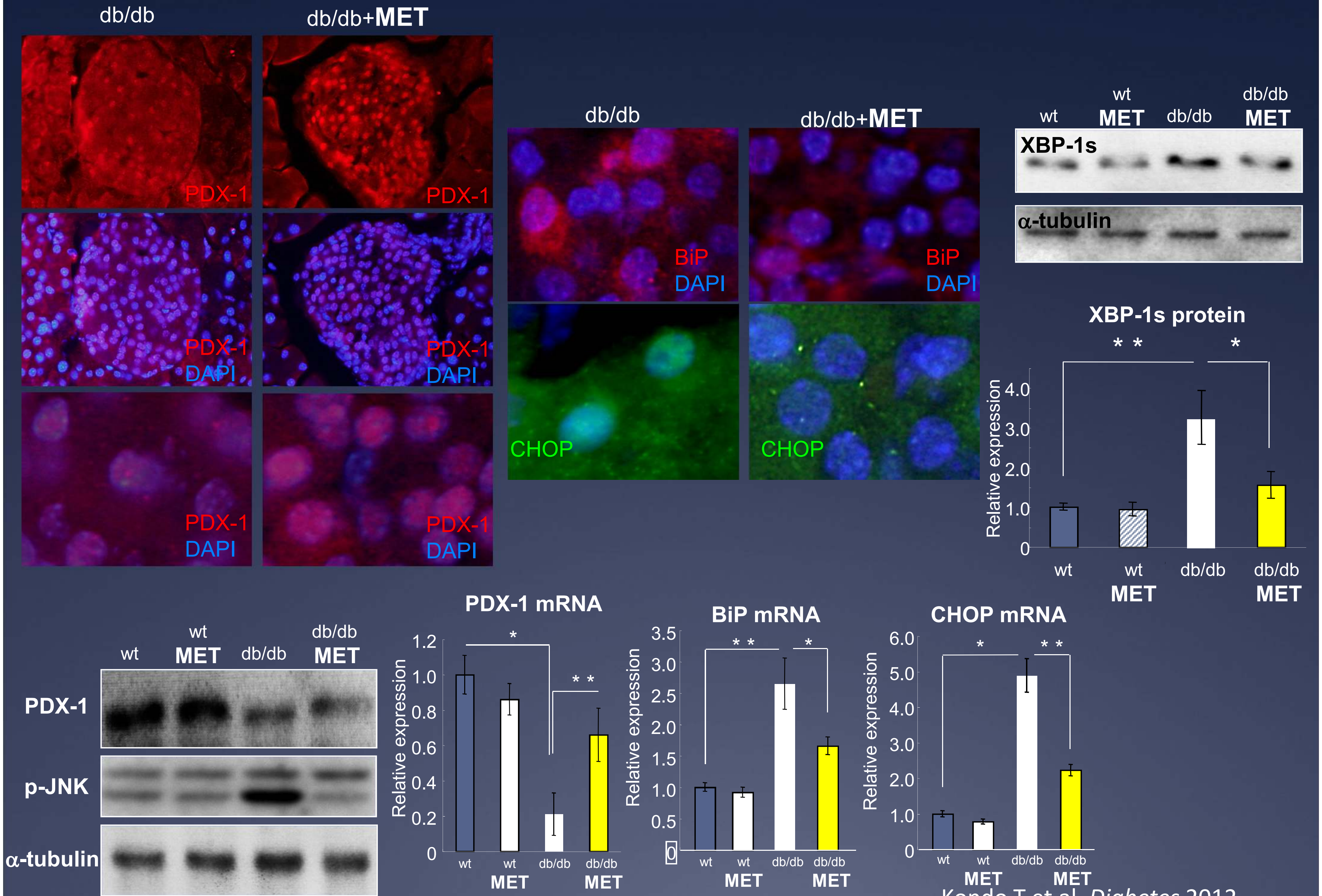






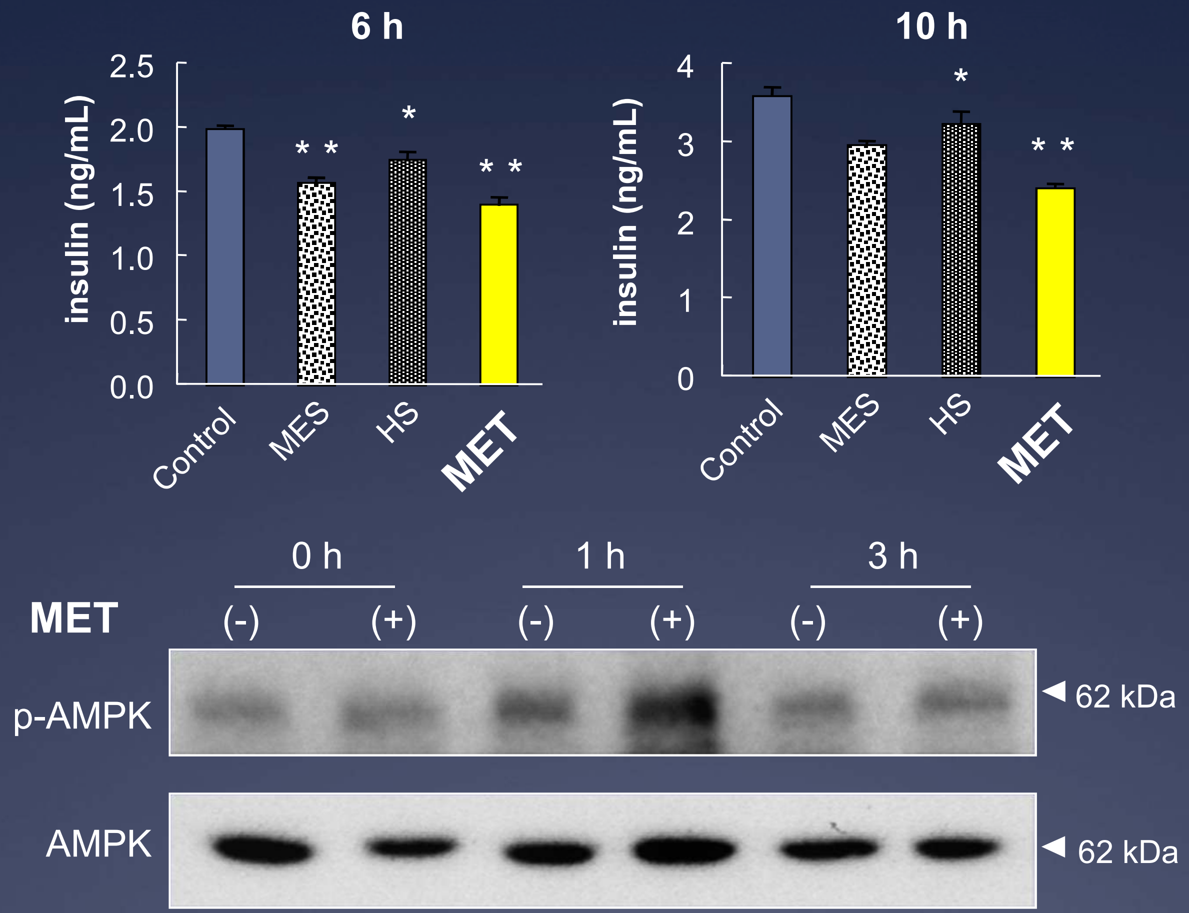
Kondo T et al. *Diabetes* 2012.

