Activation of Heat Shock Response and Insulin Resistance

Tatsuya Kondo¹, Rina Matsuyama¹, Katsutoshi Miyagawa¹, Rieko Goto¹, Hirofumi Kai², Eiichi Araki¹.

¹Department of Metabolic Medicine, Faculty of Life Sciences, Kumamoto University.

²Department of Molecular Medicine, Faculty of Life Sciences, Global COE “Cell Fate Regulation Research and Education Unit” Kumamoto University.
A New Puffing Pattern Induced by Heat Shock and DNP in D. busckii

The different puffing patterns observed in D. busckii show organ-specific stage-specificity and sometimes zygotene patterns can be explained in terms of some activity. It is known that puffing patterns are also characteristic of some D. busckii larval tissue. It is also known that puffing patterns involve the 2L chromosome bands and that the same bands are found in this region. In the

<table>
<thead>
<tr>
<th>2L 11</th>
<th>2L 15</th>
<th>2L 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The purpose of this paper is to study the effect of temperature on the puffing patterns and that the same bands are involved in the 2L chromosomes, as found in this region. In the

Fig. 1. The 2L 11 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.
• Wholebody hyperthermia improves glucose homeostasis in mice and human type 2 diabetes.

• 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.

• 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.

**Activation of HSR may contribute to improving metabolic abnormalities in type 2 diabetes.**
Hot-Tub therapy:
• 30 min a day
• 6 days a week
• 3 weeks
• Water temp. 37.8〜40.5 °C
• Oral temp. 0.8 °C ↑

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.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Duration of Diabetes</th>
<th>Medications</th>
<th>Body Weight (before/after exposure)</th>
<th>Fasting Plasma Glucose (before/after exposure)*</th>
<th>Glycosylated Hemoglobin (before/after exposure)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>yr</td>
<td>yr</td>
<td></td>
<td></td>
<td>kg</td>
<td>mg/dl</td>
<td>%</td>
</tr>
<tr>
<td>43</td>
<td>M</td>
<td>14</td>
<td>Glyburide, metformin hydrochloride</td>
<td>83.2/80.9</td>
<td>190/186</td>
<td>13.6/12.7</td>
</tr>
<tr>
<td>50</td>
<td>M</td>
<td>13</td>
<td>Glyburide, troglitazone, insulin</td>
<td>201.8/199.1</td>
<td>109/66</td>
<td>8.6/7.7</td>
</tr>
<tr>
<td>51</td>
<td>M</td>
<td>9</td>
<td>Glyburide, metformin hydrochloride, insulin</td>
<td>175.0/168.2</td>
<td>231/181</td>
<td>12.2/11.1</td>
</tr>
<tr>
<td>54</td>
<td>F</td>
<td>9</td>
<td>Metformin hydrochloride, insulin</td>
<td>60.9/61.8</td>
<td>207/156</td>
<td>17.4/14.8</td>
</tr>
<tr>
<td>57</td>
<td>F</td>
<td>8</td>
<td>Glipizide, metformin hydrochloride</td>
<td>64.5/64.5</td>
<td>197/155</td>
<td>11.0/11.1</td>
</tr>
<tr>
<td>57</td>
<td>M</td>
<td>3</td>
<td>Glyburide, troglitazone</td>
<td>75.0/73.6</td>
<td>165/162</td>
<td>8.6/7.6</td>
</tr>
<tr>
<td>63</td>
<td>M</td>
<td>11</td>
<td>Glipizide, metformin hydrochloride</td>
<td>91.8/91.8</td>
<td>158/160</td>
<td>9.1/8.1</td>
</tr>
<tr>
<td>68</td>
<td>F</td>
<td>9</td>
<td>Glyburide, metformin hydrochloride, troglitazone</td>
<td>85.5/84.1</td>
<td>197/203</td>
<td>9.5/8.9</td>
</tr>
</tbody>
</table>

*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) → 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 cardiovascular 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

**HSP72**

1. **ABD (ATP-binding domain)**
2. **PBD (Peptide-binding domain)**

- **ASK1 binding**
- **Chaperone activity**
- **JNK binding**
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
Norepinephrine
β-adrenergic signal (Isoproterenol)
Tyroxine
Insulin + Heat shock

