A Randomized Trial on the Effect of Transcutaneous Electrical Nerve Stimulator on Glycemic Control in Patients with Type 2 Diabetes

Jin-Ying Lu
National Taiwan University Hospital
Horng-Yih Ou
National Cheng Kung University Hospital
Chung-Ze Wu
Taipei Medical University
Chwen-Yi Yang
Chi Mei Medical Center
Ju-Ying Jiang
Far Eastern Memorial Hospital
Chieh-Hsiang Lu
Ditmanson Chia- Yi Christian Hospital
Yi-Der Jiang
National Taiwan University Hospital
Tien-Jyun Chang
National Taiwan University Hospital
Yi-Cheng Chang
National Taiwan University Hospital
Meng-Lun Hsieh
National Taiwan University Hospital
Wan-Chen Wu
National Taiwan University Hospital
Hung-Yuan Li
National Taiwan University Hospital
Ye-Fong Du
National Cheng Kung University Hospital
Ching-Han Lin
National Cheng Kung University Hospital
Hao-Chang Hung
National Cheng Kung University Hospital
Kai-Jen Tien

Chi Mei Medical Center
Nai-Cheng Yeh
Chi Mei Medical Center
Shang-Yu Lee
Chi Mei Medical Center
Hui-I Yu
Ditmanson Chia- Yi Christian Hospital
Lee-Ming Chuang (leeming@ntu.edu.tw)
National Taiwan University Hospital

Research Article

Keywords: Transcutaneous nerve stimulator, Type 2 diabetes, Glycemic variability, Mean amplitude of glycemic excursion

Posted Date: March 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1402009/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on February 15th, 2023. See the published version at https://doi.org/10.1038/s41598-023-29791-7.

Abstract

Transcutaneous electrical nerve stimulator (TENS) has been demonstrated to be beneficial in glycemic control in animal models, but its application in humans has not been well studied. We randomly assigned 160 patients with type 2 diabetes on oral antidiabetic drugs 1:1 to the TENS study device (n=81) and placebo (n=79). 147 (92%) randomized participants (mean [SD] age 59 [10] years, 92 men [58%], mean [SD] baseline HbA_{1c} level 8.1% [0.6%]) completed the trial. At week 20, HbA_{1c} decreased from 8.1% to 7.9% in the TENS group (-0.2% [95% CI: -0.4% to -0.1%]) and from 8.1% to 7.8% in the placebo group (-0.3% [95% CI: -0.5% to -0.2%]) (P = 0.821). Mean amplitude of glycemic excursion (MAGE) at week 20 were significantly different (66 mg/dL [95% CI: 58, 73] vs. 79 mg/dL [95% CI: 72, 87]) (P = 0.009). Besides, we noticed that hypoglycemia accounted for 6/54 (11%) of total adverse events and 14/46 (30%) in the TENS vs. the placebo groups. Our study provides the clinical evidence for the first time in humans that TENS does not demonstrate a statistically significant HbA_{1c} reduction. However, it is a safe complementary therapy to improve MAGE in patients with type 2 diabetes.

Introduction

TYPE 2 DIABETES prevalence has continued to grow in the past decades because of the obesity epidemics and modern sedentary lifestyle[1]. Patients with type 2 diabetes have higher risk of developing atherosclerotic cardiovascular disease (ASCVD) than nondiabetic populations of similar demographic characteristics[2]. Currently there is no cure for type 2 diabetes. While metformin is generally the first-line oral antidiabetic drugs (OADs) prescribed for patients with type 2 diabetes, other treatments include sulfonylureas (SU), meglitinides, thiazolidinediones (TZD), dipeptidyl peptidase-4 inhibitors (DPP-4i), α-glucosidase inhibitor (AGi), glucagon-like peptide-1 receptor agonists (GLP-1 RA), sodium glucose cotransporter 2 inhibitors (SGLT2i) and many types of insulin injections[3]. Possible side effects of the medications include hypoglycemia, body weight gain, edema, nausea, anorexia, diarrhea, pancreatitis, ketoacidosis, or genitourinary tract infections[4]. Despite numerous treatment options available, many patients with type 2 diabetes fail to achieve glycemic targets, leading to a significantly increased risk for ASCVD and related morbidity/mortality[5]. There is an urgent need to develop simple alternative or complementary therapeutic strategies for the treatment of type 2 diabetes[6].

Transcutaneous electrical nerve stimulator (TENS) is an approach that applies electrical impulses generated by specifically designed devices, and delivered through electrodes placed on the skin[7]. Scientific evidence suggest that TENS represents an effective tool to treat numerous conditions including pain relief, modulation of autonomic nervous system, and control of diabetes[8–10]. In rat models, vagus nerve stimulation has been shown to modulate glycemia by affecting glucagon and insulin secretions, and high-frequency 40 kHz stimulation might potentially be applied to the treatment of type 2 diabetes[11]. Besides, a novel physiological effect of magnetostatic and electrostatic fields has been demonstrated for the long-term, noninvasive management of type 2 diabetes in mouse models[12]. Recently, a study in STZ-induced diabetes mellitus in mouse models showed that microcurrent electrical nerve stimulation

exhibited effects similar to anti-diabetic drugs, through the peroxisome proliferator-activated receptor (PPAR), bile secretion, and insulin signaling pathways[13]. In a pre-clinical setting, we have proven that our TENS device significantly improved glucose parameters in a diabetic mouse model[14], as indicated by fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA_{1c}). After a 6-week treatment period, FPG declined significantly in diabetic mice receiving TENS. HbA_{1c} level was lower in diabetic mice treated with TENS as compared with those without treatment (5.4% vs. 7.6%) (Appendix in Supplementary Material). Besides, TENS intervention reversed the pathological changes in liver, kidney, and pancreas in mice with diabetes mellitus induced by streptozotocin. The results indicated that TENS could reduce FPG and HbA_{1c} and was safe in animal models.

However, current data in humans are scarce. The effect of TENS has been proved by a new exercise method for treating postprandial hyperglycemia in 11 patients with type 2 diabetes[15], and low-frequency neuromuscular electrical stimulation has been reported with significant positive correlation between the intensity of stimulation and changes in blood glucose in 8 participants with type 2 diabetes[16]. The role of TENS in the treatment of type 2 diabetes of human beings remains to be elucidated. Accordingly, in this phase 3 clinical study, we aim to determine the clinical efficacy of TENS by measuring glycemic parameters including HbA_{1c}, FPG, and body weight at week 0, 2, 4, 8, 12, 16, 20, and 7-point self-monitoring of blood glucose (SMBG) at week 0, 4, 12, 20 in the study period. We performed the study on patients with type 2 diabetes treated with OADs to avoid the complexity and heterogeneity caused by injectables. Mean amplitude of glycemic excursion (MAGE) was calculated using the 7-point SMBG data to represent an indicator of glycemic variability (GV). We also evaluate the safety profile of TENS, including records of any treatment-emergent adverse events (TEAEs), vital signs, rescue medication(s) for hyperglycemia, and hypoglycemic events.

