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The Modulative Effects of Microcurrent Electrical Nerve Stimulation on Diabetic Mice

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Abstract

Diabetes (one of non-communicable diseases) is serious due to its complications, such like, cardiovascular ailments, neuropathy, nephropathy, retinopathy, wound gangrene and sexual impotence. Diabetes and associated chronic conditions are rapidly emerging as major health problems. In clinical, there were different drugs for diabetes treatment on different mechanisms. However, there were limited studies on the efficacy of electric stimulations on diabetes therapeutic application. In current study, we try to evaluate the effect of microcurrent electrical nerve stimulation (MENS) on diabetes modulation as an alternative medicine. A total of 36 male ICR mice of 6 weeks old were randomly divided into 4 groups [1] Control, [2] MENS only, [3] diabetes mellitus (DM), [4] DM with MENS. During 8 weeks treatments, the diabetes-associated assessments included body weight, diet utilization, blood glucose measurement, other biochemistries and histopathological observations. The diabetes animal model induced by streptozotocin (STZ) had 180 mg/dl fasting blood glucose (GLU-AC) before MENS intervention. After 3 and 6 weeks administration, the fasting blood glucose (GLU-AC) of DM+MENS group significantly decreased 31.97% and 50.82% ($P < 0.0001$), respectively, as compared to DM group and the oral glucose tolerance test (OGTT) also demonstrated the similar significant results. The diabetic syndromes of polydipsia and polyphagia were also significantly ameliorated by MENS intervention. In other biochemical indexes, the glycated hemoglobin (HbA1c), hyperinsulinemia, liver functions (aspartate aminotransferase, AST and alanine transaminase, ALT) and kidneys function (blood urea nitrogen, BUN & creatinine) were also significantly mitigated by MENS under diabetes model. The histological observation also showed the MENS administration improved the diabetes-related pathological characteristics in liver, kidney and pancreas tissues. Our results suggest that administration of MENS could significantly improve diabetes animal model on blood sugar homeostasis, diabetic polydipsia, biochemistries, and tissue damage. In the health conditions, the MENS didn't exist obvious side effects on assessments. Therefore, the MENS could be potential on alternative medicine or supportive applications to future DM therapeutics.

Key Words: alternative medicine, blood sugar, diabetes mellitus, DM complications, microcurrent electrical nerve stimulation

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Introduction

The global diabetes mellitus (DM) prevalence in 2013, it is estimated that almost 382 million people suffer from diabetes for a prevalence of 8.3%. In difference regions, it also exhibited different prevalence with different life styles, dietary habits, genetic factors, and so on. The North America and the Caribbean was the region with the higher prevalence of 11% having 37 million people with diabetes followed by the Middle East and North Africa with a prevalence of 9.2% having 35 million people with diabetes. Western Pacific containing Mongolia, China, Southeast Asia, Australia, and New Zealand, was the region with higher number of people living with diabetes (138 million), however its prevalence is 8.6%, close to the prevalence of the World. As of 2014, there was about 387 million people suffering diabetes worldwide, with type 2 diabetes making up about 90% of the cases, and the DM patients could occupy 8.3% of the adult population with similar ratio in male or female (29). The number of DM populations could be expected for substantial increase to 592 million by 2035 and the global economic cost of diabetes in 2014 was estimated to be $612 billion USD.

DM is a king of metabolic diseases which existed two acute metabolic syndromes including hyperglycemia and ketoacidosis (17). Symptoms of diabetes include frequent urination, increased thirst and hunger. The mechanisms of DM is due to either producing insufficient insulin or improper responses to the insulin regulations and could be furthered defined as insulin-dependent diabetes mellitus (IDDM, Type I DM) and non-insulin-dependent diabetes mellitus (NIDDM, Type II DM). The DM generally was associated with many complications, such like diabetic retinopathy (37), diabetic nephropathy (23), diabetic neuropathy (14), and cardiovascular diseases (16) in the late phase of disease. Therefore, the blood glucose control is an important issue for development of therapeutic agents. For clinical use, there were several clinical drugs developed for type II DM therapy, such like, insulin sensitizers (biguanides & thiazolidinediones), secretagogues (sulfonylureas & peptide analog), and alpha-glucosidase inhibitors with different mechanism strategies. In previous studies, it showed the phytochemicals with anti-inflammatory effects could be potential against inflammatory diseases like diabetes as investigated *in vivo* (19). The other medicinal plants also exhibited the main source of organic compounds such as polyphenols, tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids which could represent a source for the discovery and development of new candidates for antidiabetic molecules with different mechanisms (5, 8).

The electrical stimulation technologies were developed to apply different medical purposes according to different specifications including frequency, wave shape, voltage, and current. The transcutaneous electrical nerve stimulation (TENS) belonged to low-frequency range (<200 Hz) with spike wave and was reported to pain relief (24). In advanced development of electrical stimulation such like functional electrical stimulation or therapeutic electrical stimulation, it elevated the frequency (>200 Hz) and combined with complex wave shapes for physiological improvements including neuromuscular rehabilitation (12), Hemiplegia motor restoration (18), and wound healing (2). The electrical stimulation could be integrated into the meridian concepts of Chinese medicine as electroacu-puncture for metabolic disease therapy (4) or improve-ment of postmenopausal osteoporosis (39). The microcurrent electrical nerve stimulation (MENS) is an emerging electrical stimulation technology by delivering current in the microampere range and it is distinct from TENS which runs at one milliamp (mA) or one thousandth of an amp. In previous studies, the MENS also was elucidated for pain management (7) and muscular growth (26).

