Mild Electrical Stimulation with Heat Shock Reduces Visceral Adiposity and Improves Metabolic Abnormalities in Subjects with Metabolic Syndrome or Type 2 Diabetes: Randomized Crossover Trials

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ABSTRACT

Background: The induction of heat shock protein (HSP) 72 by mild electrical stimulation with heat shock (MES + HS), which improves visceral adiposity and insulin resistance in mice, may be beneficial in treating metabolic syndrome (MS) or type 2 diabetes mellitus (T2DM).

Methods: Using open-label crossover trials, 40 subjects with MS or T2DM were randomly assigned using computer-generated random numbers to 12 weeks of therapeutic MES + HS followed by 12 weeks of no treatment, or vice versa. During the intervention period, physical and biochemical markers were measured.

Findings: Compared to no treatment, MES + HS treatment was associated with a significant decrease in visceral adiposity (−7.54 cm² (−8.61%), 95% CI −8.55 to −6.53 (p = 0.037)) in MS, −19.73 cm² (−10.89%) in T2DM. Fasting plasma glucose levels were decreased by 3.74 mg/dL (−5.28%; 95% CI −4.37 to −3.09 mg/dL, p = 0.029) in MS and by 14.97 mg/dL (10.40%; 95% CI −15.70 to 14.15 mg/dL, p < 0.001) in T2DM, and insulin levels were also reduced by 10.39% and 25.93%, respectively. HbA1c levels showed a trend toward reduction (−0.06%) in MS, and was significantly declined by −0.43% (95% CI −0.55 to −0.31%, p = 0.009) in T2DM. HbA1c level of less than 7.0% was achieved in 52.5% of the MES + HS-treated T2DM patients in contrast to 15% of the non-treated period. Several insulin resistance indices, inflammatory cytokines or adipokines, including C-reactive protein, adiponectin, and tumor necrosis factor-α, were all improved in both groups. In isolated monocytes, HSP72 expression was increased and cytokine expression was reduced following MES + HS treatment. Glucose excursions on meal tolerance test were lower after using MES + HS in T2DM.

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1. Introduction

Although DCCT, UKPDS and our Kumamoto Study (Shichiri et al., 2000) have shown that strict glycemic control could prevent microvascular complications, the increase of diabetes is still an important issue worldwide. The increase of type 2 diabetes mellitus (T2DM) is associated with excess visceral adiposity, which is tightly linked to metabolic syndrome (MS). MS is recognized as a cluster of cardiovascular risk factors such as hyperglycemia, dyslipidemia, elevated blood pressure and chronic inflammation (Alberti et al., 2005). Visceral fat has been demonstrated to express more pro-inflammatory cytokines than subcutaneous fat in obese states (Ohman et al., 2009). Inflammatory markers such as C-reactive protein (CRP) (Tamakoshi et al., 2003) and tumor necrosis factor (TNF)-α have been linked to MS.

Currently, there are no medications or modalities to reduce both visceral adiposity as well as chronic inflammation in MS or T2DM subjects. Recently, we (Morino et al., 2008a; Adachi et al., 2010) and others

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of the MES + HS device has been provided previously (Kondo et al., 2008; Kondo et al., 2012), a transgenic system (Chung et al., 2008), heat treatment (Gupte et al., 2009; Kavanagh et al., 2011) or chemical inducers (Adachi et al., 2010) ameliorated abnormal metabolic features in animal models of T2DM, such as insulin resistance, hyperglycemia and visceral fat accumulation. Mild electrical stimulation enhances heat induction of HSP72 (Morino et al., 2008b) and may directly activate insulin signaling by modulating the insulin receptor localization of membrane components (Yano et al., 2010; Morino-Koga et al., 2013). In this study, we investigated the effects of MES + HS on glucose homeostasis, insulin resistance, visceral adiposity and inflammatory cytokine levels in male subjects with MS or T2DM. In addition, inflammatory characteristics of circulating monocytes were examined. This novel combination therapy may provide an additional treatment strategy to improve metabolic abnormalities in lifestyle-related diseases.