MET increases PDX-1 and attenuates stress signals



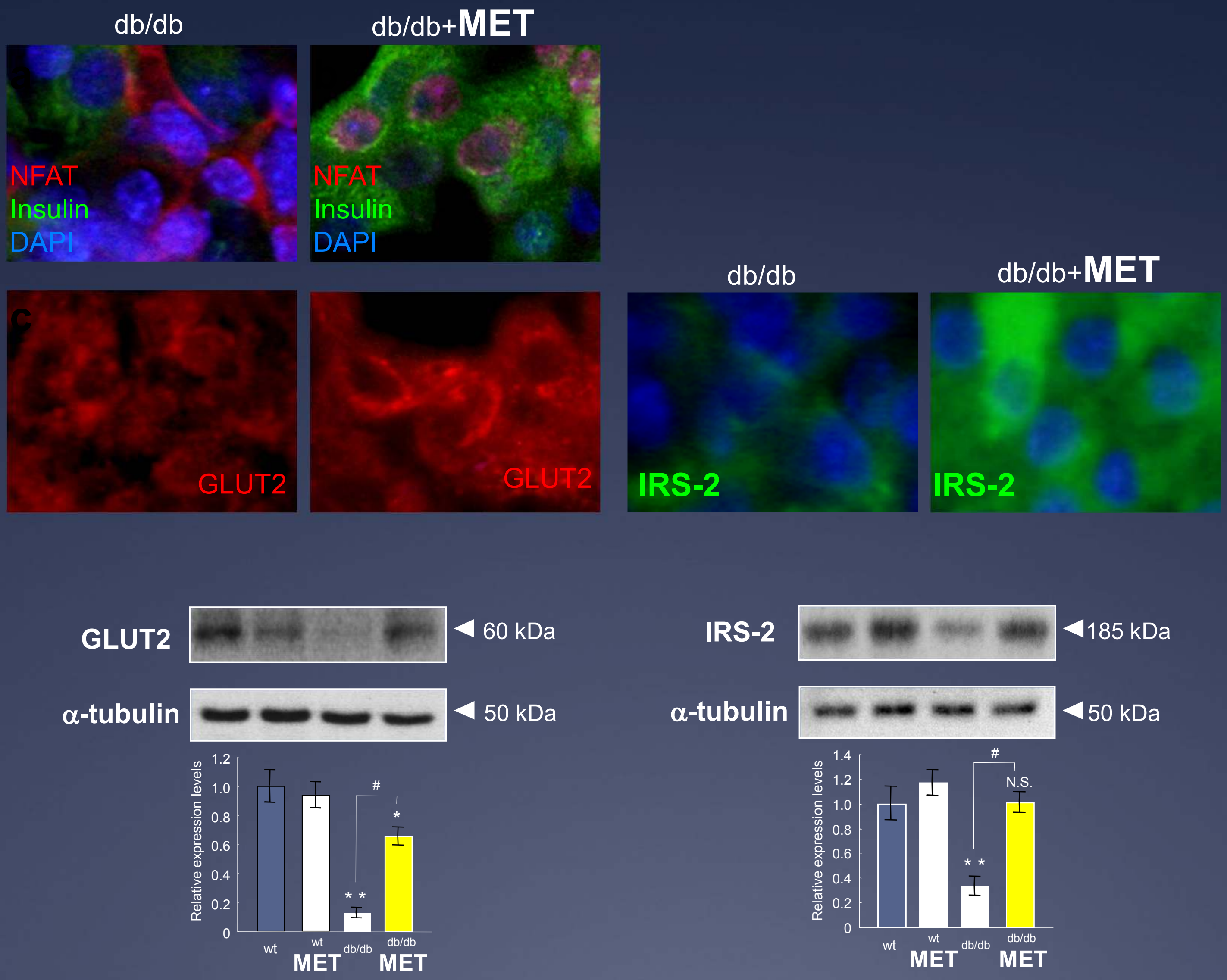
Kondo T et al. *Diabetes* 2012.

MET decreases insulin secretion accompanied by AMPK activation in MIN6 cells



Kondo T et al. *Diabetes* 2012.

MET regulates molecular markers of pancreatic β-cell integrity and function



Kondo T et al. *Diabetes* 2012.

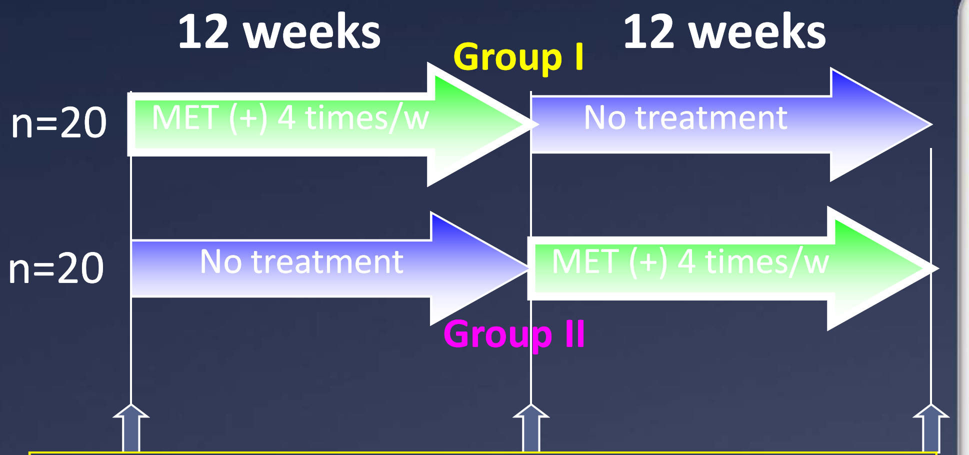
Activation of HSR by MET or GGA Improved Metabolic Abnormalities in Diabetes

Modality	MET	GGA	MET	MET
Target	Diabetic model mice		Healthy males	Metabolic syndrome
・BW	→	↓	→	?
・Abdominal fat	↓	↓	→	
・Insulin resistance	↓	↓	→	
・JNK	↓	↓	N.D.	
・Inflammatory cytokines	↓	↓	↓	
・β cell failure	↓	N.D.	→	

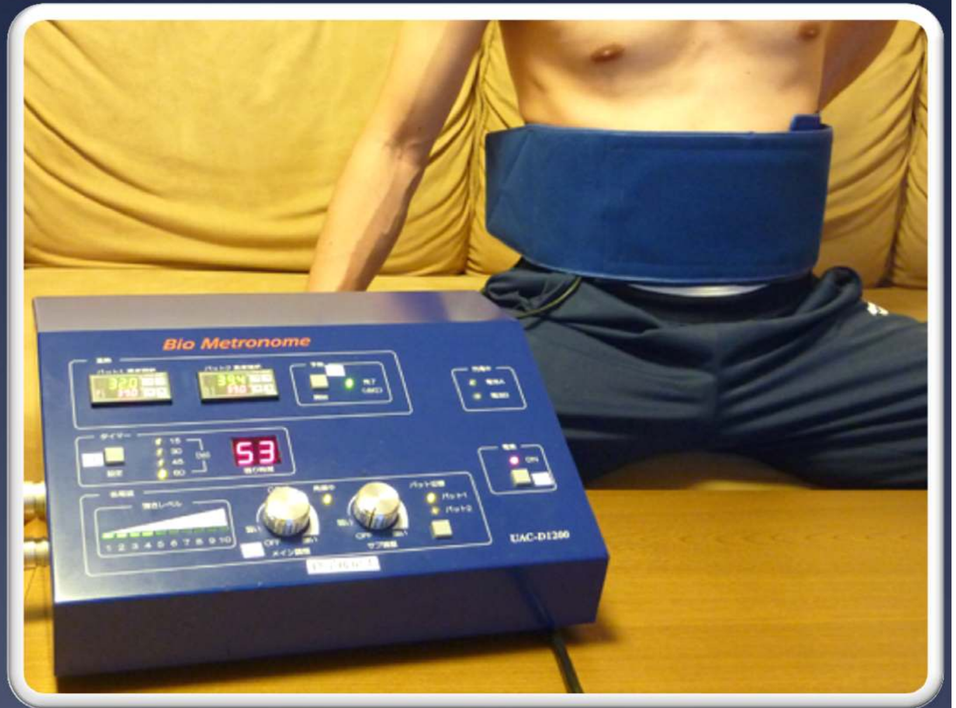
(PLoS One, 2008) (AM J PHYSIOL-ENDOC M, 2010) (ORCP, 2009)

(Diabetes, 2012)