There were limited to be reported on the effects of MENS stimulations for modulation of hyperglycemia activities. Therefore, we aimed to investigate the effects of physiological mitigations under streptozotocin (STZ) induced diabetes model with gradual MENS stimulations. We hoped we could support or assist the current pharmaceutical DM treatments by providing potential MENS treatments with concepts of alternative medicine.

Materials and Methods

MENS Device

The device (model: DW1330) was developed by Taiwan Resonant Waves Research Corp (Taipei, Taiwan, ROC). It provides adjustable frequency (1 Hz-300 KHz) with square wave output and the duty cycle could be set as 0-100% with affordable microcurrent 50 uA-1000 uA. The device was validated every two weeks to assure the sufficient indicated electromagnetic fields. In current study, the stimulation could directly transduce from a designed circuit board to organism for possible physiological functions.

Animals, and Experiment Design

The animal strains in current diabetes study were ICR mice (6 weeks old) (34) from BioLASCO Taiwan (Yi-Lan Breeding Center, Yi-Lan County, Taiwan, ROC) accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All animals were given a standard

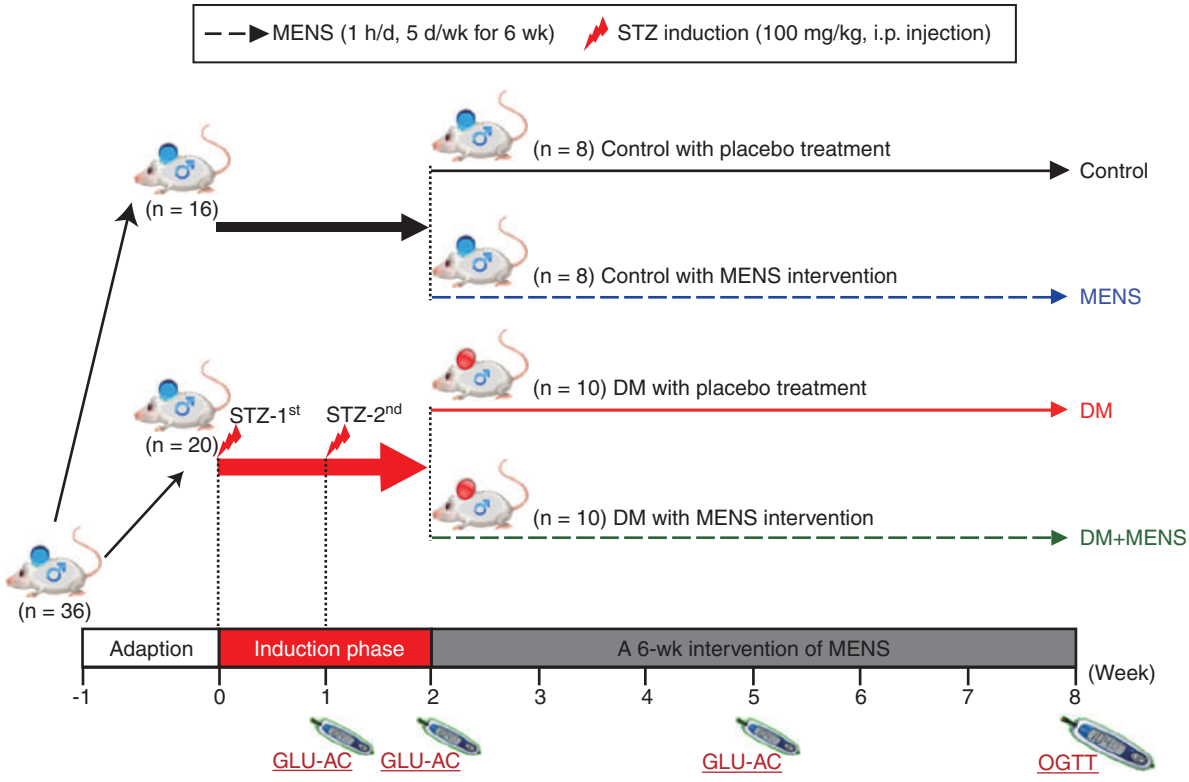

Fig. 1. Experimental designation to evaluate the effect of MENS on diabetes model.

laboratory diet (No. 5001; PMI Nutrition International, Brentwood, MO, USA) and distilled water *ad libitum*, and maintained at 12-h light/12-h dark cycle at room temperature (24 ± 2°C) and 50~60% humidity. The bedding was changed and cleaned twice per week. The mice were acclimated to environments and diets for one week before experiments. The Institutional Animal Care and Use Committee (IACUC) of National Taiwan Sport University approved all animal experiments in this study, and the study conformed to the guidelines of protocol IACUC-10316 approved by the IACUC ethics committee.

All animals (36 mice) were randomly assigned to 2 groups for normal control and diabetes induction groups (16 and 20 mice, respectively). The normal control group was further randomly assigned as normal control (Control) and normal control with MENS (8 mice/group). The diabetes induced mice were randomly separated another two group for diabetes with placebo treatment (DM) and diabetes with MENS treatment (DM+MENS) (10 mice/group). The above 4 groups were administrated with indicated MENS stimulation for 6 weeks (Fig. 1). The fasting blood glucose (GLU-AC) parameters were monitored duration of diabetes induction and MENS administration and the OGTT (oral glucose tolerance test) was evaluated at the end of experiment (Fig. 1).