2. Materials and Methods

2.1. Study Participants

A total of 40 Japanese males with MS or T2DM were recruited. MS was defined by the Examination Committee for Criteria of Metabolic Syndrome and was diagnosed on the basis of the criteria of the American Diabetes Association. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, and written informed consent was obtained from each subject. These researches were approved by the Ethics Review Committee at Kumamoto University (Advanced Ethics No. 736 and Ethics No. 514). These clinical trials were registered with an approved ICMJE clinical trial registry, UMIN (ID: UMIN 000001149, 000003210 and 000007792). Protocol details can be found in the Supplemental materials.

2.2. MES + HS Treatment

The devices (BioMetronome®) for producing MES + HS were provided by Tsuchiya Rubber Co. Ltd. (Kumamoto, Japan). The description of the MES + HS device has been provided previously (Kondo et al., 2010). Briefly, MES + HS produces electrical stimulation of 1.4 ± 0.1 V/cm: the pads were positioned on the front and back of the abdomen, 55 pulses per second, 0.1 millisecond duration with 42 °C heat. The padded area was 15 cm in length × 25 cm in width.

2.3. Randomization and Masking

Forty eligible MS or T2DM subjects were randomly assigned using computer-generated random numbers into two groups by Latin square method, each containing 20 subjects. Neither subjects nor investigators were masked to treatment allocation at the time of enrollment.

2.4. Study Design and Clinical Protocol

This study was a prospective, randomized, open-label, crossover trial. Forty eligible MS or T2DM subjects were randomly assigned into two groups, each containing 20 subjects. Group I underwent a 12-week intervention period of MES + HS followed by 12 weeks with no treatment. The order was reversed in group II. During the MES + HS-treatment period, subjects were instructed to use MES + HS 4 times a week for 60 min per session. Exercise and diet alterations were prohibited during the entire period. At 0, 12 and 24 weeks, body compositions, abdominal adiposity, metabolic and biochemical examination with a 75 g oral glucose tolerance test (OGTT) in MS or a 592 kcal meal tolerance test (MTT) in T2DM were performed. The primary endpoint is the amount of visceral adiposity and glucose control. Other outcomes include blood pressure, insulin resistance, inflammatory cytokine levels and the HbA1c achievement ratio of less than 7.0%. For the primary outcome, we estimated the need to enroll 36 subjects to detect changes in visceral fat area of 15% with MES + HS as compared to no treatment, with statistical power of 80%, allowing for a type I (α) error of 0.05. Allowing for a loss to follow-up rate of 10%, 40 subjects were required to undergo randomization.

2.5. Quantification of Adiposity

Visceral fat area (VFA) and subcutaneous fat area (SFA) at the umbilical level were measured using CT scans (HiSpeed NX/i, GE Healthcare Japan Co., Ltd., Tokyo, Japan).

2.6. Insulin Sensitivity Indices

Several indices were calculated based on the results of OGTT in MS. The quantitative insulin-sensitivity check index (QUICKI), homeostasis model assessment of insulin resistance (HOMA-IR), HOMA-%, composite whole body insulin sensitivity index (cWBISI) and insulinogenic index (II) were determined, as described previously (Yamashiro et al., 2010).

2.7. Monocytes Isolation and Analysis

To investigate the characteristics of monocyte in MS or T2DM subjects, 10 subjects were randomly selected. Before and after 4 weeks of MES + HS treatment, blood samples were collected during a fasted state. First, peripheral blood mononuclear cells (PBMCs) were isolated using BD Vacutainer™ CPT™ (BD, Franklin Lakes, NJ). Monocytes were subsequently isolated from the PBMCs magnetically by depletion technique (Miltenyl Biotec. Auburn, CA). For some experiments, monocytes were examined before and after activation with lipopolysaccharide (LPS: 160 ng/mL) overnight.

2.8. mRNA Expression Determined by qRT-PCR

Real time qRT-PCR was performed using mRNA from isolated monocytes stimulated with LPS. The protocol of qRT-PCR has been described previously (Adachi et al., 2010).