Study Design and Device of MET Treatment



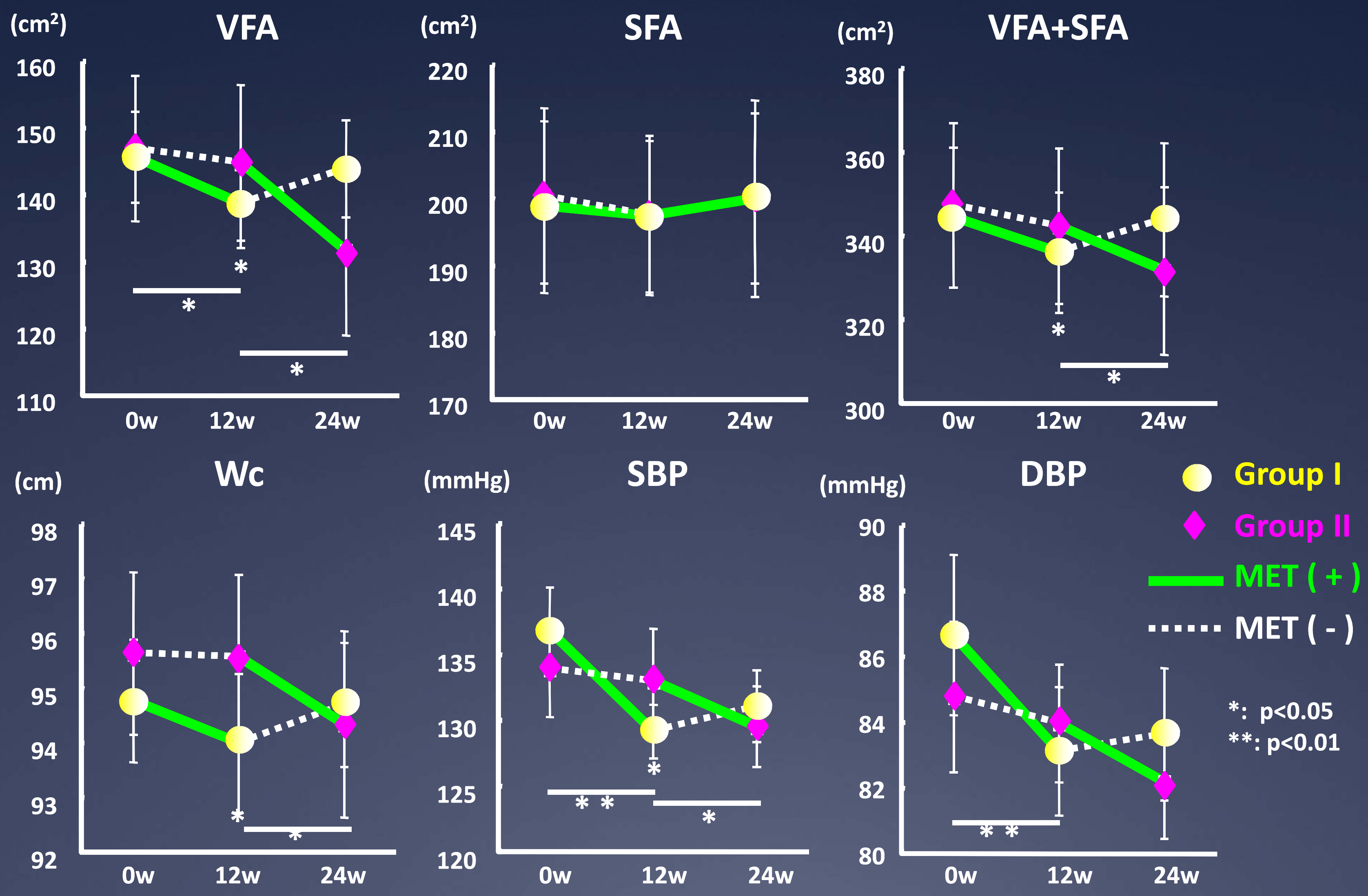
- HT, BW, Wc, %BF, BP, HR,
- Abdominal CT (Visceral fat, subcutaneous fat),
- 75g-OGTT (Blood glucose, IRI, CPR),
- Blood chemistry, Blood count,
- HbA1c,
- Lipid profile,
- Adipokines, Inflammatory cytokines.



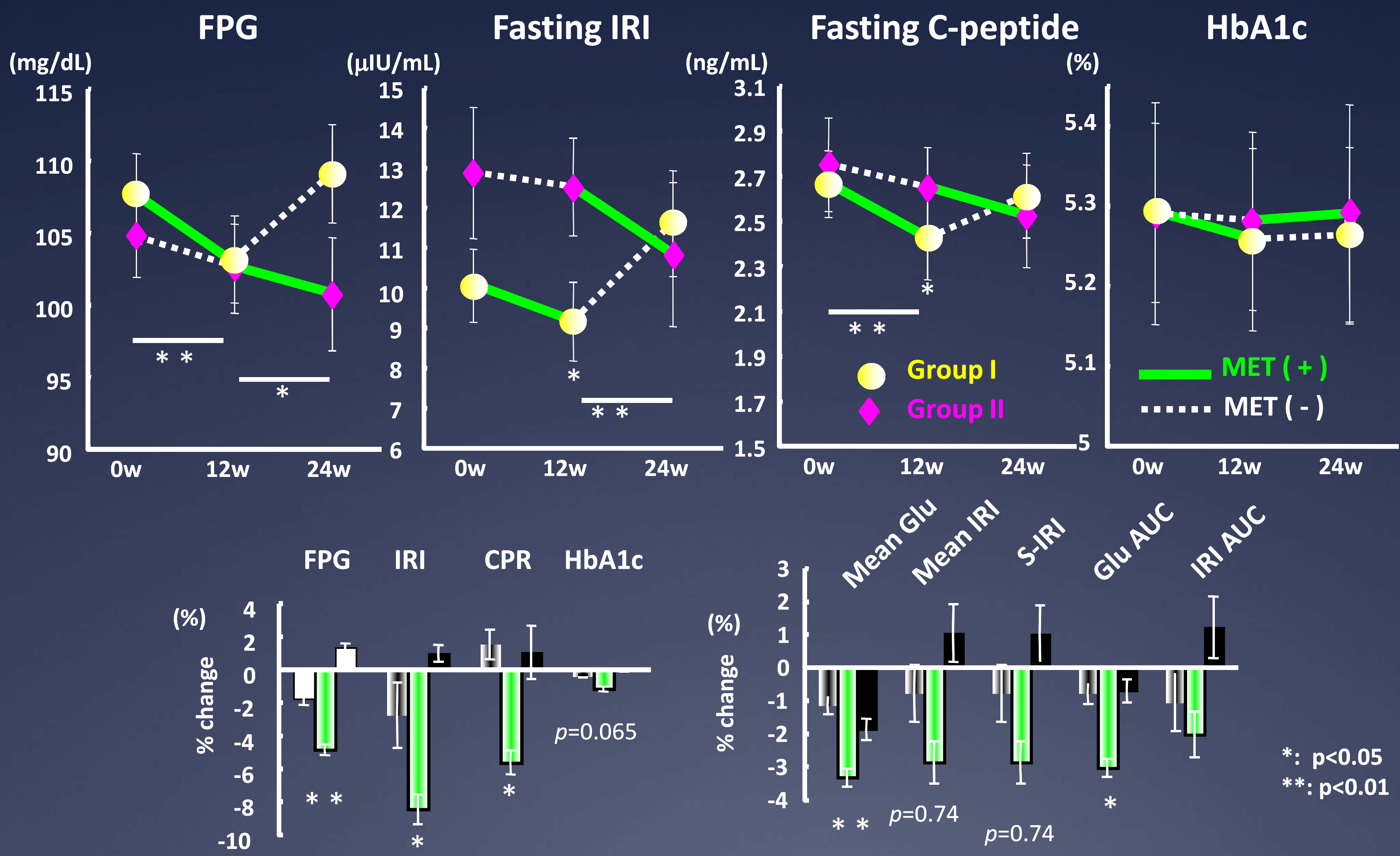
Background Characteristics of the Male Subjects with MS

