EBioMedicine 1 (2014) 80–89

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Original Article

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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ARTICLE INFO

Article history:
Received 17 September 2014
Received in revised form 4 November 2014
Accepted 4 November 2014
Available online 11 November 2014

Keywords:
Metabolic syndrome
Type 2 diabetes
Heat shock response
Insulin resistance
Chronic inflammation

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 cm^2 (−8.61%), 95% CI −8.55 to −6.53 ($p = 0.037$) in MS, −19.73 cm^2 (−10.89%), 95% CI −20.97 to −18.49 ($p = 0.003$) 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 −15.79 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.
Interpretation: This combination therapy has beneficial impacts on body composition, metabolic abnormalities, and inflammation in subjects with MS or T2DM. Activation of the heat shock response by MES + HS may provide a novel approach for the treatment of lifestyle-related diseases.
Funding: Funding for this research was provided by MEXT KAKENHI (Grants-in-Aid for Scientific Research from Ministry of Education, Culture, Sports, Science and Technology, Japan).

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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http://dx.doi.org/10.1016/j.ebiom.2014.11.001

(Chung et al., 2008; Gupte et al., 2009) have shown that the beneficial metabolic advantages of heat shock response (HSR) activation, which appears to mainly involve heat shock protein (HSP) 72. HSP72 induction by mild electrical stimulation (MES) with heat shock (HS) (Morino et al., 2008a; 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-β, composite whole body insulin sensitivity index (cWBISI) and insulinogenic index (I.I.) 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 Biotech. 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