2.9. Statistical Analysis

Statistical analysis was performed with SPSS software (IBM, Chicago, IL, USA). All values were expressed as mean ± standard deviation (S.D.). The treatment effects of MES + HS were analyzed by paired a t-test if data were normally distributed or a Wilcoxon signed-rank test if not. Sequential changes were analyzed by repeated-measures ANOVA. Two-sided p-values of less than 0.05 were considered to indicate statistical significance.

3. Results

3.1. Characteristics of MS or T2DM Subjects

Japanese males diagnosed with MS or T2DM were randomly assigned to either group I or group II. Thereafter, subjects were randomized to one of two possible group sequences to receive no treatment or MES + HS treatment (Fig. 1). The baseline values were similar between the two groups (n = 40) (Table 1).

3.2. Adverse Effects

There were no harmful or adverse events including hypoglycemia or biochemical abnormalities during these studies. As only mild electrical...
3.3. Adipose Tissue Composition and Physical Status

Visceral and subcutaneous adiposity were analyzed using abdominal CT scans. After MES + HS treatment, visceral fat area (VFA) in MS and T2DM was significantly decreased by 12.69 ± 3.25 cm² (−8.61%, p = 0.037, Table 2) and 20.88 ± 4.00 cm² (−10.89%, p = 0.003. Table 2), respectively. Although there was no change in subcutaneous fat area (SFA) as a result of MES + HS treatment, total abdominal adiposity was significantly reduced (Table 2).

Waist circumference (Wc) also showed similar changes associated with MES + HS treatment (Table 2). Although there was no significant change in MS, body mass index (BMI) was significantly decreased by 1.42% (p = 0.027) in T2DM. Systolic or diastolic blood pressure (SBP or DBP) was significantly reduced in MS and T2DM (Table 3).

3.4. Glucose Homeostasis and Insulin Resistance

Fasting plasma glucose (FPG) was significantly decreased (−5.95 mg/dL in MS, −15.45 mg/dL in T2DM, Table 2). Fasting immuno-reactive insulin (IRI) showed similar alterations (−1.08 μIU/mL in MS, −3.89 μIU/mL in T2DM, Table 2). As these FPG and IRI were both reduced, HOMA-IR was significantly decreased (Table 2). QUICKI showed a trend of reduction by 2.70% (p = 0.09), and cWBISI was significantly increased (+20.61%, p = 0.029) in MS subjects. Upon 75 g OGTT, FPG and glucose levels at 90 min were significantly lower than those at the non-treatment period (Fig. 2A). The insulin secreting indices, I.I. and HOMA-β were also evaluated. Although IRI response against glucose load was quite similar to the non-treatment period, I.I. showed a significant decrease in MS subjects (Table 2 and Fig. 2B). HOMA-β was unchanged during the study.

HbA1c (NGSP) levels showed a trend toward reduction (−0.07 ± 0.04%, p = 0.143) in MS, and this was significantly decreased (−0.49 ± 0.06%) from baseline in T2DM. Compared with no treatment period, HbA1c showed a significant reduction by 0.43% (p = 0.009). The glucose levels at 0, 120 and 300 min on meal tolerance test were significantly lower (0 min; 115.5 vs 108.7 mg/dL, p = 0.039. 120 min; 251.2 vs 225.4 mg/dL, p = 0.048. 300 min; 171.8 vs 130.1 mg/dL, p = 0.041. Fig. 2C) in T2DM. The proportion of patients who achieved the HbA1c goal of less than 7.0% was 52.5% in the MES + HS-treated period in contrast to 15.0% in the untreated period (Fig. 2D). Higher pre-HbA1c indicated greater reductions upon MES + HS treatment (HbA1c 5.9–6.4%: −0.15%, 6.5–7.5%: −0.42%, 7.6–10.0%: −0.72%. Fig. 2E)

3.5. Inflammatory Cytokines and Adipokines

Visceral adiposity influences the production of inflammatory cytokines and adipokines. High sensitivity CRP significantly decreased by

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current was used, no perceptible muscle contraction or muscle pain was generated in the subjects.