Background characteristics of the subjects	Group I	Group II	*p* value
Male/females	20 / 0	20/0	-------
Age (years)	53.5 ± 1.5	51.3 ± 1.6	0.332
Body mass index (kg/m^2)	26.1 ± 0.5	27.6 ± 0.7	0.093
% Body fat	26.7 ± 0.9	26.4 ± 0.6	0.890
Waist circumference (cm)	92.8 ± 1.1	95.6 ± 1.5	0.120
Systolic blood pressure (mmHg)	136.8 ± 3.3	133.9 ± 2.6	0.562
Diastolic blood pressure (mmHg)	86.6 ± 2.4	84.8 ± 2.3	0.588
Heart rate (beats/min)	69.2 ± 2.1	68.7 ± 1.6	0.862
Current smoking (yes/no)	7/ 13	8/ 12	N.S.
Fasting plasma glucose (mg/dL)	107.7 ± 2.7	104.7 ± 2.9	0.457
Fasting insulin (μIU/mL)	10.0 ± 0.9	12.9 ± 1.6	0.141
HOMA-IR	2.61 ± 0.2	3.36 ± 0.5	0.146
QUICKI	0.34 ± 0.01	0.33 ± 0.01	0.195
composite WBISI	3.46 ± 0.2	3.04 ± 0.2	0.253
Insulinogenic index	1.02 ± 0.2	1.15 ± 0.2	0.670
HOMA-β	92.9 ± 12.3	116.5 ± 14.4	0.231
Blood glucose AUC on OGTT (0-2h) (mg/h/dL)	270.3 ± 8.7	274.2 ± 11.6	0.788
Insulin AUC on OGTT (0-2h) (mIU/h/mL)	138.3 ± 14.1	165.4 ± 14.6	0.203
HbA1c (%) (IFCC units)	5.29 ± 0.11 (5.69)	5.19 ± 0.14 (5.59)	0.578
LDL-cholesterol (mg/dL)	138.0 ± 5.0	124.5 ± 6.9	0.125
HDL-cholesterol (mg/dL)	51.6 ± 1.8	48.7 ± 1.8	0.283
Triglyceride (mg/dL)	182.1 ± 2.1	163.4 ± 12.1	0.477
WBC (/μL)	6009.1 ± 317.9	6026.3 ± 343.7	0.972
RBC (10^4/μL)	485.7 ± 7.9	502.3 ± 8.0	0.159
Hb (g/dL)	15.2 ± 1.2	15.7 ± 0.2	0.116
Plt (10^4/μL)	24.1 ± 0.9	22.6 ± 0.7	0.087
BUN (mg/dL)	13.9 ± 0.7	13.0 ± 0.7	0.376
Creatinine (mg/dL)	0.78 ± 0.02	0.82 ± 0.03	0.312
AST (GOT) (IU/L)	20.8 ± 0.8	24.9 ± 2.5	0.117
ALT (GPT) (IU/L)	25.3 ± 1.8	35.4 ± 3.7	0.089
LDH (IU/L)	149.0 ± 4.9	163.8 ± 4.5	0.198
Adiponectin (μg/mL)	3.15 ± 0.3	2.36 ± 0.3	0.094
Leptin (ng/mL)	5.51 ± 0.9	5.77 ± 0.5	0.813
Interleukin-6 (pg/mL)	1.48 ± 0.2	1.40 ± 0.1	0.761
Tumor necrosis factor-α (pg/mL)	1.64 ± 0.2	1.23 ± 0.1	0.278
High sensitivity C-reactive protein (ng/mL)	907.14 ± 259.9	607.9 ± 107.1	0.331

