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Effects of therapeutic ultrasound on the endothelial function of patients with type 2 diabetes mellitus

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Abstract

Type 2 diabetes mellitus (T2DM) is characterized by endothelial dysfunction that causes micro- and macrovascular complications. Low intensity therapeutic ultrasound (LITUS) may improve endothelial function, but its effects have not been investigated in these patients. The aim of our study was to compare the effects of pulsed (PUT) and continuous (CUT) waveforms of LITUS on the endothelium-dependent vasodilation of T2DM patients. The present randomized crossover trial had a sample of twenty-three patients (7 men) diagnosed with T2DM, 55.6 (±9.1) years old, with a body mass index of 28.6 (±3.3) kg/m^2. All patients were randomized and submitted to different waveforms (Placebo, CUT, and PUT) of LITUS and the arterial endothelial function was evaluated. The LITUS of 1 MHz was applied in pulsed (PUT: 20% duty cycle, 0.08 W/cm$^{2\ SATA}$), continuous (CUT: 0.4 W/cm$^{2\ SPTA}$), and Placebo (equipment off) types of waves during 5 min on the brachial artery. Endothelial function was evaluated using the flow-mediated dilation (FMD) technique. PUT (mean difference 2.08%, 95% confidence interval 0.65 to 3.51) and CUT (mean difference 2.32%, 95% confidence interval 0.89 to 3.74) increased the %FMD compared to Placebo. In the effect size analysis, PUT (d=0.65) and CUT (d=0.65) waveforms presented moderate effects in the %FMD compared to Placebo. The vasodilator effect was similar in the different types of waves. Pulsed and continuous waveforms of LITUS of 1 MHz improved the arterial endothelial function in T2DM patients.

Key words: Ultrasonic therapy; Vascular endothelium; Type 2 diabetes mellitus; Endothelial function; Ultrasound; Nitric oxide

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic chronic disease characterized by hyperglycemia and altered lipid metabolism caused by inadequate secretion and/or action of insulin in response to varying degrees of over nutrition, inactivity, consequent overweight or obesity, and insulin resistance (1–3). Diabetes induces microvascular damage and macrovascular events due to atherosclerotic ischemia, such as myocardial and cerebrovascular infarction, and peripheral complications, including diabetic foot syndrome (3). Endothelial dysfunction is associated with diabetes for impaired endothelium-dependent vasodilation possibly due to an increase in superoxide anion radical ($O_2\cdot^-$) generation in mitochondria generated by the inhibition of electron transfer from mitochondrial nicotinamide adenine dinucleotide (NADH) and 1,5-dihydroflavin adenine dinucleotide ($FADH_2$) in the mitochondrial respiratory chain (4).

Diabetics experience a decrease in quality of life and life expectancy and generate excessive spending on public health (5), especially because the diabetes diagnosis is associated with the risk of heart failure (6). The functional changes of the endothelium and vascular reactivity precede histological alterations of atherosclerosis and vascular complications (4) and deregulate cardiovascular homeostasis, increasing the risk of cardiovascular events (6). They are also reliable markers of vascular complications in diabetic patients (7). Mitochondrial respiratory chain alterations promote pathological imbalance that leads to oxidative stress and inflammation (8). Among these vascular complications, long-term hyperglycemia with impaired blood vessels in patients with diabetes can lead to foot infections, and about 20% of them will develop diabetic foot ulcers during their lifetime (9). Every 30 s, an amputation due to diabetes occurs worldwide, and interventions to improve these vascular changes are extremely important (9).

Low-intensity therapeutic ultrasound (LITUS) is widely used in physical medicine and rehabilitation to manage pain and aid in the healing process of soft tissue injuries (10). According to the application parameters (intensity,

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wavelength, duty cycle, and frequency), it is possible to produce different biological responses (11) as a result of mechanical forces associated with the pressure wave and heat through thermal and non-thermal modalities (11–13). The absorption of the ultrasonic wave by the tissue produces heat at high intensities (14). However, in adequate doses (usually less than 0.5 W/cm$^{2\ SATA}$), the mechanical effects of the wave create microbubbles and shift large quantities of fluids. This forced flow hits the endothelial cell surface and may generate shear stress and stimulate NO endothelial production (12,13) by modulating cell membrane permeability, increasing protein synthesis, and activating immune response near the injury site, which may stimulate the regeneration of damaged tissue (10), making it a target for therapeutic strategies (12).