Screened MS subjects (n= 41)  
Screened T2DM subjects (n= 43)  
Not meeting inclusion criteria (n= 0)  
Not meeting inclusion criteria (n= 3)  
Eligible subjects (n= 41)  
Eligible subjects (n= 40)  
Declined participation (n= 1)  
Declined participation (n= 0)  
Randomized (n= 40)  
Randomized (n= 40)  
MES+HS (+) (n= 20)  
MES+HS (-) (n= 20)  
MES+HS (+) (n= 20)  
MES+HS (-) (n= 20)  
MES+HS (-) (n= 20)  
MES+HS (+) (n= 20)  
Washout  
Trial completion (n= 40)  
Subjects included in analysis (n= 40)  
Subjects included in analysis (n= 40)  
Fig. 1. Flowchart of study subjects.
53.85% in MS (p = 0.025) and by 4.54% in T2DM (p = 0.047) following MES + HS treatment. Adiponectin increased by 8.36% and 6.63% in MS and T2DM, respectively. Leptin tended to decrease by 8.89% in MS, and by 52.6% in 45 KDa (p = 0.0002) subunits in MS (Fig. 3A and B), and by 63.2% in T2DM (Fig. 4A and B. p = 0.017). Phosphorylation of AMP-kinase (AMPK) was also increased by 79.9% in MS (Fig. 3A and B .p= 0.001) subunits in MS (Fig. 3A and B), and by 28.0% (p = 0.006), 35.7 ± 4.5 (p = 0.085) and 3.36 ± 0.93 (p = 0.113) in MS and T2DM, respectively. Interleukin-6 showed a significant reduction in MS (−28.33%), but not in T2DM (−2.50%).

3.6. Other Metabolic Benefits

To evaluate liver fat accumulation non-invasively, the AST/ALT ratio and liver/spleen attenuation (L/S) ratio were calculated. In parallel with the reduction of visceral adiposity by MES + HS treatment, the AST/ALT ratio increased with the treatment (Fig. 3A). The L/S ratio showed a similar alteration (Table 3).

L DL-cholesterol was decreased as shown in Table 3. HDL-C was increased only in T2DM subjects (Table 3). Although BUN was not changed, serum creatinine (Cre) was slightly but significantly decreased in T2DM. Urinary excretion of albumin adjusted by urinary Cre was also reduced in T2DM. After 4 weeks of MES + HS treatment, HSP72 protein levels in monocytes were significantly increased by 34.5% compared to baseline in MS (Fig. 3A and B. p < 0.0001), and by 41.7% in T2DM (Fig. 4A and B. p = 0.001). Phosphorylation of AMP-kinase (AMPK) was also increased by 79.9% in MS (Fig. 3A and B, p = 0.0002), and by 63.2% in T2DM (Fig. 4A and B. p = 0.017). Phospho-c-Jun N-terminal kinase (p-JNK) was attenuated by 42.1% in 56 KDa (p = 0.001), and by 28.0% (p = 0.006), 35.7 ± 4.5 (p = 0.085) and 3.36 ± 0.93 (p = 0.113) in MS and T2DM, respectively. Interleukin-6 showed a significant reduction in MS (−28.33%), but not in T2DM (−2.50%).

3.7. Inflammatory Characteristics of Monocytes

To identify the effects of MES + HS on the inflammatory milieu in vivo, CD14 positive circulating monocytes were isolated, and levels of cytokine expression were examined both in MS and T2DM. After 4 weeks of MES + HS treatment, HSP72 protein levels in monocytes were significantly increased by 34.5% compared to baseline in MS (Fig. 3A and B. p < 0.0001), and by 41.7% in T2DM (Fig. 4A and B. p = 0.001). Phosphorylation of AMP-kinase (AMPK) was also increased by 79.9% in MS (Fig. 3A and B, p = 0.0002), and by 63.2% in T2DM (Fig. 4A and B. p = 0.017). Phospho-c-Jun N-terminal kinase (p-JNK) was attenuated by 42.1% in 56 KDa (p = 0.001), and by 28.0% (p = 0.006), 35.7 ± 4.5 (p = 0.085) and 3.36 ± 0.93 (p = 0.113) in MS and T2DM, respectively. Interleukin-6 showed a significant reduction in MS (−28.33%), but not in T2DM (−2.50%).