MENS Conditions and Validation

The MENS conditions of DW1330 was provided and validated by Taiwan Resonant Waves Research Corp. The MENS was conducted form printed circuit board through whole body by demonstration validation (Fig. 2, A and B). We administrated the MENS to the 4 groups treatments by printed circuit board (54 cm × 42 cm) empowered by MENS device for 1 h/day, 5 days/week with 6 weeks (Fig. 2C). The MENS placebo treatment mean the indicated groups were also put the same printed circuit board without MENS input.

Induction of Diabetes

In current studies, we applied the STZ (Sigma, St. Louis, MO, USA) to induce the diabetes animal model with slight modifications according previous references (13, 30). The ICR Mice were fasted for 16 h before diabetes STZ induction. ICR mice received a twice i.p. injection of 100 mg/kg of STZ freshly dissolved in 0.05 M citrate buffer, pH 4.5 with one week interval (Fig. 1). Normal mice of each strain were injected with the equivalent volume of citrate buffer. The GLU-AC higher than 140 mg/dl was considered successful induction (20) for following effects of MENS intervention. Subsequently, the

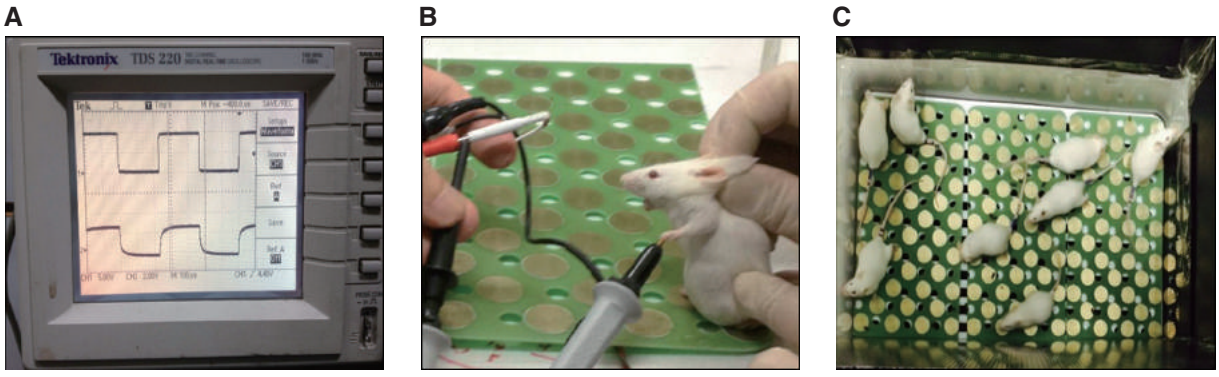

Fig. 2. MENS validation and conductions. (A) The up-panel showed the input MENS and down-panel showed the MENS detentions output from mouse body (hand). (B) The MENS could be conducted from mouse feet (input from printed circuit board) to whole body. (C) The picture showed how the MENS was administrated to experimental animals.

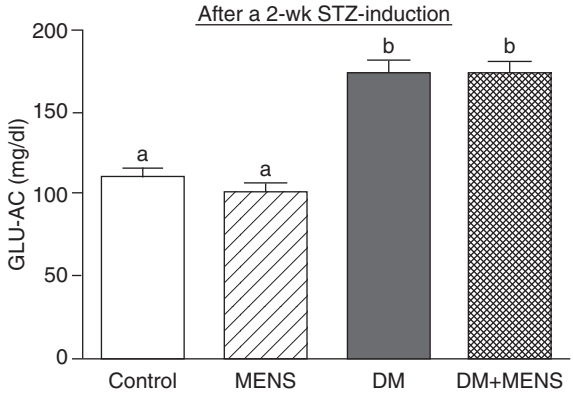

Fig. 3. The GLU-AC after successful STZ-induction. Data are mean ± SEM for n = 8-10 mice per group. Columns with different letters (a, b) are significantly different at $P < 0.05$.

diabetes-induced mice were randomly divided two groups (10 mice/group) as DM and DM+MENS described above.

Body Weight, Food Uptake, and GLU-AC Profiles

The body weights of all the mice (4 groups) were measured every week and the consumption of chow diet and water were recorded every day. The blood glucose levels of the mice were monitored after 14-h fasting. Total blood from tail vein was collected about 0.6 μl for blood glucometer measurement (Accu Chek®, Roche, Indianapolis, Indiana, USA).

OGTT

This assay was performed at the end of 6 weeks intervention on mice fasted for 14 h by administering glucose orally with a dose of 2.0 g/kg BW. Blood samples were collected from tail vein at the specific time points of 0, 15, 30, 60, and 120 min for determining plasma glucose by glucometer (Accu Chek®, Roche).

Determination of Associated Biochemical Variables

The mice were sacrificed by 95% CO_2 asphyxiation without fasting, and blood was immediately collected by cardiac puncture. Serum was separated by centrifugation (3500 rpm, 10 min, 4°C) and clinical biochemical variables, including aspartate aminotransferase (AST), alanine transaminase (ALT), glucose (GLU), insulin, blood urea nitrogen (BUN), creatinine, and glycated hemoglobin (HbA1c) were measured by use of an autoanalyzer (Hitachi 7060).