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

Hyperthermia
+ Mild electric current

Estrogen
FAS ligand
Aging
Diabetes
(Insulin resistance)

Control
Mild Electric current
Hyperthermia
Combination (MET)

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

HSP72
HSR attenuation and insulin resistance

**PI 3-kinase pathway**
- PIP3
- PIP2
- PDK1
- Akt/PKB
- GSK-3β
- IRS
- P110 catalytic
- IRS regulatory

**MAPK pathway**
- IRα
- IRβ
- GRB2
- SOS
- ERK
- JNK

**Type 2 Diabetes, MS**
- Inflammatory cytokines
- Stress signals

**PI 3-kinase pathway**
- Akt/PKB
- GSK-3β
- IRS
- PDK1
- P110 catalytic

**MAPK pathway**
- IRα
- IRβ
- GRB2
- SOS
- ERK
- JNK

**HSP72**
- HSF-1
- HRE
- NFKβ
- TNF-α
- Hs-CRP
**MES with hyperThermia (MET)**

*in vivo (muscle)*

<table>
<thead>
<tr>
<th>HSP72</th>
<th>Calnexin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MES</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

**MET**

- Treatment: twice a week (Tue, Fri)
- 55 pulses/sec, 0.1 ms duration, 0.6V/cm, 10 min, 42 °C

**Blood glucose**

<table>
<thead>
<tr>
<th>Age</th>
<th>5 w</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**i.p. GTT, i.p. ITT**

- Biochemical markers
- Inflammatory markers
- Insulin signal etc

Body Weight and Food Intake in HFD mice

**Body Weight**

- **HFD**
- **HFD+MET**

**Food Intake**

- HFD
- HFD+MET

---

Blood Glucose Levels in HFD mice

Fasting Blood Glucose (10w)

p=0.041

HFD

HFD+MET

UCP-1 mRNA Expression in BAT of HFD mice

Relative expression of UCP-1 mRNA

p=0.035

HFD
HFD+MET

Glucose Tolerance and Insulin Resistance in HFD mice

Glucose tolerance test

![Glucose tolerance test graph showing blood glucose levels over time for different treatments.]

Insulin tolerance test

![Insulin tolerance test graph showing blood glucose levels over time for different treatments.]

JNK inactivation

![JNK inactivation graph showing phosphorylation levels for different treatments.]

Intra-Abdominal Adiposity in HFD

HFD

HFD+MET

Reduction of liver and adipose tissue weight

**Liver**
- HFD: 1600 mg (p=0.02)
- HFD+MET: 800 mg

**Retroperitoneal fat**
- HFD: 1200 mg (p=0.008)
- HFD+MET: 800 mg

**Subcutaneous fat**
- HFD: 3000 mg (p=0.01)
- HFD+MET: 2000 mg

**Total intra-abdominal fat**
- HFD: 5000 mg (p=0.01)
- HFD+MET: 4000 mg

Adipocyte Size in Mesenteric Fat of HFD mice

HFD

HFD+MET

p=0.0001

Metabolic impacts of MET in db/db mice

**MET improves insulin sensitivity**

**MET improves glucose homeostasis with insulin secretion**

The effects of MET in β-cells of db/db mice

MET attenuates JNK signal in db/db mice

MET increases PDX-1 and attenuates stress signals

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

<table>
<thead>
<tr>
<th>Modality</th>
<th>MET</th>
<th>GGA</th>
<th>MET</th>
<th>MET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>Diabetic model mice</td>
<td>Healthy males</td>
<td>Metabolic syndrome</td>
<td></td>
</tr>
<tr>
<td>BW</td>
<td>→</td>
<td>↓</td>
<td>→</td>
<td></td>
</tr>
<tr>
<td>Abdominal fat</td>
<td>↓</td>
<td>↓</td>
<td>→</td>
<td></td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>↓</td>
<td>↓</td>
<td>→</td>
<td></td>
</tr>
<tr>
<td>JNK</td>
<td>↓</td>
<td>↓</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Inflammatory cytokines</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>β cell failure</td>
<td>↓</td>
<td>N.D.</td>
<td>→</td>
<td></td>
</tr>
</tbody>
</table>