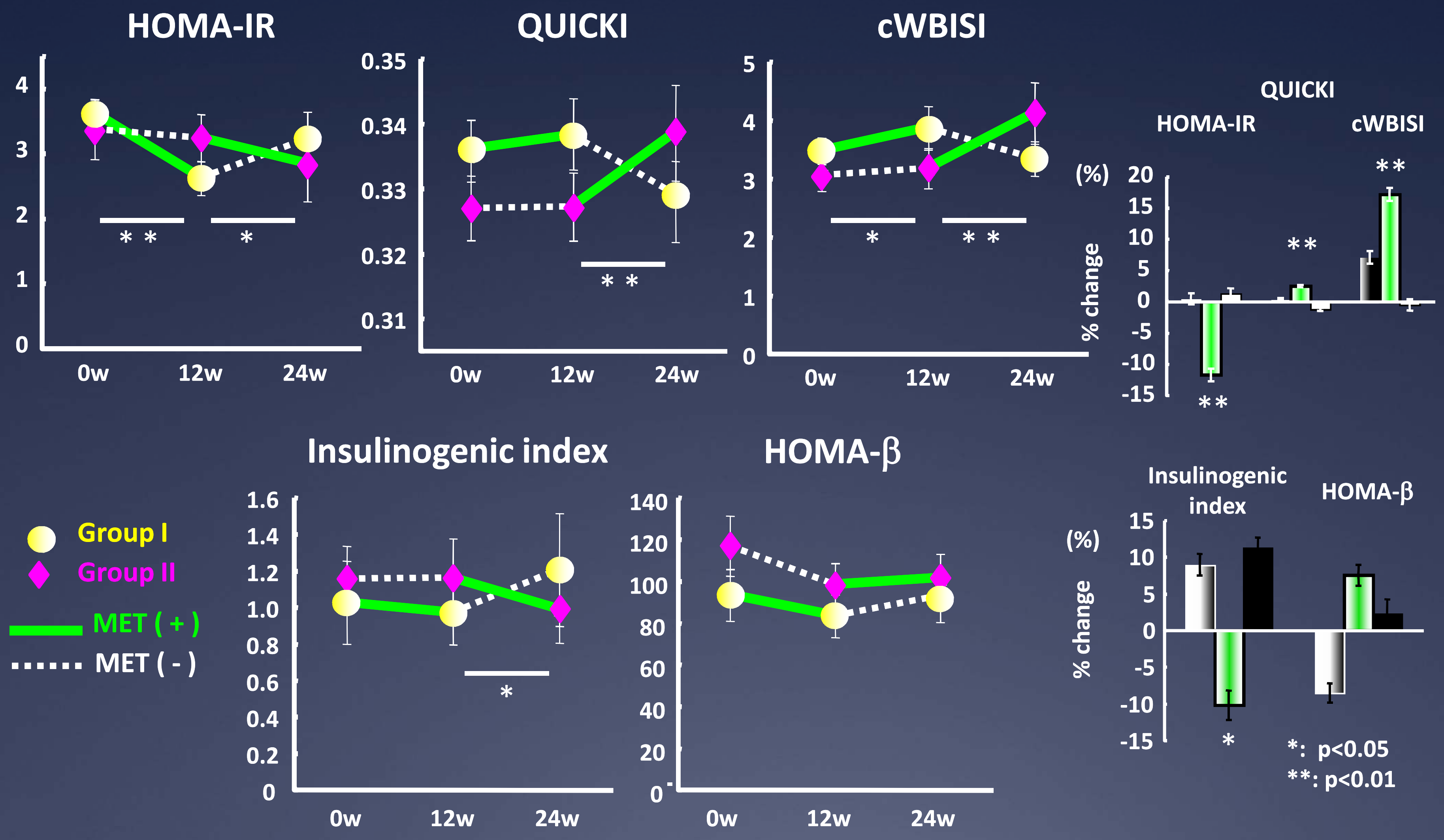
Reduction of Visceral Adiposity and BP



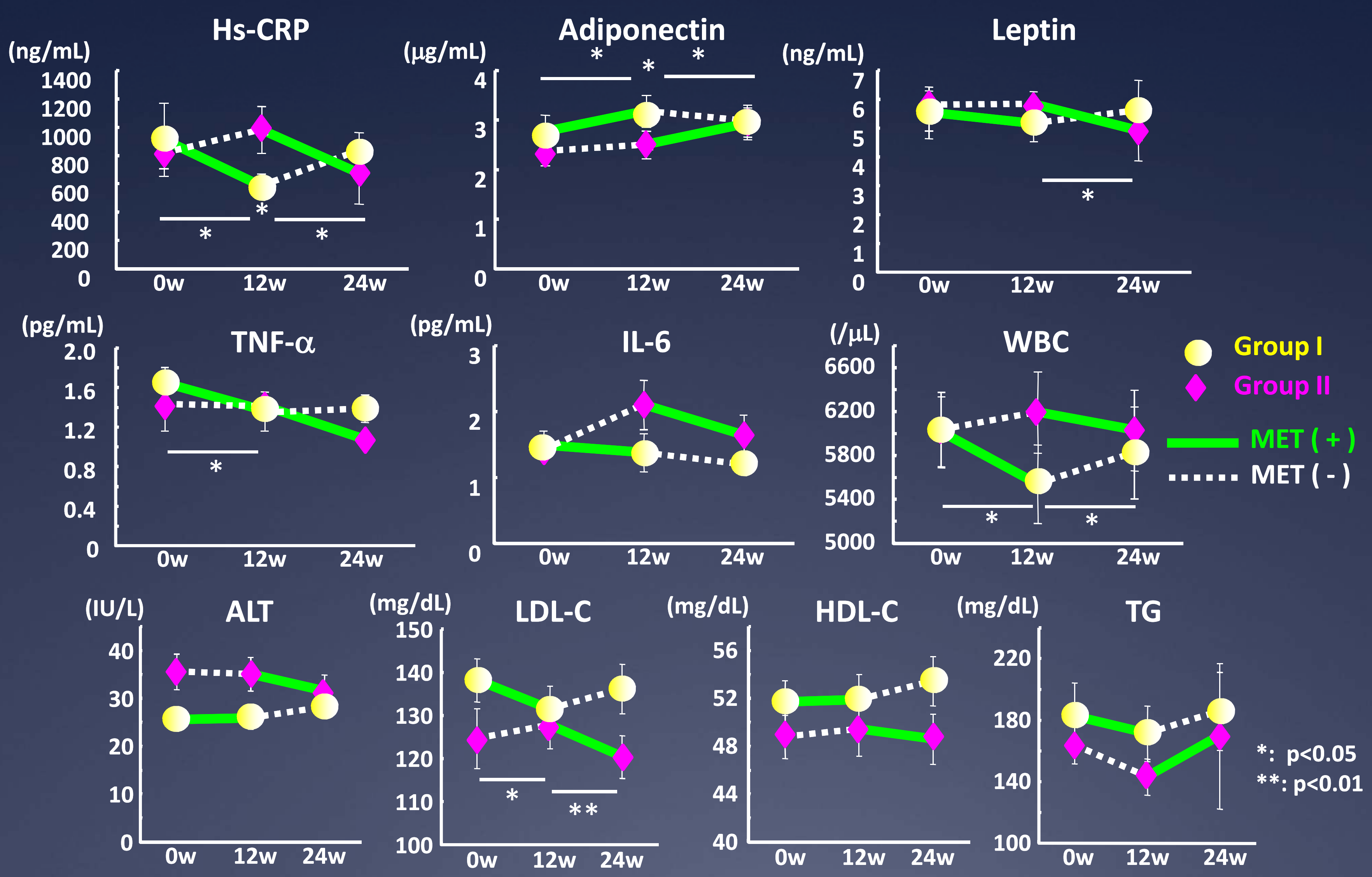
Improvement of Glucose Homeostasis



Improvement of of Insulin Sensitivity



Attenuation of Inflammatory Cytokines

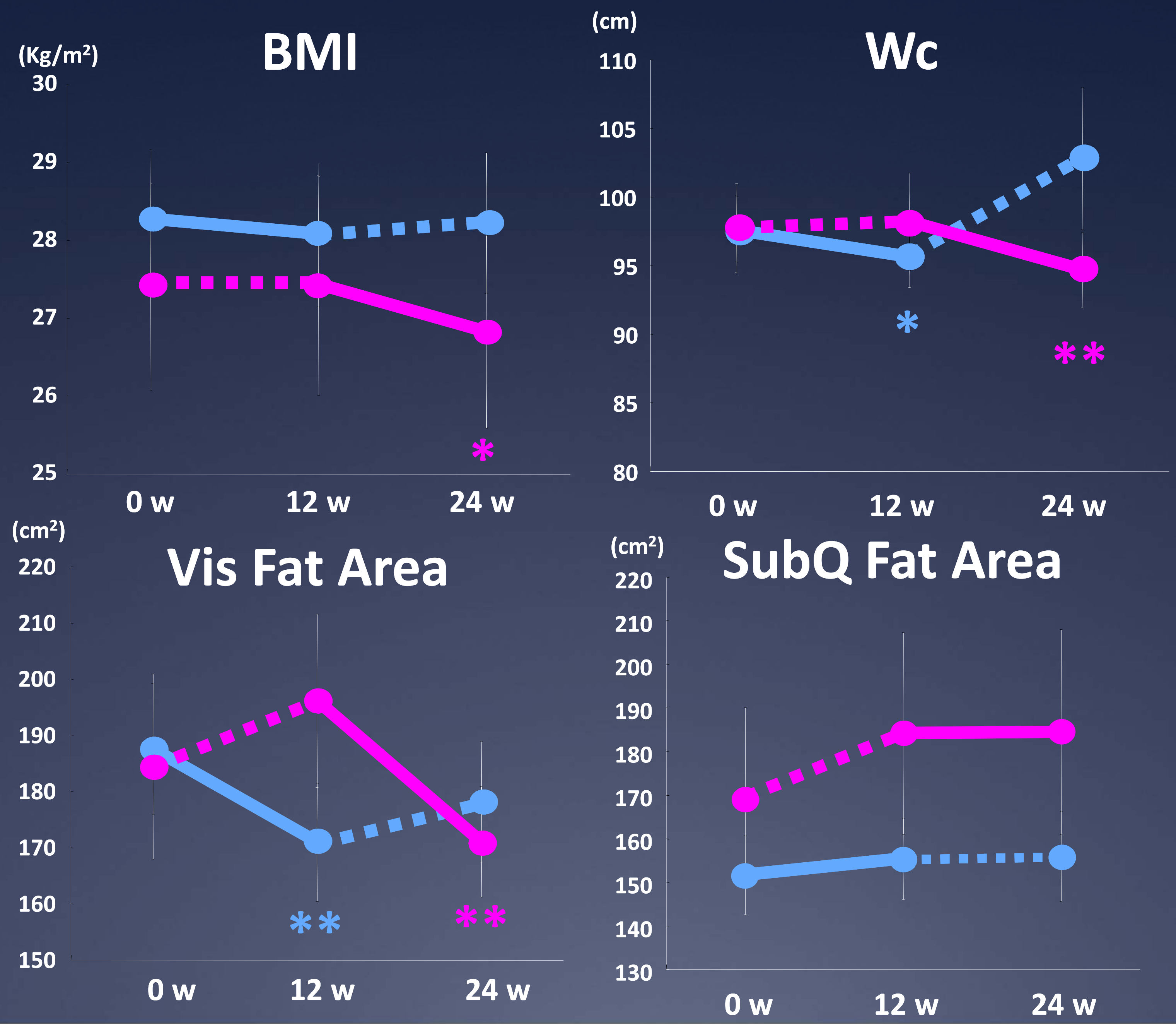


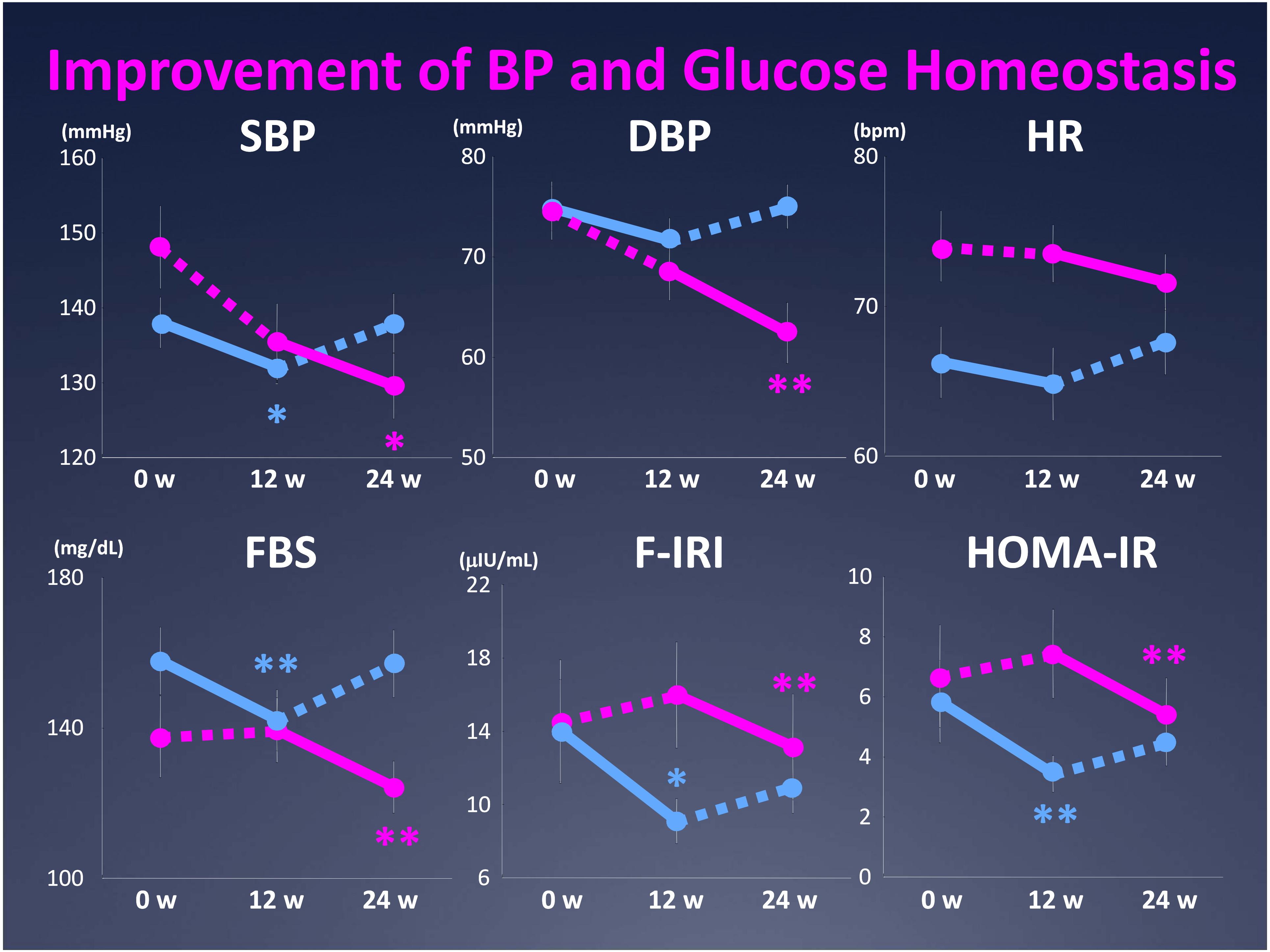
MET Treatment Improved MS Abnormalities

Modality	MET	GGA	MET	MET
Target	Diabetic model mice		Healthy males	Metabolic syndrome
▪BW	→	↓	→	→
▪Abdominal fat	↓	↓	→	↓
▪Insulin resistance	↓	↓	→	↓
▪JNK	↓	↓	N.D.	N.D.
▪Inflammatory cytokines	↓	↓	↓	↓
▪β cell failure	↓	N.D.	→	Protective (?)

What about in Type 2 diabetes ?