Body Compositions & Histology Staining of Tissues

Tissues including, liver, kidney, pancreas, epididymal fat pad (EFP), lung, heart, and muscle, were carefully removed, weighted and fixed in 10% formalin immediately after sacrifices. All samples were embedded in paraffin and cut into 4-μm thick slices for morphological and pathological evaluations. Tissue sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope equipped with a CCD camera (BX-51, Olympus, Tokyo, Japan) by a veterinary pathologist.

Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistical differences among groups were analyzed by a one-way analysis of variance (ANOVA) and Duncan's test was used to compare individual means among treatment groups. $P < 0.05$ was considered statistically significant. Statistical analyses involved use of SAS v9.0 (SAS, Cary, NC, USA).

Results

STZ-Induced Glycemia in Diabetes Animal Model

The hyperglycemia is the defining hallmark of the

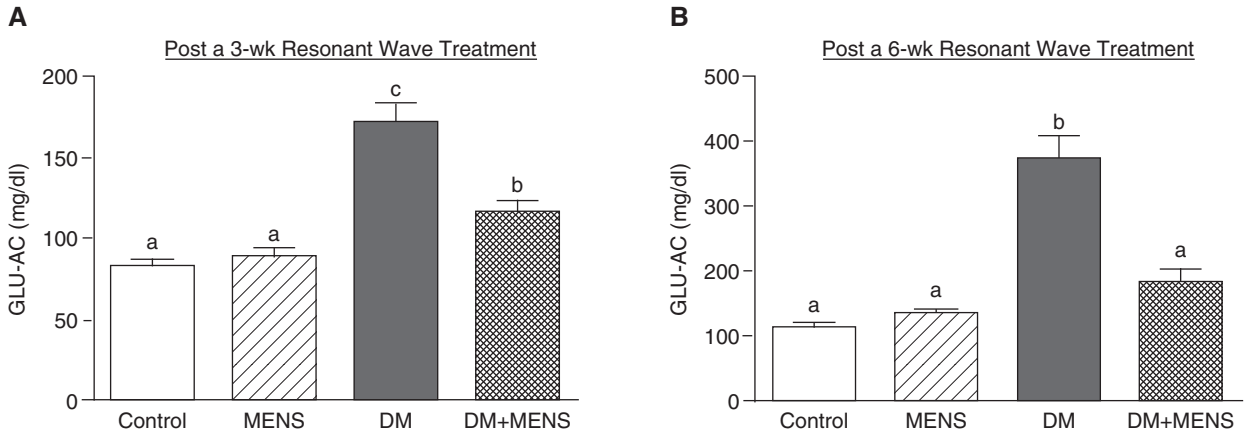

Fig. 4. Effect of MENS intervention on GLU-AC profiles of: (A) 3-week MENS intervention, and (B) 6-week MENS intervention. Data are mean ± SEM for n = 8-10 mice per group. Columns with different letters (a, b, c) are significantly different at $P < 0.05$.

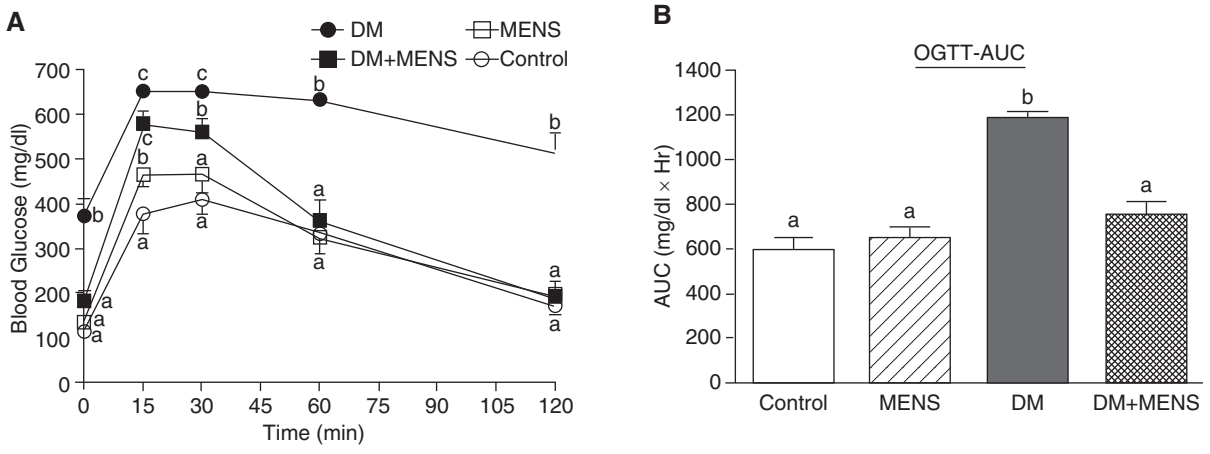

Fig. 5. Effect of MENS interventions on glucose tolerance profiles such as: (A) OGTT, and (B) the glucose area under the curve (AUC) for indicated groups. Data are mean ± SEM for n = 8-10 mice per group. Statistic symbol with different letters (a, b, c) in the same time point or on the columns are significantly different at $P < 0.05$.

diabetic state and because glucose is relatively easy to quantify, most monitoring methods have focused on glucose determinations (9). The GLU-AC was an important indicator to evaluate the regulation of blood homeostasis. In this two weeks of STZ-induction results on DM and DM+MENS, the GLU-AC of indicated groups were 173 ± 9 and 173 ± 8 mg/dl, respectively, which were significantly higher than control groups of Control and MENS (110 ± 6 and 101 ± 6 mg/dl, respectively) (Fig. 3). In previous report, the GLU-AC higher than 140 mg/dl was considered successful induction (21) for following effects of MENS intervention.