Study Design and Device of MET Treatment

- MET (+) 4 times/w
- No treatment

Group I: n=20
Group II: n=20

• 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

<table>
<thead>
<tr>
<th>Background characteristics of the subjects</th>
<th>Group I</th>
<th>Group II</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/females</td>
<td>20 / 0</td>
<td>20 / 0</td>
<td>N.S.</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.5 ± 1.5</td>
<td>51.3 ± 1.6</td>
<td>0.332</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>26.1 ± 0.5</td>
<td>27.6 ± 0.7</td>
<td>0.093</td>
</tr>
<tr>
<td>% Body fat</td>
<td>26.7 ± 0.9</td>
<td>26.4 ± 0.6</td>
<td>0.890</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>92.8 ± 1.1</td>
<td>95.6 ± 1.5</td>
<td>0.120</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>136.8 ± 3.3</td>
<td>133.9 ± 2.6</td>
<td>0.562</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>86.6 ± 2.4</td>
<td>84.8 ± 2.3</td>
<td>0.588</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>69.2 ± 2.1</td>
<td>68.7 ± 1.6</td>
<td>0.862</td>
</tr>
<tr>
<td>Current smoking (yes/no)</td>
<td>7 / 13</td>
<td>8 / 12</td>
<td>N.S.</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>107.7 ± 2.7</td>
<td>104.7 ± 2.9</td>
<td>0.457</td>
</tr>
<tr>
<td>Fasting insulin ((\mu)IU/mL)</td>
<td>10.0 ± 0.9</td>
<td>12.9 ± 1.6</td>
<td>0.141</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.61 ± 0.2</td>
<td>3.36 ± 0.5</td>
<td>0.146</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.34 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>0.195</td>
</tr>
<tr>
<td>composite WBISI</td>
<td>3.46 ± 0.2</td>
<td>3.04 ± 0.2</td>
<td>0.253</td>
</tr>
<tr>
<td>Insulinogenic index</td>
<td>1.02 ± 0.2</td>
<td>1.15 ± 0.2</td>
<td>0.670</td>
</tr>
<tr>
<td>HOMA-(\beta)</td>
<td>92.9 ± 12.3</td>
<td>116.5 ± 14.4</td>
<td>0.231</td>
</tr>
<tr>
<td>Blood glucose AUC on OGTT (0-2h) (mg/h/dL)</td>
<td>270.3 ± 8.7</td>
<td>274.2 ± 11.6</td>
<td>0.788</td>
</tr>
<tr>
<td>Insulin AUC on OGTT (0-2h) (mIU/h/mL)</td>
<td>138.3 ± 14.1</td>
<td>165.4 ± 14.6</td>
<td>0.203</td>
</tr>
<tr>
<td>HbA1c (%) (IFCC units)</td>
<td>5.29 ± 0.11 (5.69)</td>
<td>5.19 ± 0.14 (5.59)</td>
<td>0.878</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>138.0 ± 5.0</td>
<td>124.5 ± 6.9</td>
<td>0.125</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>51.6 ± 1.8</td>
<td>48.7 ± 1.8</td>
<td>0.283</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>182.1 ± 2.1</td>
<td>163.4 ± 12.1</td>
<td>0.477</td>
</tr>
<tr>
<td>WBC ((\mu)L)</td>
<td>60.09.1 ± 317.9</td>
<td>60.26.3 ± 343.7</td>
<td>0.972</td>
</tr>
<tr>
<td>RBC (10(^4)(\mu)L)</td>
<td>485.7 ± 7.9</td>
<td>502.3 ± 8.0</td>
<td>0.159</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>15.2 ± 1.2</td>
<td>15.7 ± 0.2</td>
<td>0.116</td>
</tr>
<tr>
<td>Plt (10(^4)(\mu)L)</td>
<td>24.1 ± 0.9</td>
<td>22.6 ± 0.7</td>
<td>0.087</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>13.9 ± 0.7</td>
<td>13.0 ± 0.7</td>
<td>0.376</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.78 ± 0.02</td>
<td>0.82 ± 0.03</td>
<td>0.312</td>
</tr>
<tr>
<td>AST (GOT) (IU/L)</td>
<td>20.8 ± 0.8</td>
<td>24.9 ± 2.5</td>
<td>0.117</td>
</tr>
<tr>
<td>ALT (GPT) (IU/L)</td>
<td>25.3 ± 1.8</td>
<td>35.4 ± 3.7</td>
<td>0.089</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>149.0 ± 4.9</td>
<td>163.8 ± 4.5</td>
<td>0.198</td>
</tr>
<tr>
<td>Adiponectin ((\mu)g/mL)</td>
<td>3.15 ± 0.3</td>
<td>2.36 ± 0.3</td>
<td>0.094</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>5.51 ± 0.9</td>
<td>5.77 ± 0.5</td>
<td>0.813</td>
</tr>
<tr>
<td>Interleukin-6 (pg/mL)</td>
<td>1.48 ± 0.2</td>
<td>1.40 ± 0.1</td>
<td>0.761</td>
</tr>
<tr>
<td>Tumor necrosis factor-(\alpha) (pg/mL)</td>
<td>1.64 ± 0.2</td>
<td>1.23 ± 0.1</td>
<td>0.278</td>
</tr>
<tr>
<td>High sensitivity C-reactive protein (ng/mL)</td>
<td>907.14 ± 259.9</td>
<td>607.9 ± 107.1</td>
<td>0.331</td>
</tr>
</tbody>
</table>
Reduction of Visceral Adiposity and BP