4. Discussion

Obesity due to sedentary lifestyle and/or unbalanced diet causes lifestyle-related diseases such as MS or T2DM, characterized by elevated blood glucose, often accompanied by dyslipidemia and hypertension.
with chronic inflammation resulting from an over-accumulation of visceral fat (Alberti et al., 2005). In this study, we demonstrated that MES + HS, which activates the heat shock response (Morino et al., 2008a; Kondo et al., 2012), significantly improved visceral adiposity, insulin resistance, glucose homeostasis and cytokine levels in males with MS or T2DM.

MES + HS treatment significantly improved insulin resistance in subjects with MS or T2DM. Particularly in T2DM, we have identified significant improvements in HbA1c, FPG, postprandial glucose excursion (Bruce et al., 2003; Kurucz et al., 2002). HSP72 is a cytoplasmic molecular chaperone, which is induced by several stimuli such as heat stress, viral infection or heavy metal contamination etc., and protects cells from apoptosis or cellular dysfunction caused by such cell stresses (Chung et al., 2008; Henstridge et al., 2014), and overexpression, and HSP inducers has been shown to improve insulin signaling in mice, monkeys and humans (Morino et al., 2008a; Adachi et al., 2010; Chung et al., 2008; Kondo et al., 2012; Kavanagh et al., 2011; Literati-Nagy et al., 2009).

The beneficial effects of HSP72 in insulin signaling may be partly explained by the suppression of JNK (Chung et al., 2008). Deletion of JNK1 protects mice from high-fat diet-induced insulin resistance, in part through decreased adiposity. Indeed, suppression of JNK in diabetic mice ameliorates insulin resistance and glucose intolerance (Kaneto et al., 2004). It has also been reported that an HSP72 polymorphism is linked to increased obesity and diabetes risk in humans (Zouari et al., 2004). Induction of HSP72 enhances mitochondrial capacity and/or function (Chung et al., 2008; Henstridge et al., 2014), and suppresses JNK activity (Morino et al., 2008a; Kondo et al., 2012). In addition, uncoupling protein-1 mRNA expression in brown adipose tissue was increased in MES + HS-treated diabetic animals (Morino et al., 2008a). Moreover, MES + HS treatment activates AMPK in mice (Kondo et al., 2012). In fact, AMPK α1 knockout showed increased visceral adiposity with insulin resistance (Zhang et al., 2012). As we have identified the activation of AMPK in monocytes, this may contribute to reduce visceral adiposity. In addition, testosterone treatment ameliorates metabolic abnormalities, including adipocyte hypertrophy, adipose tissue dysfunction, tissue hypoxia and insulin resistance (Maneschi et al., 2012). Heat treatment activates androgen effect especially in lipid peroxidation, indicating that activation of androgen signaling in mice, monkeys and humans (Morino et al., 2008a; Adachi et al., 2010; Chung et al., 2008; Kondo et al., 2012; Kavanagh et al., 2011; Literati-Nagy et al., 2009).

| Table 2 | Primary outcomes. |
|---------|------------------|
| **Adiposity** | No treatment | Δ no treatment | Baseline | MES + HS | ΔMES + HS | p value |
| **Visceral fat area (cm²)** | 148.4 ± 6.4 | 143.2 ± 6.8 | −5.2 | 147.4 ± 6.6 | 134.7 ± 6.4 | −12.7 | 0.037 |
| **SubQ fat area (cm²)** | 191.7 ± 9.5 | 191.0 ± 9.6 | −0.7 | 190.3 ± 9.1 | 187.4 ± 8.5 | −2.9 | 0.321 |
| **Total fat area (cm²)** | 340.1 ± 13.2 | 334.3 ± 13.0 | −5.9 | 337.7 ± 12.7 | 322.1 ± 12.0 | −15.6 | 0.019 |
| **BMI (kg/m²)** | 26.8 ± 0.5 | 26.7 ± 0.6 | −0.1 | 26.8 ± 0.4 | 25.8 ± 0.4 | −1.0 | 0.238 |
| **Waist (cm)** | 94.1 ± 1.0 | 93.9 ± 1.0 | −0.2 | 93.9 ± 1.1 | 92.9 ± 1.1 | −1.0 | 0.031 |