Results

Baseline demographics and clinical characteristics

A total of 207 subjects were screened, and 160 subjects fulfilling the eligibility criteria were enrolled and randomized and they comprised the safety population. Of these, 155 subjects were included in ITT population, while 147 subjects were included in PP population. Six subjects withdrew early from this study and did not complete the 20 weeks of treatment. Two participants violated exclusion criteria and another two participants withdrew the consent. The primary reasons for withdrawal are summarized in Supplementary Table S1. Subject disposition flowchart of each treatment group is displayed in Supplementary Fig. S2. The overall drop-out rates are 8.6% in the TENS group and 7.5% in the placebo group (both < 10%).

The demographic characteristics are summarized in Table 1 for ITT population and in Supplementary Table S2 for PP population and were well balanced between the TENS and placebo groups. In ITT population, the mean ages were 60 (10) in the TENS group and 59 (9) years in the placebo group. More

than half of the subjects were men (54% in the TENS group and 61% in the placebo group). FPG levels were higher in the TENS group when compared to the placebo group (168 (51) vs. 156 (39) mg/dL, P = 0.086). More patients in the TENS group used SU when compared to placebo (9/78 vs. 3/77, P = 0.130). The demographic characteristics of PP was like those of ITT population.

Table 1
Baseline Demographics, Clinical Characteristics and Prescribed Oral Antidiabetic Drugs

	No. (%)		
	TENS (n = 78)	Placebo (n = 77)	*P* value
Age, mean (SD), y	59 (10)	59 (9)	0.615
Sex			0.365
Female	36 (46)	30 (39)	
Male	42 (54)	47 (61)	
Height, mean (SD), cm	163 (10)	164 (8)	0.574
Weight, mean (SD), kg	72 (16)	73 (14)	0.515
BMI, mean (SD), kg/m^2	27 (4)	27 (4)	0.594
HbA_{1c}, mean (SD), %	8.1 (0.5)	8.1 (0.5)	0.989
FPG, mean (SD), mg/dL	168 (50)	155 (39)	0.086
Mean 7-point SMBG, mean (SD), mg/dL	184 (33)	180 (28)	0.746
MAGE, mean (SD), mg/dL	85 (33)	88 (30)	0.354
SBP, mean (SD), mmHg	128 (15)	127 (13)	0.746
DBP, mean (SD), mmHg	77 (10)	78 (9)	0.611
TC, mean (SD), mg/dL	160 (31)	162 (31)	0.588
TG, mean (SD), mg/dL	158 (94)	154 (95)	0.863
LDL-C, mean (SD), mg/dL	90 (24)	93 (23)	0.230
HDL-C, mean (SD), mg/dL	46 (13)	46 (12)	0.790
OADs prescribed			
Metformin	73 (94)	75 (97)	0.442*

Abbreviations:

TENS, the study device "Dragon Waves Resonant Home Care" Electronic Nerve Stimulator; BMI, body mass index; HbA_{1c}, glycosylated hemoglobin; FPG, fasting plasma glucose; SMBG, self-monitoring blood glucose; MAGE, mean amplitude of glycemic excursion; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; OADs, oral anti-hyperglycemic drugs; SU, sulfonylurea; DPP4i, dipeptidyl peptidase 4 inhibitor; SGLT2i, sodium-glucose cotransporter 2 inhibitor; TZD, thiazolidinedione; AGi, -glucosidase inhibitor. Data are presented in means ± SD unless otherwise indicated. Two-sample *t*-test was conducted for continuous variable; Chi-square test or Fisher's exact test were conducted for categorical variable.

	No. (%)		
SU	9 (12)	3 (4)	0.130*
DPP4i	24 (31)	25 (33)	0.956
SGLT2i	16 (21)	24 (31)	0.183
TZD	17 (22)	13 (17)	0.568
AGi	7 (9)	6 (8)	1.000

Abbreviations:

TENS, the study device "Dragon Waves Resonant Home Care" Electronic Nerve Stimulator; BMI, body mass index; HbA_{1c}, glycosylated hemoglobin; FPG, fasting plasma glucose; SMBG, self-monitoring blood glucose; MAGE, mean amplitude of glycemic excursion; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; OADs, oral anti-hyperglycemic drugs; SU, sulfonylurea; DPP4i, dipeptidyl peptidase 4 inhibitor; SGLT2i, sodium-glucose cotransporter 2 inhibitor; TZD, thiazolidinedione; AGi, -glucosidase inhibitor. Data are presented in means ± SD unless otherwise indicated. Two-sample *t*-test was conducted for continuous variable; Chi-square test or Fisher's exact test were conducted for categorical variable.

Study treatment compliance in ITT and PP populations are summarized in Supplementary Table S3. The study expected subjects to use the study device 60 minutes per day, at least 5 days per week for consecutive 20 weeks (overall 6,000 minutes). All treatments administration were recorded by each study subject, and the treatment compliance rate achieved over 100% in both groups.

Primary outcome

The primary efficacy endpoint was HbA_{1c} at baseline and after 20 weeks of treatment, which is shown in Table 2 and Supplementary Table S4. HbA_{1c} at each visit for ITT and PP populations is shown in Supplementary Table S5. In general, HbA_{1c} gradually decreased from week 0 to week 12 and remained stable afterwards in both the TENS and the placebo groups. The primary endpoint analysis of HbA_{1c} at week 20 did not demonstrate a statistically significant difference between the TENS and the placebo groups ($P = 0.540$ in ITT, $P = 0.679$ in PP population). In ITT population, HbA_{1c} decreased 2.4 (6.4)% in the TENS group and 3.3 (8.2)% in placebo group at week 20 compared to baseline. In PP population, HbA_{1c} decreased 2.7 (6.6)% in the TENS group and 3.4 (8.4)% in the placebo group at week 20 compared to baseline.