**VFA**

- Group I: 12w < 0w
- Group II: 12w < 0w

**SFA**

- Group I: 12w < 0w
- Group II: 12w < 0w

**VFA+SFA**

- Group I: 12w < 0w
- Group II: 12w < 0w

**Wc**

- Group I: 12w < 0w
- Group II: 12w < 0w

**SBP**

- Group I: p<0.05
- Group II: p<0.01

**DBP**

- Group I: p<0.05
- Group II: p<0.01
Improvement of Glucose Homeostasis

FPG

- Group I: MET (+)
- Group II: MET (-)

Fasting IRI

- Group I: MET (+)
- Group II: MET (-)

Fasting C-peptide

- Group I: MET (+)
- Group II: MET (-)

HbA1c

- Group I: MET (+)
- Group II: MET (-)

**FPG**

- 0w: 110 mg/dL
- 12w: 105 mg/dL
- 24w: 100 mg/dL

**Fasting IRI**

- 0w: 15 µU/mL
- 12w: 13 µU/mL
- 24w: 11 µU/mL

**Fasting C-peptide**

- 0w: 3.1 ng/mL
- 12w: 2.9 ng/mL
- 24w: 2.7 ng/mL

**HbA1c**

- 0w: 5.4%
- 12w: 5.3%
- 24w: 5.2%

**Group I**

- MET (+)
- P<0.05

**Group II**

- MET (-)
- P<0.01

**% change**

- FPG: -5%
- IRI: -2%
- CPR: -3%
- HbA1c: -1%

**% change**

- Mean Glu: 0%
- Mean IRI: 0%
- S-IRI: 0%
- Glu AUC: 0%
- IRI AUC: 0%

*: p<0.05
**: p<0.01
Improvement of Insulin Sensitivity

**HOMA-IR**

- Group I: 3.1, 3.0, 2.9
- Group II: 3.2, 3.1, 3.0

**QUICKI**

- Group I: 0.34, 0.33, 0.32
- Group II: 0.35, 0.34, 0.33

**cWBISI**

- Group I: 4.0, 4.1, 4.2
- Group II: 4.0, 4.1, 4.2

**Insulinogenic index**

- Group I: 1.6, 1.5, 1.4
- Group II: 1.7, 1.6, 1.5

**HOMA-β**

- Group I: 140, 130, 120
- Group II: 145, 135, 125

Group I: MET (+)  Group II: MET (-)