Effect of MENS Interventions on GLU-AC

We stared to perform the indicated MENS administration after successful diabetes induction. The data showed that the GLU-AC of 3-weeks MENS intervention were 83 ± 3, 88 ± 6, 171 ± 12, and 116 ± 8 mg/dl in Control, MENS, DM, and DM+MENS, respectively (Fig. 4A). The GLU-AU of DM+MENS group significantly decreased 31.97% as compared to DM group ($P < 0.0001$) but was still significantly higher than Control and MENS groups. The effects of MENS intervention was extended to 6 weeks and the GLU-AC of 6-weeks MENS intervention were 115 ± 6, 135 ± 7, 373 ± 36, and 183 ± 21 mg/dl in Control, MENS, DM, and DM+MENS, respectively (Fig. 4B). The GLU-AC of DM group was significantly elevated by 3.24-fold as compared to Control group ($P < 0.0001$). The GLU-AC in DM+MENS group was significantly 50.82% lower than DM group ($P < 0.0001$) and also exhibited not-sig-nificant difference as compared to Control and MENS group. There was also no significant difference between Control and MENS group in 3- or 6-weeks MENS interventions.

Table 1. Effects of MENS intervention on growth curve, diet, and body compositions in indicated treatments

Characteristics	Control	MENS	DM	DM+MENS
Initial BW (g)	25.6 ± 0.3^{a}	25.5 ± 0.2^{a}	25.5 ± 0.3^{a}	25.7 ± 0.3^{a}
Final BW (g)	41.7 ± 0.4^{c}	39.2 ± 0.6^{b}	37.5 ± 0.4^{a}	39.2 ± 0.8^{b}
Food intake (g/mouse/day)	7.4 ± 0.1^{a}	7.2 ± 0.1^{a}	13.5 ± 0.3^{c}	10.8 ± 0.2^{b}
Water intake (mL/mouse/day)	9.6 ± 0.1^{a}	9.0 ± 0.1^{a}	34.5 ± 1.1^{c}	22.4 ± 0.8^{b}
Relative liver (%)	5.42 ± 0.13^{a}	5.32 ± 0.13^{a}	6.43 ± 0.14^{c}	5.99 ± 0.06^{b}
Relative kidney (%)	1.53 ± 0.06^{c}	1.54 ± 0.09^{c}	1.21 ± 0.14^{b}	0.83 ± 0.01^{a}
Relative pancreas (%)	0.82 ± 0.06^{a}	0.96 ± 0.07^{a}	0.89 ± 0.03^{a}	0.84 ± 0.03^{a}
Relative EFP (%)	1.41 ± 0.08^{b}	1.43 ± 0.34^{b}	0.75 ± 0.27^{a}	0.47 ± 0.10^{a}
Relative lung (%)	0.95 ± 0.06^{a}	0.96 ± 0.04^{a}	0.86 ± 0.04^{a}	0.85 ± 0.04^{a}
Relative heart (%)	0.55 ± 0.04^{a}	0.62 ± 0.09^{a}	0.63 ± 0.03^{a}	0.52 ± 0.01^{a}
Relative muscle (%)	0.94 ± 0.02^{b}	0.96 ± 0.03^{b}	0.90 ± 0.02^{b}	0.83 ± 0.01^{a}

Values in the same row with different superscript letters (a, b, c) differ significantly, $P < 0.05$, by one-way ANOVA. The tissue relative weight (%) was calibrated by individual body weight.

Effect of MENS Interventions on Glucose Tolerance Profiles

The tail vein blood from different time points including 0, 15, 30, 60, and 120 min were sampled for glucometer measurement to characterize the status of glucose tolerance profiles. We administrated 2 g/kg BW glucose dosage to mice of indicated treatments by oral gavage after 14-h fasting (Fig. 5A). The 15 time point showed the GLU-AC values were 377 ± 44, 463 ± 25, 650 ± 0, 578 ± 27 mg/dl in Control, MENS, DM, and DM+MENS, respectively. Values for the MENS, DM, and DM+MENS groups were significantly higher, by 1.23- ($P = 0.0388$), 1.73- ($P < 0.0001$), and 1.54-folds ($P < 0.0001$), respectively, than Control group and the DM+MENS group decreased 11.08% ($P = 0.0541$) as compared to DM group.

The peak of glucose assimilation was 30 min in Control group. In this time point, the GLU-AC values were 409 ± 31, 464 ± 40, 650 ± 0, and 559 ± 30 mg/dl in Control, MENS, DM, and DM+MENS, respectively. Values for the DM and DM+MENS groups were significantly higher, by 1.59- ($P < 0.0001$), and 1.37-folds ($P = 0.0006$), respectively, than Control group and the DM+MENS group significantly decreased 13.95% ($P = 0.0216$) as compared to DM group.

The GLU-AC values were 336 ± 46, 322 ± 22, 632 ± 13, and 362 ± 46 mg/dl in Control, MENS, DM, and DM+MENS, respectively, after 60 min of glucose administration. There were no significant difference amount Control, MENS, and DM+MENS groups. However, the DM group still existed significant higher glucose content than Control group by 1.88-fold ($P < 0.0001$) and the DM+MENS group significantly decreased 42.69% ($P < 0.0001$) as compared to DM group. In the 120 min time point, the GLU-AC values were 171 ± 18, 197 ± 18, 514 ± 44, and 193 ± 34 mg/dl in Control, MENS, DM, and DM+MENS, respectively. The blood glucose of DM still maintained significantly higher concentration as compared to Control by 3.01-fold ($P < 0.0001$) and the DM+MENS group significantly decreased 62.53% ($P < 0.0001$) as compared to DM group. There were also no significant difference amount Control, MENS, and DM+MENS groups.