Table 2
Glycemic Outcomes

	Mean (SD)				***P* value**
	Baseline		20 weeks		
	TENS	Placebo	TENS	Placebo	
Primary outcome					
No.	78	77	77	73	
HbA_{1c}, %,	8.1 (0.5)	8.1 (0.5)	7.9 (0.8)	7.8 (0.8)	
Change from baseline, %, [95% CI]			-0.2 (0.6) [-0.4, -0.1]	-0.3 (0.8) [-0.5, -0.2]	0.821
Secondary outcomes					
No.	78	77	77	73	
HbA_{1c} < 7%, No. (%)	0	0	8 (10)	8 (11)	0.910
No.	78	77	77	73	
FPG, mg/dL	168 (50)	155 (39)	157 (45)	151 (42)	
Change from baseline, mg/dL, [95% CI]			-11 (45) [-22, -1]	-4 (56) [-16, 9]	0.369
No.	78	76	77	73	
Mean 7-point SMBG	184 (33)	180 (28)	173 (34)	172 (34)	
Change from baseline, mg/dL, [95% CI]			-11 (34) [-19, -3]	-8 (36) [-17, 0]	0.730
Additional outcome					
No.	78	76	77	73	
MAGE, mg/dL	85 (33)	88 (30)	66 (33)	79 (33)	
Change from baseline, mg/dL, [95% CI]			-19 (39) [-28, -10]	-9 (43) [-19, 2]	0.087

Abbreviations: SD, standard deviation; CI, confidence interval; HbA_{1c}, glycosylated hemoglobin; FPG, fasting plasma glucose; SMBG, self-monitoring blood glucose; MAGE, mean amplitude of glycemic excursion; CRP, C-reactive protein; FGF-21, fibroblast growth factor-21. Two-sample *t*-test (continuous variable) or Chi-square Test (categorical variable) was conducted. If normal assumption was violated, Wilcoxon rank sum test was applied.

	Mean (SD)				*P* value
Exploratory outcomes					
No.	78	77	77	73	
CRP, mg/dL	0.22 (0.30)	0.24 (0.07)	0.16 (0.23)	0.38 (1.12)	
Change from baseline, mg/dL, [95% CI]			-0.06 (-0.02) [-0.13, 0.01]	0.13 (-0.003) [-0.14, 0.40]	0.181
No.	78	77	77	73	
FGF-21, ng/mL	0.29 (0.25)	0.35 (0.33)	0.24 (0.17)	0.32 (0.30)	
Change from baseline, ng/mL, [95% CI]			-0.05 (-0.01) [-0.09, 0.00]	-0.03 (-0.01) [-0.08, 0.02]	0.789

Abbreviations: SD, standard deviation; CI, confidence interval; HbA_{1c}, glycosylated hemoglobin; FPG, fasting plasma glucose; SMBG, self-monitoring blood glucose; MAGE, mean amplitude of glycemic excursion; CRP, C-reactive protein; FGF-21, fibroblast growth factor-21. Two-sample *t*-test (continuous variable) or Chi-square Test (categorical variable) was conducted. If normal assumption was violated, Wilcoxon rank sum test was applied.

Secondary outcome

The proportion of subjects achieving HbA_{1c} of < 7% at each visit for ITT and PP populations are shown in Supplementary Table S6. The proportion of subjects who achieved HbA_{1c} of < 7% gradually increased since week 4 in both groups. In ITT population, there were 10.4% of subjects in the TENS group and 11% of subjects in placebo group achieved HbA_{1c} of < 7% at week 20. The proportion of subjects achieving HbA_{1c} of < 7% in both groups in PP population was like that in ITT population.

FPG at each visit for ITT and PP populations are shown in Supplementary Table S7. FPG gradually decreased since week 0 in both groups. No statistically significant difference between groups was observed. In ITT population, FPG decreased 11 (45) mg/dL in the TENS group and 4 (56) mg/dL in the placebo group at week 20 compared to baseline. In PP population, FPG decreased 12 (46) mg/dL in the TENS group and 4 (57) mg/dL in the placebo group at week 20 compared to baseline.

According to subgroup analysis (Supplementary Table S8), female in the TENS but not the placebo group exhibited a statistically significant HbA_{1c} reduction from baseline in ITT ($P = 0.0320$) and PP population ($P = 0.0049$). HbA_{1c} and FPG from baseline to week 20 significantly decreased in the TENS subgroup of subjects with body mass index (BMI) ≥ 26.9 kg/m^2 (the median) in both ITT and PP populations (HbA_{1c} in ITT: $P = 0.0309$, PP: $P = .0218$; FPG in ITT: $P = 0.0425$; PP: $P = 0.0325$). The change was not statistically significant in the corresponding placebo subgroup. Subjects having DM duration for more than 9 years in

the TENS group also displayed a significant HbA_{1c} reduction from baseline to week 20 in both populations (ITT: $P = 0.0282$; PP: $P = 0.0282$); again, the change was not statistically significant in the corresponding placebo subgroup.

Other outcomes

The 7-point SMBG was measured immediately before breakfast, lunch, and dinner, two hours after each meal, and at bedtime, at week 0, 4, 12 and 20. The 7-point SMBG level at each visit for ITT and PP populations are shown in Supplementary Table S9. The mean SMBG level slightly decreased since week 0 in both the TENS and the placebo groups without significant difference between the two groups. In ITT population, the mean 7-point SMBG decreased 11 (34) mg/dL in the TENS group and 8 (36) mg/dL in the placebo group at week 20 compared to baseline (Table 2 and Supplementary Table S4). In PP population, the mean 7-point SMBG decreased 11 (34) mg/dL in the TENS group and 8 (37) mg/dL in the placebo group at week 20 compared to baseline (Supplementary Table S4).

In the TENS group, the 7-point SMBG maintained relatively more stable GV compared to the placebo group, as reflected by the significant difference of MAGE at week 20 between the TENS group and the placebo (66 (33) mg/dL [95% CI: 58, 73] vs. 79 (33) mg/dL [95% CI: 72, 87]) (Table 2, Supplementary Table S10, and Fig. 1A) (two-sample *t*-test showed a significant post-treatment difference with $P = 0.009$; mixed model 2-way ANOVA showed a significant group effect with $P = 0.033$ and a significant time effect with $P = 0.0008$). Similar trends of greater MAGE decline were observed in the TENS group as compared to the placebo group in both genders, in patients with either higher or lower HbA_{1c} and BMI, and in patients with either longer or shorter duration of DM (Fig. 1B). Statistically significant differences of MAGE after treatment in the TENS group versus the placebo group were noted in female ($P = 0.042$), in patients with $HbA_{1c} \geq 8.0\%$ ($P = 0.002$), in patients with BMI < 26.9 kg/m^2 ($P = 0.047$).