**Glucose control**

| Blood glucose at 0 min (mg/dL) | 112.3 ± 1.8 | 110.1 ± 2.4 | −2.2 | 112.7 ± 1.8 | 106.7 ± 2.5 | −6.0 | 0.029 |
| Insulin at 0 min (μIU/mL) | 11.4 ± 1.0 | 11.7 ± 1.0 | 0.3 | 10.4 ± 0.8 | 9.3 ± 0.7 | −1 | 0.049 |
| HOMA-IR | 3.2 ± 0.3 | 3.2 ± 0.3 | 0.05 | 2.9 ± 0.2 | 2.5 ± 0.2 | −0.4 | 0.024 |
| Insulinogenic index | 1.7 ± 0.4 | 1.4 ± 0.3 | −0.3 | 1.7 ± 0.4 | 1.0 ± 0.1 | −0.7 | 0.042 |
| BMI (kg/m²) | 87.8 ± 9.0 | 88.8 ± 7.3 | 1.0 | 78.7 ± 6.5 | 84.0 ± 6.7 | 5.3 | 0.732 |
| QUICKI | 0.33 ± 0.01 | 0.33 ± 0.01 | −0.003 | 0.33 ± 0.01 | 0.34 ± 0.01 | 0.01 | 0.09 |
| Compositional WRI | 3.2 ± 0.2 | 3.5 ± 0.2 | 0.3 | 3.3 ± 0.2 | 4.0 ± 0.3 | 0.7 | 0.029 |
| Glucose AUC | 334.2 ± 10.6 | 330.4 ± 12.3 | −3.8 | 337.2 ± 12.4 | 325.1 ± 12.9 | −12.1 | 0.150 |
| IR AUC | 152.6 ± 10.6 | 148.1 ± 12.2 | −4.5 | 145.3 ± 9.6 | 139.4 ± 9.7 | −5.9 | 0.385 |
| Hba1c (%) | 5.24 ± 0.09 | 5.23 ± 0.08 | −0.01 | 5.29 ± 0.08 | 5.22 ± 0.09 | −0.07 | 0.143 |

**Type 2 diabetes mellitus**

| Adiposity | No treatment | Δ no treatment | Baseline | MES + HS | ΔMES + HS | p value |
| Visceral fat area (cm²) | 186.0 ± 9.8 | 187.1 ± 9.3 | 1.1 | 191.8 ± 9.5 | 179.9 ± 7.0 | −12.0 | 0.003 |
| SubQ fat area (cm²) | 160.4 ± 11.1 | 170.0 ± 12.6 | 9.6 | 167.3 ± 12.4 | 169.9 ± 12.5 | 2.6 | 0.021 |
| total fat area (cm²) | 346.4 ± 19.2 | 357.1 ± 20.7 | 10.7 | 359.6 ± 20.6 | 340.8 ± 17.6 | −18.8 | 0.005 |
| BMI (kg/m²) | 27.8 ± 0.8 | 27.8 ± 0.8 | −0.02 | 27.8 ± 0.8 | 27.4 ± 0.8 | −0.4 | 0.027 |
| Waist (cm) | 97.7 ± 2.0 | 100.5 ± 3.1 | 2.8 | 97.9 ± 2.1 | 95.2 ± 1.8 | −2.7 | 0.021 |

The results of MES + HS intervention data compared to no treatment period. Values are expressed as mean ± S.D. Other abbreviations are the same as Table 1.
visceral adiposity by MES + HS treatment, which need further investigation.

Increased production of pro-inflammatory cytokines is associated with impaired insulin sensitivity (Tamakoshi et al., 2003). We previously reported that activation of the HSR by MES + HS in diabetic mice was associated with metabolic benefits, accompanied by improvements in inflammatory cytokine productions (Morino et al., 2008a). In this study, we observed similar cytokine changes in human subjects with MS and T2DM. Although this may be partly explained by the reduction in visceral adiposity, we also reported significant reductions in CRP and TNF-α levels by MES + HS in healthy subjects without any body composition changes (Kondo et al., 2010). These results suggest a possible direct association between HSR activation and suppression of systemic inflammation. High serum CRP levels are associated with reduced HSP72 levels (Armutcu et al., 2008), and are positively regulated by NF-κB, which can be attenuated by HSP72. Indeed, we observed a