AUC of in-dicated treatments were calculated from individual glucose profiles (Fig. 5B). It showed that AUC index of DM group was significantly higher than Control by 1.98-fold ($P < 0.0001$) and DM+MENS group significantly decreased 37.06% ($P < 0.0001$) as compared to DM group.

Effect of MENS Interventions on the Growth, Diet, and Body Compositions

We evaluated general characteristics of mice with indicated treatments, such as growth, diet intake, and organ weight, with gradual MENS effects on diabetic animals. BW did not differ with all treatments in initial stage but the intervention groups could cause the significant difference at the end of experiments (Table 1). The body weight significantly differed among indicated groups and it was lower, by 5.85% ($P = 0.0104$), 10.07% ($P < 0.0001$) and 5.96% ($P = 0.0063$), with

Table 2. Effects of MENS treatment on biochemical analysis in diabetic mice at the end of experiment

Parameters	Control	MENS	DM	DM+MENS
HbA1c (%)	3.7 ± 0.1^{a}	3.8 ± 0.2^{a}	7.6 ± 0.2^{c}	5.4 ± 0.4^{b}
Glucose (mg/dl)	162 ± 5^{a}	176 ± 9^{a}	663 ± 31^{c}	467 ± 62^{b}
Insulin (μIU/ml)	2.37 ± 0.59^{a}	2.28 ± 0.38^{a}	4.64 ± 0.75^{b}	1.61 ± 0.19^{a}
AST (U/l)	63 ± 6^{a}	65 ± 5^{a}	104 ± 6^{b}	77 ± 7^{a}
ALT (U/l)	39 ± 3^{a}	43 ± 4^{ab}	98 ± 5^{c}	56 ± 5^{b}
BUN (mg/dl)	26.6 ± 1.1^{b}	20.3 ± 0.7^{a}	28.5 ± 0.9^{b}	19.5 ± 0.5^{a}
Creatinine (mg/dl)	0.22 ± 0.02^{a}	0.24 ± 0.01^{ab}	0.36 ± 0.02^{c}	0.28 ± 0.02^{b}

Values in the same row with different superscript letters (a, b, c) differ significantly, $P < 0.05$, by one-way ANOVA.

MENS, DM, and DM+MENS groups than Control group at the final experiment. However, the DM+MENS group could still maintain the body weight with significant 4.56% ($P = 0.0404$) increase as compared to DM group. The food intake in DM and DM+MENS groups were significantly higher 1.82- and 1.74-fold ($P < 0.0001$) than Control group and DM+MENS group could significantly improve the abnormal polyphagia by 19.57% ($P < 0.0001$). The water intake in DM and DM+MENS groups were significantly higher 3.6- and 2.34-fold ($P < 0.0001$) than Control group and DM+MENS group could also significantly ameliorate the abnormal polydipsia by 19.57% ($P < 0.0001$) as compared to DM group.

In body composition analysis of indicated treatments (Table 1), the relative EFP weight of DM and DM+MENS groups were significantly lower 46.8% and 66.6% than Control group and the relative kidney weight of DM and DM+MENS groups were significantly lower 20.9% and 45.8% than Control group. The relative liver weight of DM and DM+MENS groups were significantly higher than Control group by 20.9% and 45.8%. The relative weights of the other three tissues including pancreas, lung, and heart, were not significant difference among groups.

Effect of MENS Interventions on Biochemical Variables

After 6-weeks gradual indicated MENS interventions, the mice were sacrificed without fasting and the blood was immediately collected for HbA1c and the other biochemical assessments (Table 2). The HbA1c is a form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. The HbA1c of DM and DM+MENS groups was significantly higher than Control, by 2.05- and 1.46-folds, respectively ($P < 0.0001$) and the DM+MENS group was significantly improved by 28.6% as compared to DM group. The values of random blood glucose also reflected the corresponding results as well as HbA1c trend. The random glucose of DM and DM+MENS groups were significantly higher than Control, by 4.1- and 2.89-folds, respectively ($P < 0.0001$) and the DM+MENS group was significantly decreased by 29.57% as compared to DM group. The insulin levels of the DM group significantly increased by 1.96-fold ($P = 0.0054$), as compared to Control, and the DM+MENS group could significantly decrease the insulin over-secretion for 65.27% ($P = 0.0002$) as compared to DM group.

In the liver functional parameters, the AST and ALT were important indexes for evaluate status of liver injury. The DM+MENS group could significantly mitigate for 25.97% ($P = 0.0025$) and 42.92% ($P < 0.0001$) decrease, as compared to DM group in AST and ALT levels, respectively. BUN and creatinine are the primary indexes used to check kidney functions able to filter waste products from blood. The DM group didn't show significant difference as compared to Control in BUN index and the DM+MENS group could be lower 31.6% ($P = 0.0025$) than DM group. For creatinine index, the DM and DM+MENS groups showed significantly higher, by 1.61-fold ($P < 0.0001$) and 1.27-fold ($P = 0.0270$), than Control group and the DM+MENS group could significantly decrease for 22.2% ($P < 0.0001$) as compared to DM group.