Following 20 weeks of treatment, most subjects in the TENS and the placebo groups maintained stable OAD regimens in the maintenance period of the study. Overall, four subjects had OAD regimen change: two in the TENS group, and two in the placebo group (Supplementary Table S11). The vital signs including blood pressure, pulse, respiratory rate, and body temperature in both treatment groups remained stable over the study period with no significant difference from baseline (Supplementary Table S12). Comparisons of lipid profile between groups are shown in Supplementary Table S13. Overall, total cholesterol (TC) and triglyceride (TG) showed significant decline in the placebo group, but high-density lipoprotein cholesterol (HDL-C) showed significant elevation in the TENS group.

Exploratory biomarkers including CRP, adiponectin, TNF-α, and FGF-21 were performed at week 0 and 20. Figure 2, Table 2, and Supplementary Table S14 summarize the levels and mean change from week 0 to 20 in exploratory laboratory parameters. CRP decreased 0.06 (0.02) in the TENS group ($P = 0.022$) and increased 0.13 (0.003) mg/dL ($P = 0.848$) in the placebo group from week 0 to week 20 (two sample *t*-test $P = 0.095$; mixed model 2-way ANOVA showed a significant group effect with $P = 0.0486$). TNF-α decreased 0.03 (0.45) pg/mL in the TENS group and increased 0.03 (0.55) pg/mL in the placebo group from week 0 to week 20 (two sample *t*-test $P = 0.454$; mixed model 2-way ANOVA showed a non-

significant group effect $P = 0.7376$). Adiponectin increased 0.33 (2.48) μg/mL in the TENS group and 0.25 (4.44) μg/mL in the placebo group (two sample *t*-test $P = 0.886$; mixed model 2-way ANOVA group showed a non-significant group effect $P = 0.1390$). FGF-21 decreased 0.05 (0.19) ng/mL in the TENS group and 0.04 (0.21) ng/mL in the placebo group (two sample *t*-test $P = 0.752$; mixed model 2-way ANOVA showed a significant group effect with $P = 0.0142$).

Safety outcome

Adverse events are summarized in Table 3 and Supplementary Table S15. About one-third of subjects reported at least one AE during the trial. Number of subjects experiencing at least one AE were 25 (31%) in the TENS group and 28 (35%) in the placebo group. Most reported AEs were mild in severity (TENS: 83%, placebo: 80%). A total of nine serious adverse events (SAEs) were reported during the study, including four events reported from three subjects in the TENS group and five events reported from two subjects in placebo group. None of the SAEs were related to the study device, and no death was reported in this study.

Table 3
Adverse events, serious adverse events, and unanticipated adverse device effects

	TENS (n = 81)	Placebo (n = 79)
Patients with adverse events	25 (31)	28 (35)
Patients with serious adverse events	3 (4)	2 (3)
Patients discontinued due to serious adverse events	0	1 (1)
Total number of adverse events	54	46
Hypoglycemia*	6/54 (11)	14/46 (30)
Severity		
Mild	5/54 (9)	13/46 (28)
Moderate	1/54 (2)	1/46 (2)
Severe	0	0
Hyperglycemia*		
Rescue medication usage	1 (1)†	3 (4)‡
Unanticipated device adverse effects*		
Redness, swelling, pain	1 (1)	
Hypoglycemia/ Pseudo-hypoglycemia		1 (1)

See Supplementary Table S15 for a full listing of adverse events. Data are presented as n (%). *The number of hypoglycemic events, hyperglycemic events or unanticipated adverse device effects/total adverse events is presented; the percentage of hypoglycemic events, hyperglycemic events or unanticipated adverse device effects/total adverse events is shown in parentheses. †Add-on OAD was the rescue medication in this patient; ‡insulin injections were the rescue medications in these patients.

Hypoglycemia was the most frequent AE reported, occurring in five (6%) patients in the TENS group versus nine (11%) patients in the placebo group. Of 100 AEs reported in total, 20 hypoglycemia events were reported by 14 subjects, including 14 events by nine subjects in placebo group (30%) and six events by five subjects in the TENS group (11%). Five subjects had experienced hypoglycemia more than once, including four subjects in placebo group and one subject in the TENS group. Three (4%) subjects in the placebo group encountered hyperglycemia requiring rescue insulin therapy, and one (1%) subject in the TENS group had hyperglycemia being rescued by add-on OAD. One patient in the TENS group

experienced redness, swelling and pain on abdominal wall which completely resolved spontaneously without specific treatment.

Data availability

The data that support the findings of this study are available from Bestat Pharmaservices Corporation, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Bestat Pharmaservices Corporation.

Discussion

This was a phase 3, prospective, double blind, randomized, placebo-controlled, multi-center, pivotal study to investigate the efficacy and safety of TENS in improvement of glycemic control in patients with type 2 diabetes. The primary efficacy endpoint is to compare the HbA_{1c} following a 20-week treatment of TENS versus placebo. We found that the final mean HbA_{1c} at week 20 is 7.9% in the TENS group and 7.8% in the placebo group, and the mean change of HbA_{1c} from the baseline to week 20 was − 0.2% and − 0.3% in the TENS group and the placebo group, respectively. The secondary endpoints, including the proportions of subject who achieved HbA_{1c} of < 7% at week 20, were similar between the two groups. The mean changes of FPG and 7-point SMBG from baseline to week 20 was greater in the TENS group. However, this relation did not hold on for the primary endpoint, i.e., HbA_{1c} reductions during the study. The disappointing result was possibly related to the already strong effectiveness of current OADs targeting multiple pathogenesis pathways of type 2 diabetes[17, 18], thus leading to the inability of the study to detect the efficacy of TENS in HbA_{1c} reduction on top of the present medications.

However, according to the subgroup analysis, we found that female patients in the TENS group but not the placebo group exhibited a significant HbA_{1c} reduction from baseline. Additionally, HbA_{1c} and FPG from baseline to week 20 significantly decreased in the TENS subgroup of subjects with BMI ≥ 26.9 kg/m^2. The change was not statistically significant in the corresponding placebo subgroup. Subjects that have had DM duration for more than 9 years in the TENS group had also displayed a significant HbA_{1c} reduction from baseline to week 20. These data suggest TENS might be beneficial for HbA_{1c} and FPG lowering in certain specific clinical conditions.

We further found that the 7-point SMBG maintained a relatively more stable GV in the TENS group compared to the placebo group, as reflected by MAGE reduction, with a greater decline found throughout the study in the TENS group compared to the placebo group. From week 0 to week 20, MAGE decreased 19 mg/dL in the TENS group and 9 mg/dL in the placebo group and became significantly different at week 20 (Fig. 1A and Supplementary Table S10). We further carried out subgroup analysis and found that the trends of greater MAGE decline in the TENS group hold on in different patient subgroups; while female, patients with HbA_{1c} ≥ 8% (the median), and patients with BMI < 26.9 kg/m^2 (the median) were even more likely to have MAGE reduction than their counterparts.