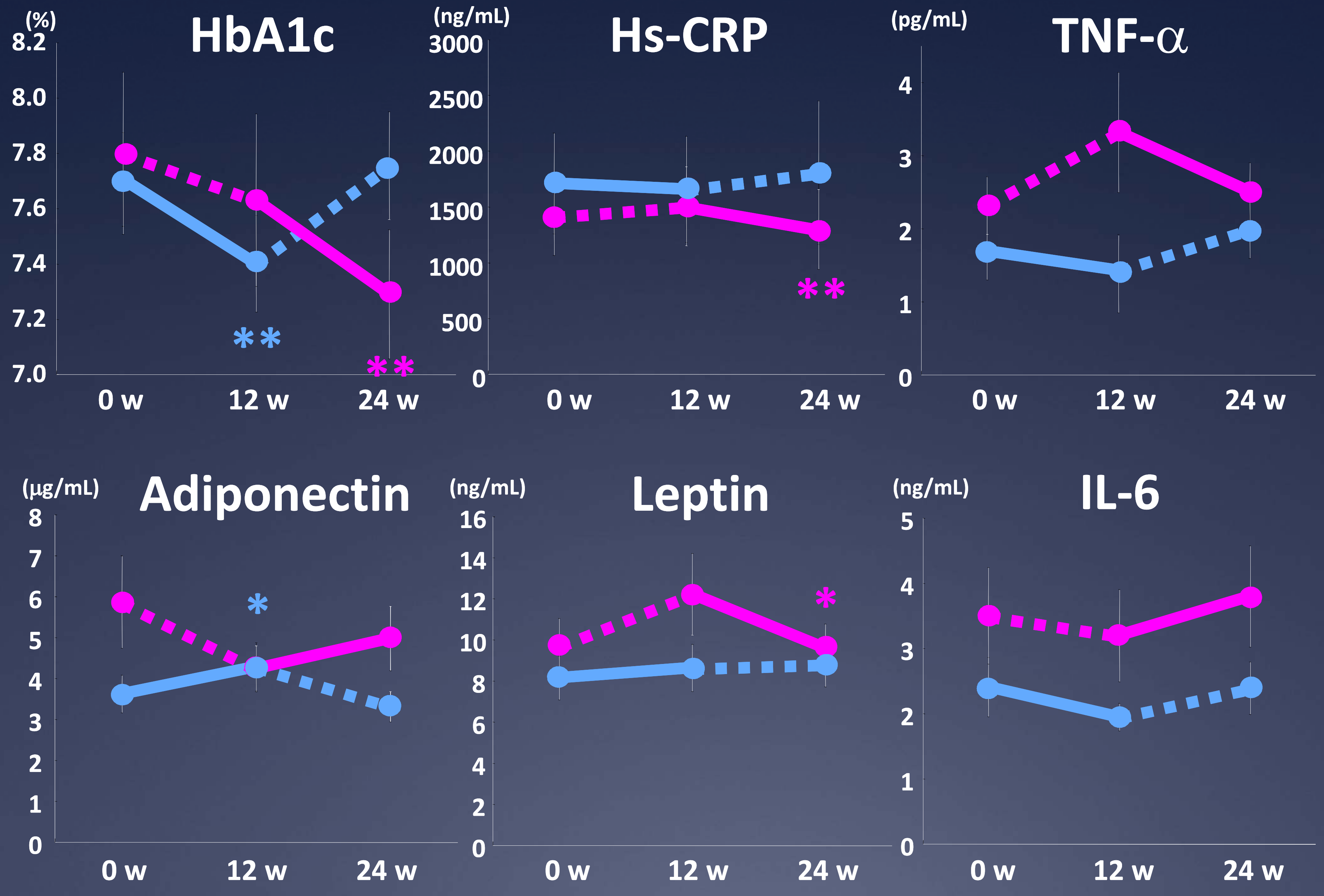
Reduction of Visceral Adiposity



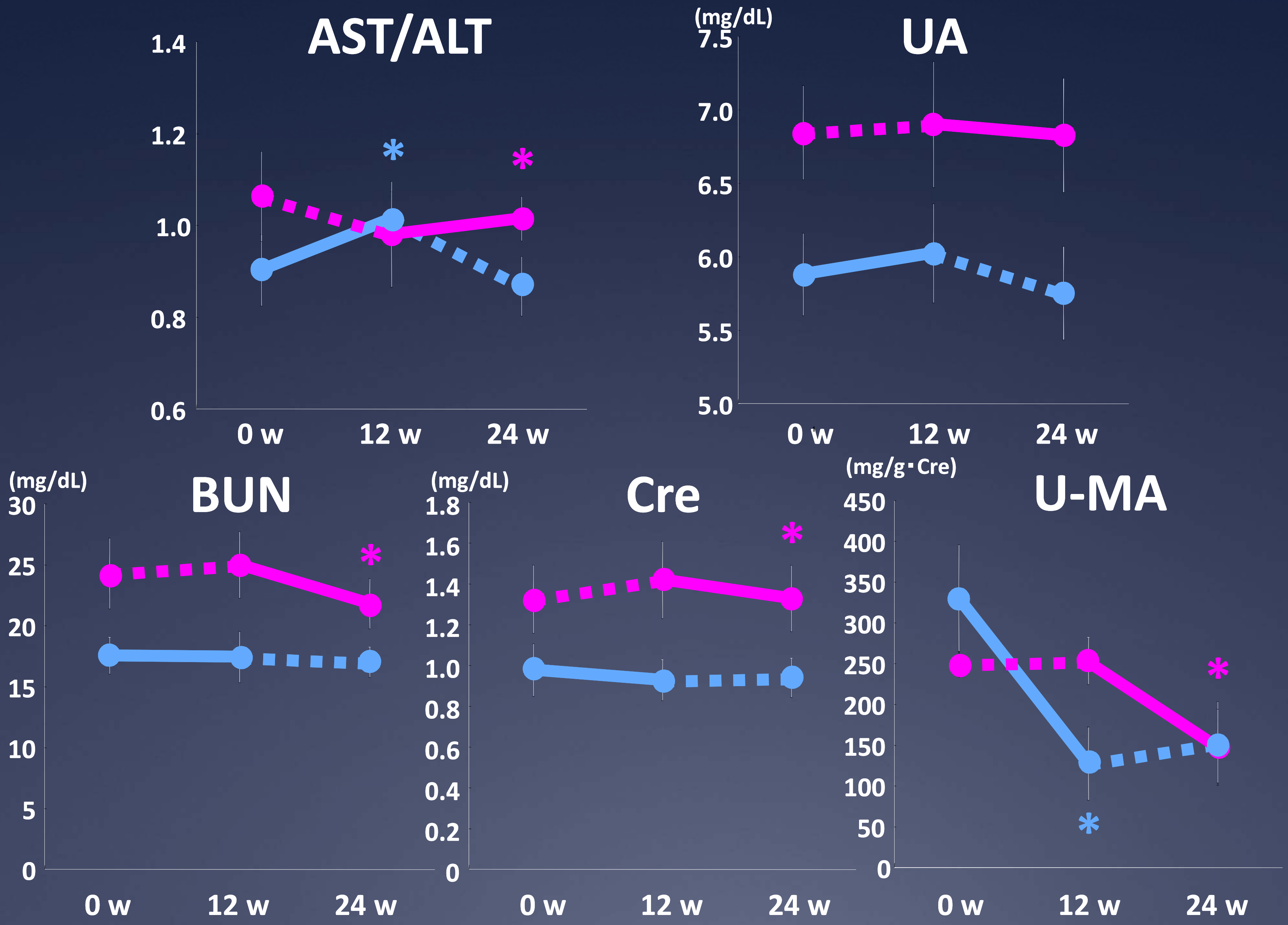


Improvement of BP and Glucose Homeostasis
SBP
(mmHg)
160
150
140
130
120
0 w
12 w
24 w
*
*
DBP
(mmHg)
80
70
60
50
0 w
12 w
24 w
**
HR
(bpm)
80
70
60
0 w
12 w
24 w
FBS
(mg/dL)
180
140
100
0 w
12 w
24 w
**
**
F-IRI
(μIU/mL)
22
18
14
10
6
0 w
12 w
24 w
*
**
HOMA-IR
10
8
6
4
2
0
0 w
12 w
24 w
**
**

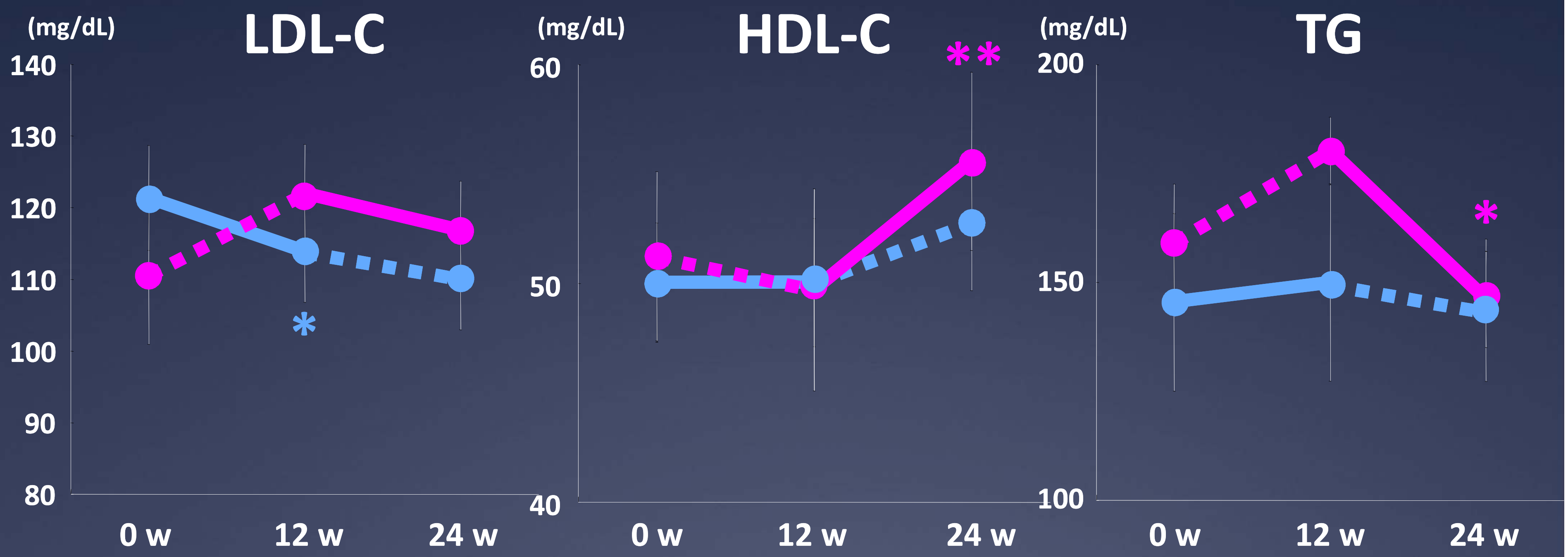
Attenuation of Inflammatory Cytokines

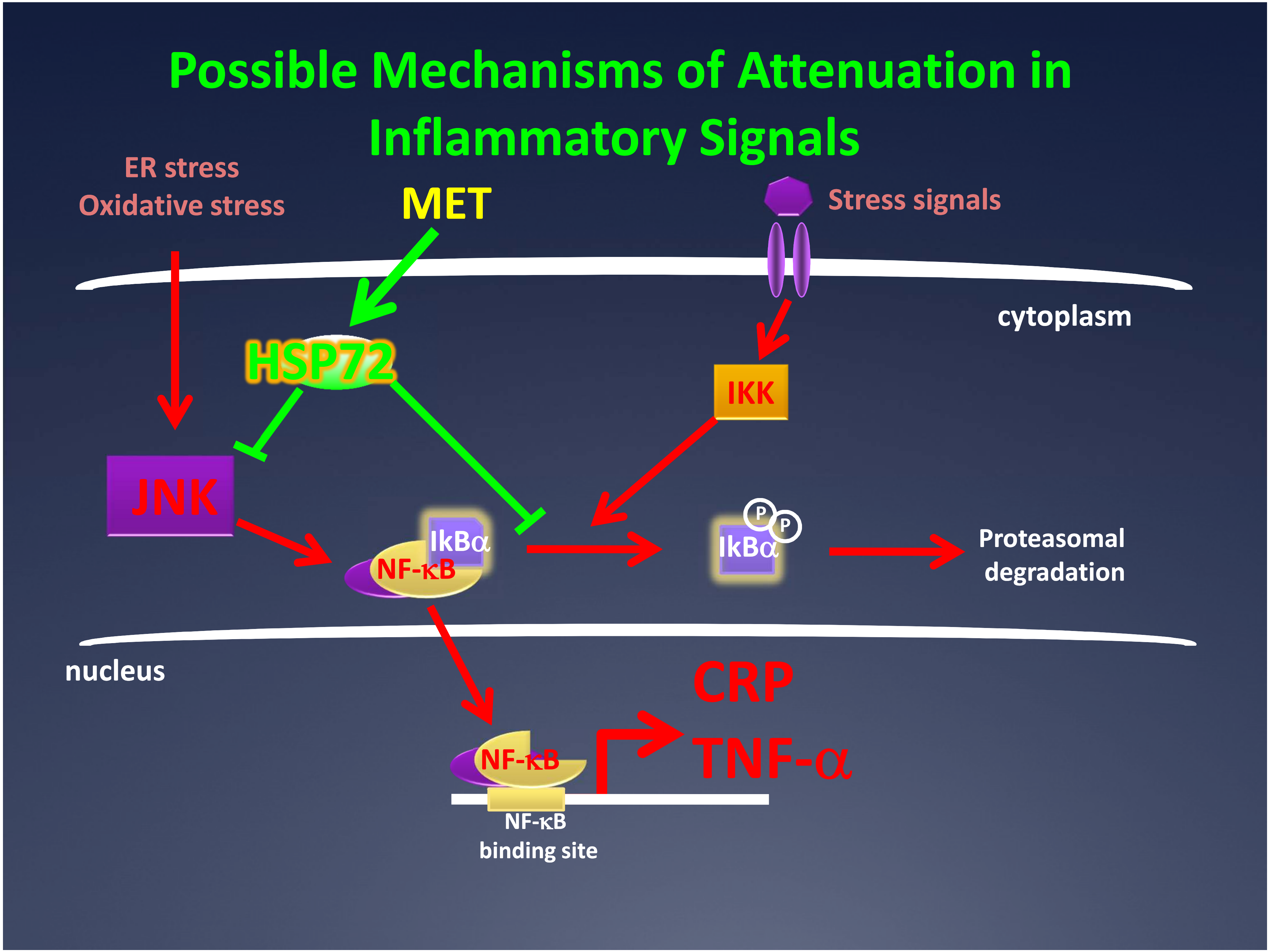


Improvement of Fatty Liver and Kidney function



Improvement of Lipid Profile




Possible Mechanisms of Attenuation in Inflammatory Signals
ER stress
Oxidative stress
MET
Stress signals
cytoplasm
HSP72
IKK
JNK
IkBα
NF-κB
P
P
IkBα
Proteasomal degradation
nucleus
CRP
TNF-α
NF-κB
NF-κB binding site