Pathological Evaluation of Diabetic Mice with MENS Interventions

All slides were observed by pathological veterinarian. In liver tissues, the Control group showed normally obvious glycogen storage or distributions in hepatocyte cytosol but this phenomenon couldn't be clearly observed in DM group. The DM+MENS group still existed partially glycogen distributions in hepatocytes near the central vein (Fig. 6A). The morphology of glomerulus was also important pathological char-

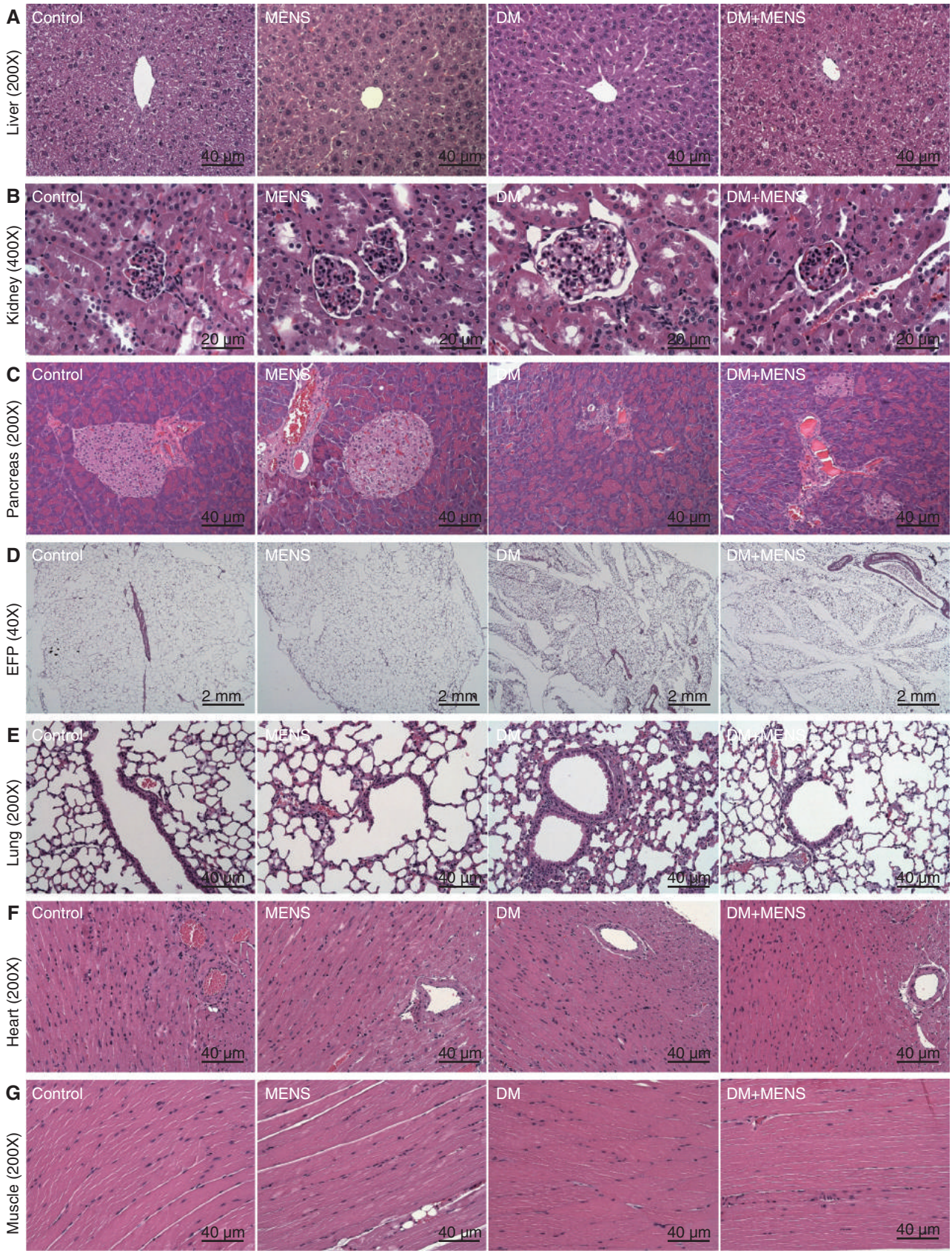

Fig. 6. Effects of MENS intervention on pathological morphology of: (A) liver, (B) kidney, (C) pancreas, (D) EFP, (E) lung, (F) heart, and (G) muscle. Specimens were photographed by light microscopy. (H & E stain, magnification: ×40, ×200 or ×400).

acteristics in DM nephropathy. In Fig. 6B, we found DM group showed pathological processes with early diabetes glomerulus, such like glomerular hypertrophy and basement membrane thickening, majorly happened in kidney cortex. The pathological features were significantly ameliorated in DM+MENS group.

In STZ-induced diabetes model, the islet cells will absorb and accumulate the STZ molecules to cause irreversible injury. Therefore, the DM group showed islet cells were significant atrophy and loss in pancreas lobules. On the DM+MENS group, the islet cells were mitigated significantly on quantities and morphology as compared to DM group (Fig. 6C). The fat tissue, EFP, was composed of adipocytes enriched with lipid contents. We observed the DM group showed adipocytes atrophy and slight inflammatory infiltration phenomena (Fig. 6D). The DM+MENS group was also significantly improved as compared to DM group. The inflammatory features were also found at bronchial and alveolar wall (mainly in neutrophils) but the other three didn't observe the similar inflammatory phenomena (Fig. 6E). In Fig. 6F and 6G, the hypertrophy and hyperplasia were not observed in heart cardiomyocytes and rhabdomyocytes of gastrocnemius muscle in four groups.