The significant effect of TENS on MAGE reduction is a post-hoc novel finding of our study. MAGE has long been recognized as an indicator of GV and thus represented as an important part of diabetes control because of the need to reach target HbA_{1c} level while avoiding hypoglycemia[19]. In this study, we measure MAGE by using the 7-point SMBG data to derive the average of each blood glucose increase or decrease (nadir-peak or vice versa)[20]. Several OADs have been shown to decrease GV; most of them belong to DPP4i[21], SGLT2i[22], and AGi,[23, 24] when compared to SU[21, 23]. In our study the use of OADs is well balanced in both groups, except that slightly more patients in the TENS group used SU, which excluded the possibility that differential preference of medication usage results in the beneficial MAGE reduction in the TENS group. Besides, the OADs described above all have respective side effects which might be intolerable or unacceptable by individual patients. On the contrary, our study device exhibited good safety profile and is well tolerated by most patients. Furthermore, GV has been linked to several acute and chronic micro- and macro-vascular complications related to diabetes[14, 25–27]. Whether the beneficial effects of TENS on MAGE reduction can be translated to amelioration of short and long-term diabetic complications as well as improvement in quality of life in patients with type 2 diabetes needs to be investigated in the future.

Regarding the safety aspect of the study device, over 80% of TEAEs were mild, and no serious adverse device effects were reported. Hypoglycemia was the most common AE presented with six events occurring in five subjects receiving TENS and 14 events occurring in nine subjects receiving placebo, while hyperglycemia requiring rescue medication was encountered in one subject in the TENS group versus three subjects in placebo group. Overall, patients in the TENS group experienced less hypoglycemic and hyperglycemic events when compared to placebo, which corroborates with the previous finding that TENS is associated with significant reduction in GV. We propose the underlying mechanism might involve the harmonious regulation of glucagon and insulin secretions associated with TENS, leading to maintenance of optimal glucose homeostasis[28–30].

Throughout the study, we also found significant decline of CRP from baseline in the TENS group, but not in the placebo group. In addition, CRP and FGF-21 showed significant group effects in the TENS group versus the placebo group. Although the other biomarkers examined in this study did not show significant change, the decreases in CRP and FGF-21 in the TENS group demonstrated potential improvement in systemic inflammation[31, 32], and fibrosis[33, 34], during the study period. Taken together, we suggest that TENS has the potential benefit to induce reduction of inflammatory cytokine release and end-organ fibrotic damage through avoidance of hypoglycemic and hyperglycemic events, thus might be a safe alternative therapeutic strategy to prevent diabetic complications.

The limitations of this study are listed as below. First, the duration of the study was only 20 weeks, and it is not known whether the MAGE reduction would be sustained or even better for a longer duration as revealed in this study in a time-dependent manner. An extension of the study period to 40 weeks might help to answer the question. Second, since continuous glucose monitoring (CGM)-derived MAGE has been shown to be more accurate than that derived from self-monitoring of blood glucose[23], further study

using CGM to measure MAGE is required to confirm the beneficial effects of TENS on GV. Third, the beneficial results about the exploratory parameters CRP and FGF-21 warrant further study of the underlying mechanisms. Whether this effect is directly from TENS treatment or secondary to the improvement of GV and thus amelioration of oxidative stress needs to be investigated in the future. Finally, this randomized clinical trial was designed to evaluate the potential benefits of TENS on glycemic control; however, we found no statistically significant reduction in major glycemic variables (the primary and secondary endpoints). The significant finding was the difference in MAGE calculated by using SMBG at the end of the trial. Thus, it is important to carry out another randomized clinical trial to confirm the beneficial effects of TENS on GV evaluated by a more elaborate MAGE obtained using CGM before claiming the generalizability including external validity and applicability of the trial findings.

In conclusion, the domestic use of both "Dragon Waves Resonant Home Care" Transcutaneous Electronic Nerve Stimulator (TENS) and placebo (sham TENS ineffective pulse wave) demonstrated a modest effect in glycemic control and were well tolerated without safety concerns in patients with type 2 diabetes. Nonetheless, for the primary endpoint, TENS did not demonstrate a statistically difference in the HbA_{1c} reduction as compared to placebo. The borderline significant reduction of MAGE and the markedly significant difference in MAGE at the end of the study derived from the 7-point SMBG, and of the exploratory biomarkers CRP and FGF-21 in the TENS group as compared to the placebo group warrants further follow-up study to confirm the potential beneficial effects of TENS on GV, inflammation, and fibrosis in patients with type 2 diabetes.

Methods

Study Design and participants

This was a multi-center, prospective, double blind, randomized, placebo-controlled trial of transcutaneous electrical nerve stimulator (TENS) to improve glycemic control in patients with type 2 diabetes (ClinicalTrials.gov Identifier: NCT03102424). The study and all experimental protocols were approved by Institutional Review Boards of the six joining hospitals (National Taiwan University Hospital, National Cheng Kung University Hospital, Taipei Medical University, Chi Mei Medical Center, Far Eastern Memorial Hospital, and Ditmanson Chia-Yi Christian Hospital), and written informed consent was obtained from each study participant. All experiments were carried out in accordance with relevant guidelines and regulations.

Subjects with type 2 diabetes who met all the inclusion but none of the exclusion criteria were randomized with a 1:1 allocation into either of the 2 groups below: 1. TENS, 2. placebo (sham TENS delivering ineffective pulse wave). The inclusion criteria, exclusion criteria and sample size determination are detailed in the Methods Section in Supplementary Material. A schematic diagram of study design is depicted in Supplementary Fig. S1. The name of the trial registry was “A Prospective, Double Blind, Randomized, Placebo-controlled Trial of Transcutaneous Electrical Nerve Stimulator (DW1330) to

Improve Blood Glucose Control in Patients with Type 2 Diabetes". The full date of first registration is 05/04/2017, and the registration number of the clinical trial is TRWRDM1604001.

Study intervention

Study visits occurred every 2 or 4 weeks depending on the study phase. The study device "Dragon Waves Resonant Home Care" Transcutaneous Electronic Nerve Stimulator (Taiwan Resonant Waves Research Corporation, Taipei, Taiwan) is a small portable, battery-operated device equipped with wires and 2 patches, and the impulses are sent through wires to patches which are placed on bilateral abdominal wall. The study device used full-frequency wave resonant technology with mixed frequencies ranging from 1 to 20,000 Hz in the TENS group, and from 1 to 30 Hz in the placebo group, respectively. Both the mixed frequency sets were predefined and composed of monophasic square pulse wave with 50% duty cycle. The amplitude of the pulse wave was 7.2 Vpp in average (for further details please refer to eAppendix in Supplementary Material).