Diet or Exercise Interventions Fail to Attenuate Inflammation

TABLE 1 Effects of different dietary and exercise interventions on plasma levels of CRP and TNFα from recent studies

Subjects and design	Treatment	Design	CRP	TNFα	Time	Weight loss	Reference
41 diabetic patients	Soy protein	Randomized, parallel	↓[1]	ND[2]	4 y	No	15
6 men and 6 women	(n-3) fatty acids	Longitudinal	↓	ND	8 wk	No	16
15 overweight men	Low fat/ low carbohydrate	Cross-over	↓	↓	12 wk	Yes	26
28 overweight men	Carbohydrate restriction + eggs	Randomized, parallel	↓	↔	12 wk	Yes	17
210 men and women	Exercise	Randomized parallel	↔	ND	12 mo	Yes	19
13 men with low back pain	Exercise	Longitudinal	↓	ND	8 wk	No	18
11 healthy 11 multiple sclerosis	Exercise	Parallel	ND	↑	8 wk	No	30
87 Obese subjects with knee pain	Hypocaloric diet	Parallel	↔	↔	6 mo	No	28
44 women	Polyphenols in grapes	Randomized, cross-over	↔	↓	4 wk	No	31
12 men and 12 women	Polyphenols in raisins	Longitudinal	ND	↓	6 wk	No	29
16 obese subjects	Exercise	Parallel	↓	↔	12 wk	No	27

[1] All decreases and increases indicated by the arrows are significant.
[2] ND, not determined.

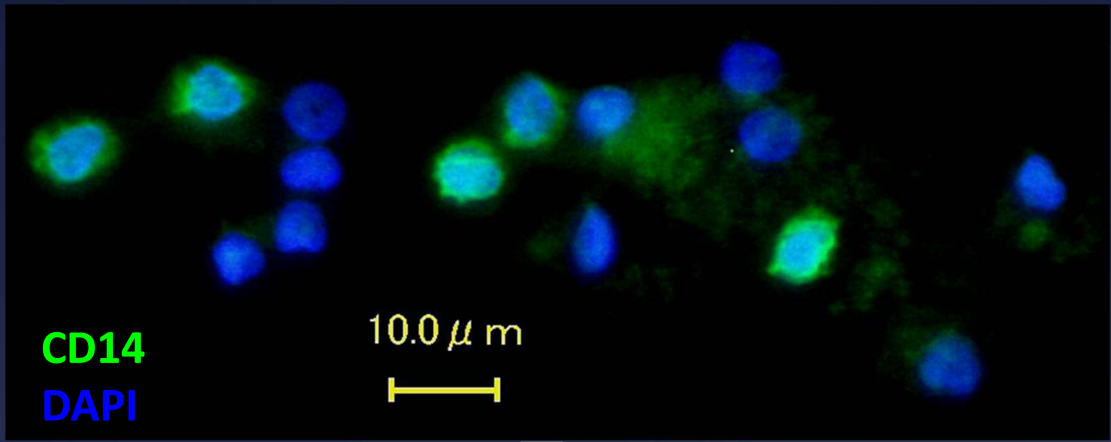
2008 American Society for Nutrition. J. Nutr. 138: 2293–2296, 2008.

MET could be Beyond Life-Style Interventions

MET could be beyond life-style interventions

BW →	FBS ↓	hs-CRP ↓
Wc ↓	F-IRI ↓	Adiponectin ↑
	CPR ↓	Leptin ↓
VFA ↓	HOMA-IR ↓	TNF-α ↓
SFA →		
VFA+SFA ↓	QUICKI ↑	WBC ↓
	cWBISI ↑	LDL-C ↓
SBP ↓		
DBP ↓	Insulinogenic index ↓	
HR →		

PBMC (Peripheral Blood Mononuclear Cells)

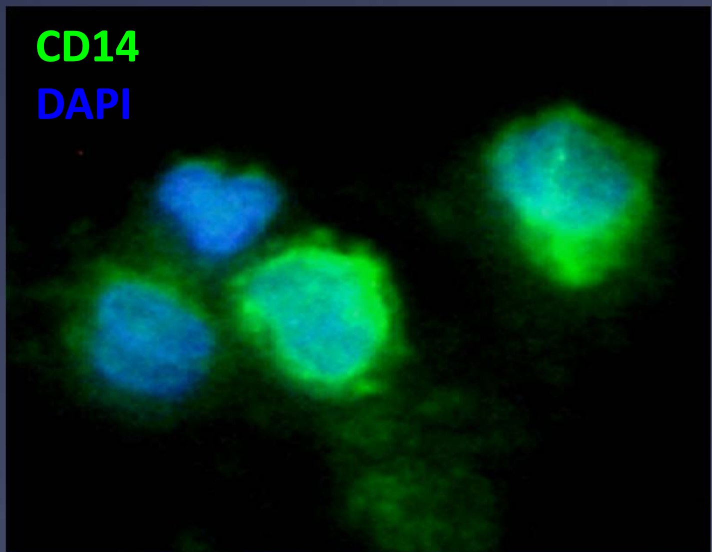


CD14(+): Monocytes
CD14(-): Non-monocytes, i.e. T cells, NK cells, B cells, dendritic cells and basophils

MACS : magnetic labeling selection

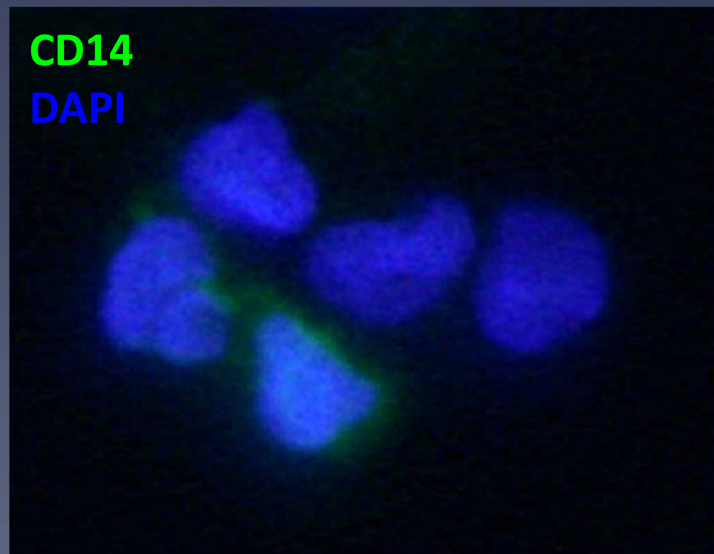
Monocyte isolation

CD14(+)



NF-κB
CRP
TNF-α
IL-6

CD14(-)



Summary

- Suppression of chronic inflammation is one of the principal mechanism of MET action to improve glucose homeostasis in MS and T2DM.

- Activation of HSR may promise a novel therapeutic alternative to treat life style-related diseases.

Acknowledgement

Metabolic Medicine, Kumamoto University

Kazunari Sasaki, Hironori Adachi, Rina Matsuyama, Katsutoshi Miyagawa, Rieko Goto, Eiichi Araki

Molecular Medicine, Kumamoto University

Saori Morino-Koga, Mary Ann Suico, Hirofumi Kai

Kumamoto Red Cross Society, Health Care Center

Yoko Wakaki, Yoshiko Nishida, Toru Marubayashi

Tsuchiya Rubber (Co. Ltd)

Yuhei Kurata, Hidetoshi Furushima, Shintaro Tsuda
Akifumi Matsuda