Discussion

The diabetic animal model could be established by genetic modifications (38) or diabetogenic activity of STZ administrations combined with high fat diet (22) and the induction of type I or II DM models depended on STZ dosages, diet supplementations or induced durations (6). In current study, we adapted previous reported conditions with slight modifications to induce the type II DM ICR mice model (13) for functional evaluations of MENS interventions. After two weeks inductions, the GLU-AC was about 170 mg/dl which is more the previous DM GLU-AC criteria in ICR strain (20). The diabetes physiological features, such like GLU-AC became more severe (higher) over the time prolonged after STZ induction (36) and the GLU-AC also sub-stantially increase from 171 ± 12 mg/dl (the 3rd week after induction) to 373 ± 36 mg/dl (the 6th week after induction) (Fig. 4, A and B) which is consistent to previous report. After gradual MENS intervention, the irregular GLU-AC could be ameliorated at 3rd or 6th week and it didn't showed significant difference between Control and MENS groups. On the oral glucose tolerance assay, previous study showed 8 Hz electrical stimulation could significantly modulate the glucose intolerance from 60 min to 120 min in type II DM (15). Our data showed the optimized MENS condition could be more effective than previous from 30 min as well as in glucose tolerance AUC (Fig. 5).

The other physiological syndromes of STZ-induced diabetes showed significant differences in body weights loss, liver glycogen depletion, polydipsia, polyphagia, and so on (1, 27) which were consistent with our DM group in our results. We found the MENS intervention (DM+MENS) could significantly alleviate the syndromes of weight loss and the phenomena of polydipsia and polyphagia, as compared to DM group (Table 1). The glycogen content of liver group could was preserved and still existed partial glycogen distribution and storage near central vein in DM+MENS group. The Hamada *et al*. demonstrated electrical stimulation could substantially enhance energy consumption, carbohydrate oxidation, and whole body glucose uptake at low intensity of exercise in health subjects (10). In our data, we found the MENS group exhibited significant decrease in body weight as compared to Control group (Table 1) and it could be caused by more energy consumption with possible effects of MENS intervention. However, we also observed the MENS intervention could alleviate the diabetes-induced weight loss and liver glycogen depletion but the mechanisms behind the MENS on diabetes could be further investigated.

The hallmark of diabetes is hyperglycemia which duration is best predicted by elevated HbA1c levels which may contribute, as a causative factor, to the progression of atherosclerosis in diabetics (28). A higher HbA1c level represents a poorer control of blood glucose and a higher prevalence of diabetic complications. The previous report also showed the type 2 diabetic mice mortality rate could be significantly decreased by decrease of HbA1c and blood glucose levels (21). In current results, the DM group exhibited significantly higher HbA1c and random blood glucose level than Control group but the MENS intervention (DM+MENS group) could significantly decrease the both of indicators (Table 2). In previous physiological results, the low-frequency electrical stimulation of quadriceps muscles alone significantly enhanced glucose disposal rate (GDR) during euglycemic clamp (10, 11). The mechanism studies have shown that electrical stimulation combined with exercise could increase muscle glucose transporter 4 (GLUT4) expression and improved insulin sensitivity in patients with spinal cord injury (25). There-fore, we believed the diabetes glucose homeostasis (HbA1c, fasting or random blood glucose) could be effectively regulated by current MENS interventions (Fig. 5A and Table 2).

The diabetes complication could be also observed in pathological features as early stage diabetes nephropathy and we also found the pathological syndromes were mitigated by MENS intervention (DM+MENS group) (Fig. 6B). The functional biochemical variables, BUN, creatinine, AST, and ALT, associated

with kidney and liver functions were significantly higher than normal individuals in diabetes-induced model (3, 32). The biochemical functional indicators of kidney, BUN and creatinine, showed also significant amelioration as corresponding to pathological observations in DM+MENS group. The protective effects of electrical stimulation were also proved by previous studies. The low-frequency electric stimulation with acupuncture was effective in counteracting diabetes-induced skeletal muscle atrophy by increasing IGF-1 and muscular regeneration (33). The IGF-1 was also reported for beneficial effect on glucose tolerance (35). The electrical stimulation also improved electrophysiological and behavioral indices of nerve regrowth in a chronic diabetic model of mice with pre-existing neuropathy *via* PI3K-PTEN regulation (31). In current result, we demonstrated the MENS could also ameliorate the early diabetes nephropathy in DM+MENS group (Fig. 6B) which also reflected the similar mitigations on related biochemical variables (Table 2).

Clinically, the use of MENS has been shown to potentially improve or compensate for disadvantages in disabled or chronic patients with non-invasive method. We demonstrated the MENS intervention could significantly improve the diabetes-induced glucose tolerance, homeostasis, physiological syndromes, and potential pathology and complications. Thus percutaneous MENS could be potential to become an important part of complementary and alternative therapy on diabetes disease.

Ethics Approval and Consent to Participate

The Institutional Animal Care and Use Committee (IACUC) of National Taiwan Sport University approved all animal experiments in this study, and the study conformed to the guidelines of protocol IACUC-10316 approved by the IACUC ethics committee.

Competing Interests

The authors declare no conflict of interest. Taiwan Resonant Waves Research Corp. (Taipei, Taiwan, ROC) had no role in the analysis or writing of this article.

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