In pre-clinical setting, DW1330 has been tested in a mouse model of diabetes mellitus. The study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). A pilot study on 40 human subjects with type 2 diabetes mellitus has been conducted. Both the animal and human studies are detailed in eAppendix in Supplementary Material.

Outcome assessments

Primary efficacy variable was the change in HbA_{1c} after 20 weeks of treatment. Secondary efficacy variables included 1. percentage of subjects who achieved HbA_{1c} target of < 7%; 2. change in FPG; 3. changes in mean 7-point SMBG. Safety assessments were as follows, 1. incidence of TEAEs; 2. change in lipid profile and vital signs; 3. percentage of subjects who used rescue medication(s); 4. time to hyperglycemia rescue; 5. frequency and severity of hypoglycemia at each scheduled visit. For post-hoc analysis after the data were seen, as an additional outcome, MAGE was calculated by using the SMBG data to represent the glycemic variability (GV).

We also measured the changes in biomarkers C-reactive protein (CRP), adiponectin, tumor necrosis factorα (TNF-α), fibroblast growth factor-21 (FGF-21), as the exploratory assessments. CRP was determined by latex enhanced immunoturbidimetric assay (ADVIA 1800, ADVIA Chemistry XPT, Dimension EXL, SIEMENS). Adiponectin and TNF-α were measured by enzyme-linked immunosorbent assay (R&D Systems). FGF-21 was quantified by using enzyme-linked immunosorbent assay kits according to manufacturer's instructions (Duoset human FGF21 ELISA kit, R&D systems, Inc., Minneapolis, MN, USA).

Measurements and definitions

There were three analysis populations: intention-to-treat (ITT) population, per-protocol (PP) population, and safety population. Both ITT and PP populations were applied to efficacy analyses, while safety

population was used in the analysis of safety variables. Definition of the three populations is detailed in the Methods section in Supplementary Material.

Statistical analysis

The statistical analysis and data management including double data entry, data verification, data validation was performed periodically after receiving case report forms from sites. The statistical analyses were conducted using SAS® software (SAS® Institute Inc., USA, version 9.4) and GraphPad® software (GraphPad Prism® Inc., San Diego, California, USA, version 9.1.0). Two-sample *t*-test was conducted for continuous variable; if normal assumption was violated, Wilcoxon rank sum test would be applied. Chi-square test or Fisher's exact test were conducted for categorical variable. After the data were disclosed, because of the apparently lower rate of hypoglycemia and hyperglycemia requiring rescue medications experienced by the TENS group when compared to placebo, we also carried out post-hoc analyses on MAGE in addition to the prespecified primary and secondary outcomes. Mixed model two-way analysis of variance (2-way ANOVA) was used to assess the effects of group allocation (TENS versus placebo), time (before versus after treatment), and their interaction (group*time) on MAGE and the exploratory assessments. All values are given as means (standard deviation, SD). unless stated otherwise. After the data were disclosed, because of the apparently lower rate of hypoglycemia and hyperglycemia requiring rescue medications experienced by the TENS group when compared to placebo, we also carried out post-hoc analyses on MAGE in addition to the prespecified primary and secondary outcomes.

Declarations

Authors' contributions

J.Y.L, H.Y.O., and L.M.C. developed the concept for this article. C.Z.W., C.Y.Y., J.Y.J., C.H.L., Y.D.J., T.J.C., Y.C.C., W.C.W., H.Y.L., Y.F.D., C.H.L., H.C.H., K.J.T., N.C.Y., S.Y.L., N.C.Y., S.Y.L., H.I.Y. enrolled their patients into this study. M.L.H. performed the FGF-21 assay. J.Y.L., H.Y.O., and L.M.C. analyzed the data and wrote the manuscript. All authors were responsible for reviewing and revising this article and assume responsibility and accountability for the results.

Acknowledgements

We thank Professors June-Tai Wu, Pei-Lung Chen, Chi-Tai Fang, and Dr. Yu-Ling Huang for their critical review and valuable discussion about this manuscript. We sincerely thank the clinical trial participants for their precious contribution of time and effort to the study.

Competing interests

The authors declared that no competing financial conflicts of interests exist in the study.

Funding information

This clinical trial was funded by the Liver Disease Prevention and Treatment Research Foundation, the Taiwan Resonant Waves Research Corporation and a grant (106-EC-17-A-22-I5-0-005) from the Ministry of Economics, Taiwan. The study machines and glucose meters used in this trial were provided by the Taiwan Resonant Waves Research Corporation.

References

1. Jiang Y. D., Chang C. H., Tai T.Y., Chen J. F., & Chuang L. M. Incidence and prevalence rates of diabetes mellitus in Taiwan: analysis of the 2000-2009 Nationwide Health Insurance database. *J. Formos. Med. Assoc.* **111**(11):599-604 (2012).
2. Rosenzweig J. L. *et al*. Primary Prevention of ASCVD and T2DM in Patients at Metabolic Risk: An Endocrine Society* Clinical Practice Guideline. *J. Clin. Endocrinol. Metab.* 3939-3985 (2019).
3. Chawla R., *et al*. RSSDI-ESI Clinical Practice Recommendations for the Management of Type 2 Diabetes Mellitus. *Indian J. Endocrinol. Metab.* **24**(1):1-122 (2020).
4. American Diabetes A. 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2021. *Diabetes Care* **44**(Suppl 1):S111-S124 (2021).
5. Chiang C. E., *et al*. 2020 Consensus of Taiwan Society of Cardiology on the pharmacological management of patients with type 2 diabetes and cardiovascular diseases. *J. Chin. Med. Assoc.* **83**(7):587-621 (2020).
6. Felton A. M., LaSalle J., McGill M., & Global Partnership for Effective Diabetes M. Treatment urgency: The importance of getting people with type 2 diabetes to target promptly. *Diabetes Res Clin. Pract.* **117**:100-103 (2016).
7. Gibson W., Wand B. M., & O'Connell N. E. Transcutaneous electrical nerve stimulation (TENS) for neuropathic pain in adults. *Cochrane Database Syst. Rev.* **9**:CD011976 (2017).
8. do Amaral Sartori S., Stein C., Coronel C. C., Macagnan F. E., & Plentz R. D. M. Effects of Transcutaneous Electrical Nerve Stimulation in Autonomic Nervous System of Hypertensive Patients: A Randomized Controlled Trial. *Curr. Hypertens. Rev.* **14**(1):66-71 (2018).
9. Borges M. R., et al. Transcutaneous electrical nerve stimulation is superior than placebo and control for postoperative pain relief. *Pain Manag.* **10**(4):235-246 (2020).
10. Khan M. U. Is there a role for TENS application in the control of diabetes mellitus in insulin-dependent patients? *Singapore Med. J.* **53**(11):e249-250 (2012).
11. Payne S. C., *et al*. Differential effects of vagus nerve stimulation strategies on glycemia and pancreatic secretions. *Physiol. Rep.* **8**(11):e14479 (2020).
12. Carter C. S., *et al*. Exposure to Static Magnetic and Electric Fields Treats Type 2 Diabetes. *Cell Metab.* **32**(6): 561-574 (2020).
13. Luo Y. C., Huang S. H., Pathak N., Chuang Y. H., & Yang J. M. An integrated systematic approach for investigating microcurrent electrical nerve stimulation (MENS) efficacy in STZ-induced diabetes mellitus. *Life Sci.* **279**:119650 (2021).