* * * *: p<0.05  **: p<0.01
Attenuation of Inflammatory Cytokines

Hs-CRP

Adiponectin

Leptin

TNF-α

IL-6

WBC

ALT

LDL-C

HDL-C

TG

*: p<0.05
**: p<0.01
## MET Treatment Improved MS Abnormalities

<table>
<thead>
<tr>
<th>Modality</th>
<th>MET</th>
<th>GGA</th>
<th>MET</th>
<th>MET</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target</strong></td>
<td>Diabetic model mice</td>
<td>Healthy males</td>
<td>Metabolic syndrome</td>
<td></td>
</tr>
<tr>
<td>• BW</td>
<td>→</td>
<td>↓</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>• Abdominal fat</td>
<td>↓</td>
<td>↓</td>
<td>→</td>
<td>↓</td>
</tr>
<tr>
<td>• Insulin resistance</td>
<td>↓</td>
<td>↓</td>
<td>→</td>
<td>↓</td>
</tr>
<tr>
<td>• JNK</td>
<td>↓</td>
<td>↓</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>• Inflammatory cytokines</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>• β cell failure</td>
<td>↓</td>
<td>N.D.</td>
<td>→</td>
<td>Protective (?)</td>
</tr>
</tbody>
</table>
What about in Type 2 diabetes?
Reduction of Visceral Adiposity

BMI

Vis Fat Area

SubQ Fat Area

Wc
Improvement of BP and Glucose Homeostasis

- **SBP**
  - 0 w: 150 (mmHg)
  - 12 w: 130 (mmHg)
  - 24 w: 120 (mmHg)

- **DBP**
  - 0 w: 80 (mmHg)
  - 12 w: 70 (mmHg)
  - 24 w: 60 (mmHg)

- **HR**
  - 0 w: 70 (bpm)
  - 12 w: 70 (bpm)
  - 24 w: 70 (bpm)

- **FBS**
  - 0 w: 100 (mg/dL)
  - 12 w: 80 (mg/dL)
  - 24 w: 60 (mg/dL)

- **F-IRI**
  - 0 w: 6 (µIU/mL)
  - 12 w: 4 (µIU/mL)
  - 24 w: 2 (µIU/mL)

- **HOMA-IR**
  - 0 w: 3
  - 12 w: 2
  - 24 w: 1

* and ** indicate statistical significance at the 0.05 and 0.01 levels, respectively.
Attenuation of Inflammatory Cytokines

- **HbA1c**
  - 0 w: 7.0%
  - 12 w: 7.2%
  - 24 w: 7.4%

- **Hs-CRP**
  - 0 w: 500 ng/mL
  - 12 w: 1000 ng/mL
  - 24 w: 1500 ng/mL

- **TNF-α**
  - 0 w: 4 pg/mL
  - 12 w: 3 pg/mL
  - 24 w: 2 pg/mL

- **Adiponectin**
  - 0 w: 8 µg/mL
  - 12 w: 6 µg/mL
  - 24 w: 4 µg/mL

- **Leptin**
  - 0 w: 16 ng/mL
  - 12 w: 12 ng/mL
  - 24 w: 10 ng/mL

- **IL-6**
  - 0 w: 5 ng/mL
  - 12 w: 3 ng/mL
  - 24 w: 2 ng/mL

*Significance markers: **p < 0.01, *p < 0.05*
Improvement of Fatty Liver and Kidney function

**AST/ALT**
- 0 w: 0.6
- 12 w: 0.8
- 24 w: 1.0

**UA**
- 0 w: 7.0
- 12 w: 6.5
- 24 w: 6.0

**BUN**
- 0 w: 0
- 12 w: 5
- 24 w: 10

**Cre**
- 0 w: 0.2
- 12 w: 0.4
- 24 w: 0.6

**U-MA**
- 0 w: 0
- 12 w: 50
- 24 w: 100

*Significant improvement:*
Improvement of Lipid Profile

- **LDL-C**
  - 0 w: 130 (mg/dL)
  - 12 w: 120 (mg/dL)
  - 24 w: 110 (mg/dL)

- **HDL-C**
  - 0 w: 50 (mg/dL)
  - 12 w: 60 (mg/dL)
  - 24 w: 70 (mg/dL)

- **TG**
  - 0 w: 150 (mg/dL)
  - 12 w: 140 (mg/dL)
  - 24 w: 130 (mg/dL)
Possible Mechanisms of Attenuation in Inflammatory Signals