| Table 3 |
| --- |
| Secondary outcomes. |

| Metabolic syndrome | Inflammation | No treatment | MES + HS |
| --- | --- | --- | --- |
| | | Baseline | No treat | Δ no treat | Baseline | MES + HS | ΔMES + HS | p value |
| hs-CRP (ng/mL) | 3923.3 ± 1779.0 | 4206.5 ± 1932.1 | 283.2 | 4158.1 ± 1920.7 | 3969.5 ± 1882.4 | −1886.6 | 0.047 |
| Adiponectin (μg/mL) | 4.7 ± 0.6 | 3.8 ± 0.3 | −0.9 | 3.9 ± 0.3 | 4.2 ± 0.5 | 0.3 | 0.003 |
| Leptin (μg/mL) | 8.9 ± 0.9 | 10.5 ± 1.1 | 1.6 | 10.2 ± 1.1 | 9.1 ± 0.8 | 1.3 | 0.013 |
| TNF-α (pg/mL) | 2.0 ± 0.3 | 2.7 ± 0.5 | 0.7 | 2.5 ± 0.5 | 2.0 ± 0.3 | 0.5 | 0.001 |
| IL-6 (pg/mL) | 3.0 ± 0.4 | 2.8 ± 0.4 | −0.2 | 2.8 ± 0.4 | 2.9 ± 0.4 | 0.1 | 0.230 |

| Blood pressure | Systolic blood pressure (mm Hg) | 143.0 ± 7.3 | 142.4 ± 3.0 | −0.6 | 136.7 ± 3.0 | 129.1 ± 2.5 | −7.6 | 0.013 |
| Diastolic blood pressure (mm Hg) | 74.7 ± 1.8 | 73.6 ± 1.9 | −1.1 | 71.7 ± 1.9 | 66.5 ± 2.0 | −5.2 | 0.005 |
| Heart rate (bpm) | 70.1 ± 1.8 | 70.6 ± 1.5 | 0.5 | 69.9 ± 1.6 | 68.2 ± 1.8 | −1.7 | 0.110 |

| Type 2 diabetes mellitus | Inflammation | No treatment | MES + HS |
| --- | --- | --- | --- |
| | | Baseline | No treat | Δ no treat | Baseline | MES + HS | ΔMES + HS | p value |
| hs-CRP (ng/mL) | 4206.5 ± 1932.1 | 4158.1 ± 1920.7 | −283.2 | 3969.5 ± 1882.4 | −1886.6 | 0.047 |
| Adiponectin (μg/mL) | 3.8 ± 0.3 | 3.9 ± 0.3 | 0.9 | 4.2 ± 0.5 | 0.3 | 0.003 |
| Leptin (μg/mL) | 10.5 ± 1.1 | 10.2 ± 1.1 | −0.3 | 9.1 ± 0.8 | 1.3 | 0.013 |
| TNF-α (pg/mL) | 2.7 ± 0.5 | 2.5 ± 0.5 | −0.2 | 2.0 ± 0.3 | 0.5 | 0.001 |
| IL-6 (pg/mL) | 2.8 ± 0.4 | 2.8 ± 0.4 | 0.0 | 2.9 ± 0.4 | 0.1 | 0.230 |

The results of MES + HS intervention data compared to no treatment period. Values are expressed as mean ± S.D.
L/S ratio = Liver to spleen ratio on computed tomography.
L-ACR = Urinary albumin creatinine ratio. Other abbreviations are the same as Table 1.
significant increase in HSP72 with reduction of NF-κB nuclear accumulation in monocytes after MES + HS treatment. HSP72 also decreases TNF-α levels by suppression of NF-κB as well. Therefore, induction of HSP72 by MES + HS could decrease inflammatory cytokines through suppression of NF-κB activation. It is also possible that an anti-inflammatory effect of MES + HS could be achieved by AMPK activation, because AMPK deficiency in macrophages markedly increases the pro-inflammatory status (Zhang et al., 2012). As chronic activation of inflammatory status is associated with atherogenesis, anti-inflammatory effect of MES + HS may contribute to limit future vascular complications.