14. Huang W. C., *et al*. The modulative effects of microcurrent electrical nerve stimulation on diabetic mice. *Chin. J. Physiol.* **60**(1):62-72 (2017).
15. Miyamoto T., *et al*. Effect of percutaneous electrical muscle stimulation on postprandial hyperglycemia in type 2 diabetes. *Diabetes Res. Clin. Pract.* **96**(3):306-312 (2012).
16. Jabbour G., *et al*. Effect of Low Frequency Neuromuscular Electrical Stimulation on Glucose Profile of Persons with Type 2 Diabetes: A Pilot Study. *Diabetes Metab. J.* **39**(3):264-267 (2015).
17. Galicia-Garcia U., *et al*. Pathophysiology of Type 2 Diabetes Mellitus. *Int. J. Mol. Sci.* **21**(17):6275 (2020).
18. Banday M. Z., Sameer A. S., & Nissar S. Pathophysiology of diabetes: An overview. *Avicenna J. Med.* **10**(4):174-188 (2020).
19. Kovatchev B., & Cobelli C. Glucose Variability: Timing, Risk Analysis, and Relationship to Hypoglycemia in Diabetes. *Diabetes Care* **39**(4):502-510 (2016).
20. Jung H. S. Clinical Implications of Glucose Variability: Chronic Complications of Diabetes. *Endocrinol. Metab. (Seoul)* **30**(2):167-174 (2015).
21. Lee S., Lee H., Kim Y., & Kim E. Effect of DPP-IV Inhibitors on Glycemic Variability in Patients with T2DM: A Systematic Review and Meta-Analysis. *Sci. Rep.* **9**(1):13296 (2019).
22. Torimoto K., Okada Y., Koikawa K., & Tanaka Y. Early effects of sodium-glucose co-transporter 2 inhibitors in type 2 diabetes: study based on continuous glucose monitoring. *Diabetol. Metab. Syndr.* **9**:60 (2017).
23. Umpierrez G. E., & B PK. Glycemic Variability: How to Measure and Its Clinical Implication for Type 2 Diabetes. *Am. J. Med. Sci.* **356**(6):518-527 (2018).
24. Derosa G., Franzetti I., Querci F., D'Angelo A., & Maffioli P. Effect of Acarbose on Glycemic Variability in Patients with Poorly Controlled Type 2 Diabetes Mellitus Receiving Stable Background Therapy: A Placebo-Controlled Trial. *Pharmacotherapy* **35**(11):983-990 (2015).
25. Martinez M., Santamarina J., Pavesi A., Musso C., & Umpierrez G. E. Glycemic variability and cardiovascular disease in patients with type 2 diabetes. *BMJ Open Diabetes Res. Care* **9**(1): e002032 (2021).
26. Sun B., Luo Z., & Zhou J. Comprehensive elaboration of glycemic variability in diabetic macrovascular and microvascular complications. *Cardiovasc. Diabetol.* **20**(1):9 (2021).
27. Papachristoforou E., Lambadiari V., Maratou E., & Makrilakis K. Association of Glycemic Indices (Hyperglycemia, Glucose Variability, and Hypoglycemia) with Oxidative Stress and Diabetic Complications. *J. Diabetes Res.* **2020**:7489795 (2020).
28. Ahren B., & Taborsky G. J., Jr. The mechanism of vagal nerve stimulation of glucagon and insulin secretion in the dog. *Endocrinology* **118**(4):1551-1557 (1986).
29. Meyers E. E., Kronemberger A., Lira V., Rahmouni K., & Stauss H. M. Contrasting effects of afferent and efferent vagal nerve stimulation on insulin secretion and blood glucose regulation. *Physiol. Rep.* **4**(4): e12718 (2016).

30. Chunchai T., *et al*. Vagus Nerve Stimulation Exerts the Neuroprotective Effects in Obese-Insulin Resistant Rats, Leading to the Improvement of Cognitive Function. *Sci. Rep.* **6**:26866 (2016).
31. Quispe R., *et al*. High-Sensitivity C-Reactive Protein Discordance With Atherogenic Lipid Measures and Incidence of Atherosclerotic Cardiovascular Disease in Primary Prevention: The ARIC Study. *J. Am. Heart Assoc.* **9**(3):e013600 (2020).
32. Fu Y., Wu Y., & Liu E. C-reactive protein and cardiovascular disease: From animal studies to the clinic (Review). *Exp. Ther. Med.* **20**(2):1211-1219 (2020).
33. Ferrer-Curriu G., *et al*. Fibroblast growth factor-21 protects against fibrosis in hypertensive heart disease. *J. Pathol.* **248**(1):30-40 (2019).
34. Wang R., Yi X., Li X., & Jiang X. Fibroblast growth factor-21 is positively associated with atrial fibrosis in atrial fibrillation patients with rheumatic heart disease. *Int. J. Clin. Exp. Pathol.* **8**(11):14901-14908 (2015).