- JNK
- HSP72
- MET
- ER stress
- Oxidative stress
- NF-κB
- IkBα
- NF-κB binding site
- CRP
- TNF-α
- Stress signals
- Proteasomal degradation
- Cytoplasm
- Nucleus
- IKK
- MET
- HSP72
**Diet or Exercise Interventions Fail to Attenuate Inflammation**

<table>
<thead>
<tr>
<th>Subjects and design</th>
<th>Treatment</th>
<th>Design</th>
<th>CRP</th>
<th>TNF-α</th>
<th>Time</th>
<th>Weight loss</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>41 diabetic patients</td>
<td>Soy protein</td>
<td>Randomized, parallel</td>
<td>$\uparrow^1$</td>
<td>ND$^2$</td>
<td>4 y</td>
<td>No</td>
<td>15</td>
</tr>
<tr>
<td>6 men and 6 women</td>
<td>(n-3) fatty acids</td>
<td>Longitudinal</td>
<td>$\downarrow$</td>
<td>ND</td>
<td>8 wk</td>
<td>No</td>
<td>16</td>
</tr>
<tr>
<td>15 overweight men</td>
<td>Low fat/ low carbohydrate</td>
<td>Cross-over</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
<td>12 wk</td>
<td>Yes</td>
<td>26</td>
</tr>
<tr>
<td>28 overweight men</td>
<td>Carbohydrate restriction + eggs</td>
<td>Randomized, parallel</td>
<td>$\downarrow$</td>
<td>$\leftrightarrow$</td>
<td>12 wk</td>
<td>Yes</td>
<td>17</td>
</tr>
<tr>
<td>210 men and women</td>
<td>Exercise</td>
<td>Randomized parallel</td>
<td>$\leftrightarrow$</td>
<td>ND</td>
<td>12 mo</td>
<td>Yes</td>
<td>19</td>
</tr>
<tr>
<td>13 men with low back pain</td>
<td>Exercise</td>
<td>Longitudinal</td>
<td>$\downarrow$</td>
<td>ND</td>
<td>8 wk</td>
<td>No</td>
<td>18</td>
</tr>
<tr>
<td>11 healthy 11 multiple sclerosis</td>
<td>Exercise</td>
<td>Parallel</td>
<td>ND</td>
<td>$\uparrow$</td>
<td>8 wk</td>
<td>No</td>
<td>30</td>
</tr>
<tr>
<td>87 Obese subjects with knee pain</td>
<td>Hypocaloric diet</td>
<td>Parallel</td>
<td>$\leftrightarrow$</td>
<td>$\leftrightarrow$</td>
<td>6 mo</td>
<td>No</td>
<td>28</td>
</tr>
<tr>
<td>44 women</td>
<td>Polyphenols in grapes</td>
<td>Randomized, cross-over</td>
<td>$\leftrightarrow$</td>
<td>$\downarrow$</td>
<td>4 wk</td>
<td>No</td>
<td>31</td>
</tr>
<tr>
<td>12 men and 12 women</td>
<td>Polyphenols in raisins</td>
<td>Longitudinal</td>
<td>ND</td>
<td>$\downarrow$</td>
<td>6 wk</td>
<td>No</td>
<td>29</td>
</tr>
<tr>
<td>16 obese subjects</td>
<td>Exercise</td>
<td>Parallel</td>
<td>$\downarrow$</td>
<td>$\leftrightarrow$</td>
<td>12 wk</td>
<td>No</td>
<td>27</td>
</tr>
</tbody>
</table>

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

**MET could be Beyond Life-Style Interventions**

**MET could be beyond life-style interventions**

<table>
<thead>
<tr>
<th>BW</th>
<th>FBS</th>
<th>hs-CRP</th>
<th>Wc</th>
<th>F-IRI</th>
<th>Adiponectin</th>
<th>CPR</th>
<th>Leptin</th>
<th>VFA</th>
<th>HOMA-IR</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td></td>
<td></td>
<td>VFA</td>
<td></td>
<td>QUICKI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SFA</td>
<td></td>
<td>cWBISI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VFA+SFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WBC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LDL-C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Insulinogenic index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PBMC (Peripheral Blood Mononuclear Cells)

CD14(+) CD14(-):
- Monocytes
- 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(-):
- DAPI
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 lifestyle-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