Because visceral fat produces angiotensinogen and increases sympathetic nervous activity, MS and T2DM are often associated with hypertension. In this regard, reduction in blood pressure as a result of MES + HS treatment could be explained, at least in part, by a reduction in visceral adiposity. Recently, it has been shown that heat treatment in high fat-fed mice augmented angiotensin (Shichiri et al., 2000; Alberti et al., 2005; Ohman et al., 2009; Tamakoshi et al., 2003; Morino et al., 2008a; Adachi et al., 2010; Chung et al., 2008), which counteracts against angiotensin II, through Mas receptor/eNOS axis that may improve blood pressure and endothelial dysfunction (Karpe and Tikoo, 2014). Therefore, both reductions in visceral fat and eNOS activation may contribute to ameliorate hypertension upon MES + HS treatment.

Hepatic steatosis is considered to be one of the phenotypes of lifestyle-related diseases. Our results indicate that activation of the HSR
by MES + HS improved surrogate markers of fatty liver. Although precise interaction between HSR activation and improvement of fatty liver is still unknown, naringin, a bioflavonoid isolated from grapefruit, activates HSP72 and attenuates hepatic steatosis (Sharma et al., 2011). Alternatively, endoplasmic reticulum (ER) stress attenuation by HSR activation may be involved in this process, because HSP72 enhances ER capacity, and hepatic lipid accumulation is correlated with ER stress (Gupta et al., 2010).

The molecular mechanisms involved in metabolic benefits of MES + HS appear to be similar to those observed in exercise, particularly in HSP72 induction and AMPK activation (Zhang et al., 2009). Indeed, 8 weeks of endurance training in diabetic rats increased HSP72 expression (Atalay et al., 2004). It is also proposed that HSR activators share metabolic pathways associated with exercise with activation of AMPK (Hooper et al., 2014).

**Fig. 3.** Inflamatory characteristics in CD14 positive monocytes in MS subjects. Ten MS subjects were selected, and 4 weeks of MES + HS treatment was performed. Before and after the MES + HS treatment period, blood sampling was performed on a fasted state. Circulating monocytes were isolated, then HSP72 protein, p-AMPK, p-JNK and actin were determined by Western blot analysis. Representative results are shown in A. The relative intensity calculated using actin is shown in B. (C) Isolated monocytes were subjected to immunofluorescent staining after LPS stimulation using antibodies against NF-κB p65 (red) and CD14 (green). Representative results are shown under low (upper panels) or high (lower panels) magnifications. (D) Isolated monocytes after LPS stimulation were subjected to mRNA measurements of CRP, IL-6, NF-κB and TNF-α. Averages of each mRNA level with or without MES + HS treatment are shown. *p < 0.05, **p < 0.01.
Thus, MES + HS treatment may mimic exercise training sharing similar mechanisms and results.

4.1. Limitations

This study was conducted in relatively small number, and the participants were limited to male only in order to preclude menstruation cycle, which may disturb metabolic parameters.

5. Conclusion

MES + HS treatment exerts anti-visceral obesity, anti-hypertensive, anti-diabetic and anti-inflammatory effects with no harmfulness, possibly through HSP72 induction and AMPK activation. Moreover, it can also be used in physically handicapped or bed-ridden patients who are unable to follow an exercise regimen. MES + HS could be analogous to exercise training in terms of HSP72 induction and AMPK activation, and might confer additional benefits, such as anti-atherogenic effect. HSR
activation by physical MES + HS system may be an alternative and benefical therapeutic approach to the treatment of lifestyle-related diseases.

Conflict of interest

The authors report no potential conflicts of interest relevant to this article.

Author contributions

T.K. designed the study, researched data, contributed to discussion and wrote the manuscript. K.O. and S.Ki. researched data, and contributed to discussion. R.M. and R.G., researched data. M.A.S., S.Ka., M.I., J.K., H.M., and T.M., contributed to the discussion. H.K. reviewed and edited the manuscript. E.A. designed the study, reviewed and edited the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jbi.2014.11.001.

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