Figures

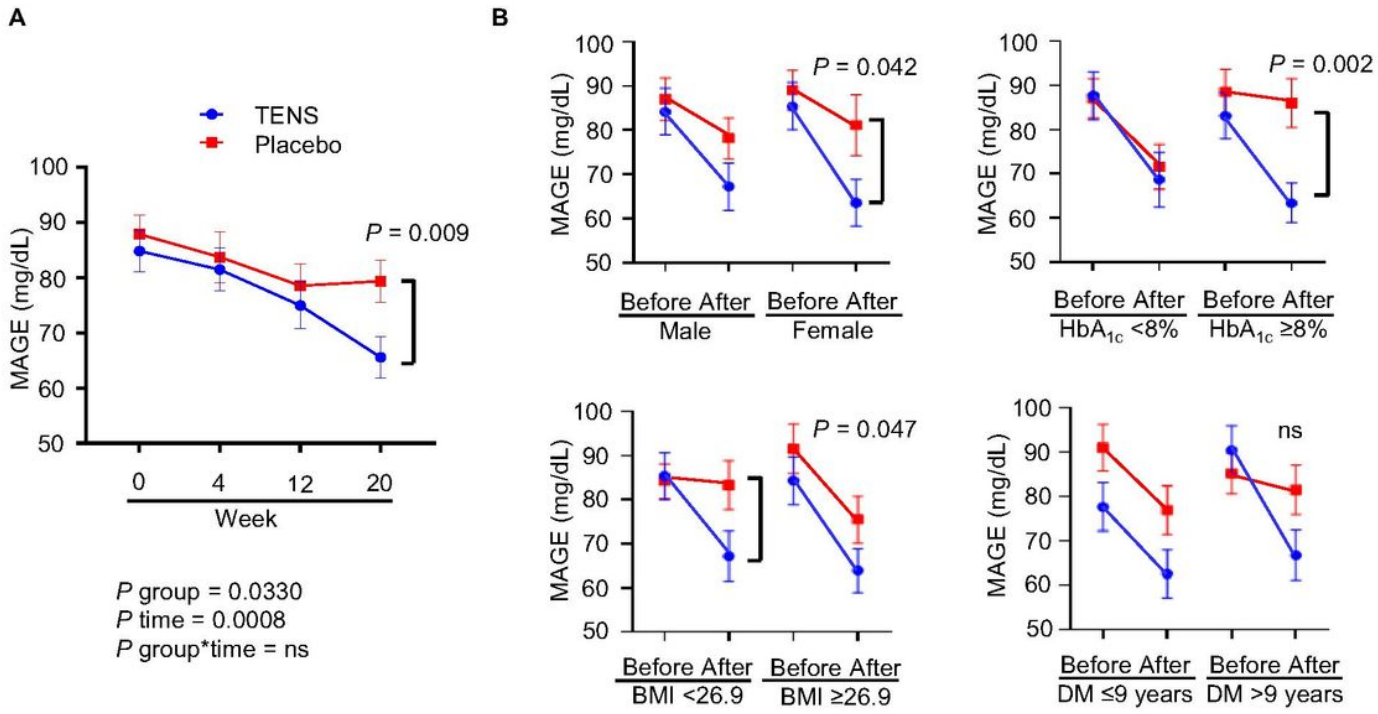

Figure 1

(A) Mean amplitude of glycemic excursion (MAGE) from week 0 to week 20 in the Transcutaneous Electrical Nerve Stimulator (TENS) vs. the placebo group. (B) Subgroup analysis of MAGE before and after treatment by gender, HbA_{1c} (%), BMI (kg/m^2) and duration of DM. Mixed model two–way ANOVA

and two-sample *t*-test were carried out and presented with means ± standard error of mean (SEM), ns, non-significant.

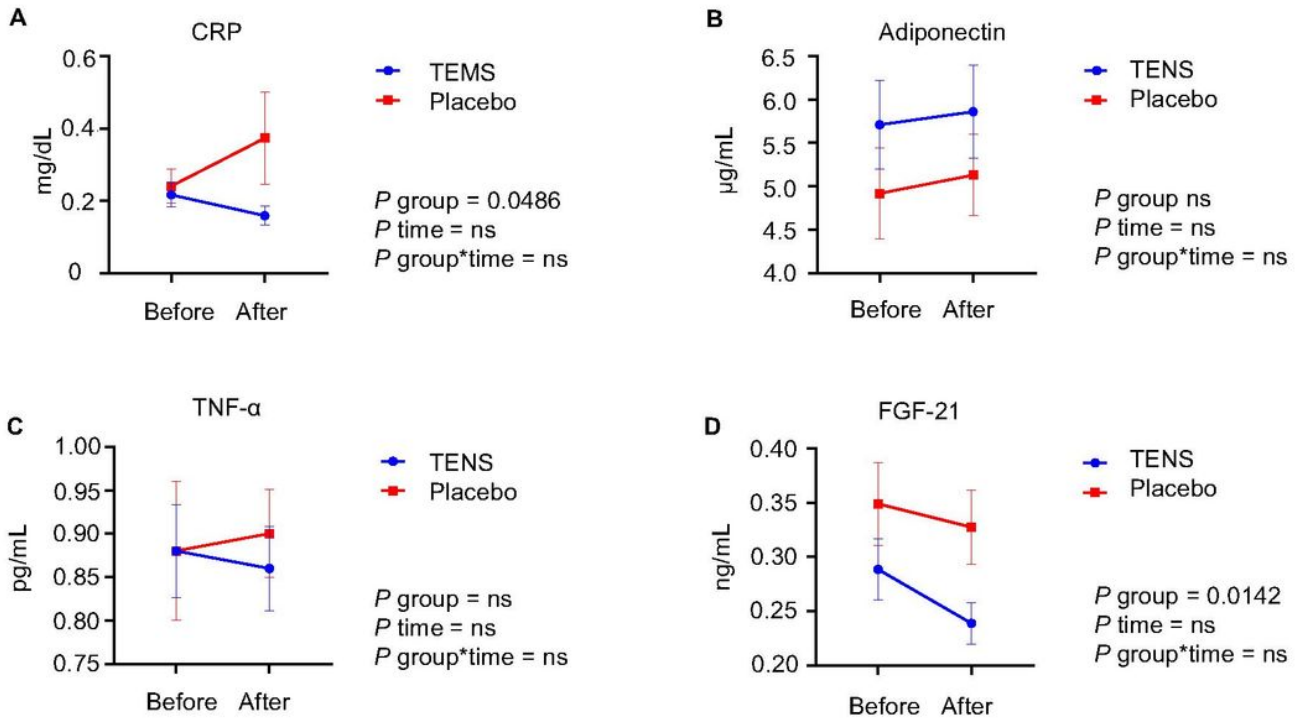

Figure 2

Change of exploratory parameters from before (week 0) and after (week 20) treatment in Transcutaneous Electrical Nerve Stimulator (TENS) vs. the placebo group. Statistical analysis with mixed model two–way ANOVA was carried out. Values are means ± standard error of mean (SEM), ns, non-significant. CRP, C-reactive protein; TNF-α, tumor necrosis factor-α; FGF-21, fibroblast growth factor-21.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- LuSupplementaryMaterial.docx