In humans, pulsed waveform (1.4 W/cm$^{2\ SATA}$, 30% duty cycle) of low-frequency LITUS (29 kHz) improved the endothelium-dependent vasodilation of the brachial artery (15). Other clinical trials strengthened this result, in which continuous (0.4 W/cm$^{2\ SPTA}$) and pulsed (20% duty cycle) waveforms of the LITUS (1 MHz (16) and 3 MHz (17)) wave, at different intensities (1 MHz, between 0.1 to 1.6 W/cm$^{2\ SPTA}$) (18), enhanced the endothelial function of healthy individuals. We hypothesized that the mechanical stress produced by therapeutic ultrasound improves the function of the endothelial cells in patients with T2DM, and it could contribute to the treatment of vascular complications in these patients. The aim of our study was to compare the effects of continuous and pulsed waveforms of 1 MHz therapeutic ultrasound on the endothelium-dependent vasodilation of T2DM.

Material and Methods

The present study was approved by the Ethics Committee for Health Research from the Federal University of Rio Grande (CEPAS-FURG, No. 115/2013) and protocolled in the Brazilian Clinical Trials Registry (protocol: U1111-1146-1663). The evaluations were carried out at the University Hospital Dr. Miguel Riet Corrêa Jr. (Brazil). The methodological design was based on determinations of the 2010 CONSORT Statement, with extension to randomized crossover trials (19).

Eligibility criteria

The subjects included in the study were patients with a previous diagnosis of T2DM. Patients were literate, aged between 25 and 65 years, had a body mass index (BMI: kg/m^2) lower than 40, were non-smokers, had no symptoms of skeletal muscle disorders, no previous cardiovascular surgery, no previous diagnosis of rheumatic, neurological, oncological, immune or hematologic disease, and no evidence of psychiatric and/or cognitive diseases. The patients were advised not to do physical activity, and not to drink alcoholic or caffeinated beverages, nor citrus juices before evaluations. Medications were suspended until the end of the exams on the days of interventions, and patients were in a fasting state of 8 h. Exclusion criteria on intervention days were leukocytosis ($>11.000\times10^3$/mm^3), impaired fasting glycemia (<70 and >300 mg/dL), and brachial artery diameter less than 2.5 mm and larger than 5.0 mm.

Sample calculation

Based on previous studies (16,17), it was estimated that the sample size of 20 volunteers in the study groups would be sufficient to identify a difference of 2% in mean and 2% in standard deviation of %FMD, with a power of 80% for α=0.05. The sample was composed of 23 volunteers, and %FMD was measured before (basal) and after the interventions.

Outcomes and follow-up

The primary outcome was the endothelial function, which was measured by the percentage of flow-mediated dilation (%FMD). The secondary outcome was endothelium-independent vasodilation, which was evaluated by the vascular response to nitroglycerin.

The sample was composed of 23 patients and the evaluations of endothelial function were performed before (basal: data presented by the average of three baseline measurements) and after the interventions were applied (Placebo, pulsed (PUT), and continuous (CUT)). The randomization of interventions (crossover) was made by software (www.random.org), and the information was sealed in a brown envelope, with patients and evaluators blinded (M.H., L.U.S., and F.S.P.) to the type of intervention. Patients were evaluated on three different days, with a 24 h interval between evaluations (washout). During the interventions, the evaluator would leave the room. The flowchart is shown in Figure 1.

Interventions

Calibrations of ultrasound equipment (Ultrasound Therapy, Sonopulse III 1/3 M, IBRAMED, Brazil) were performed before and after the study, ensuring the scale linearity. A commercially-available ultrasound gel was used as a conduction agent, and the head of the transducer was positioned over the brachial artery at the same place evaluated by the FMD's method. The continuous waveform was applied in a stationary manner for 5 min, at an intensity of 0.4 W/cm$^{2\ SPTA}$ (SPTA: spatial peak-temporal average), using a transducer with a 3-cm diameter (No. TR3CCE02) and an effective radiating area of 5 cm (16–18,20). Pulsed waveform was applied with a 20% duty cycle (2 ms on, 8 ms off), which represents spatial averaged-temporal intensity (SATA) of 0.08 W/cm$^{2\ SATA}$ (16–18,20). In Placebo interventions, all procedures above were repeated but with the ultrasound equipment powered off.

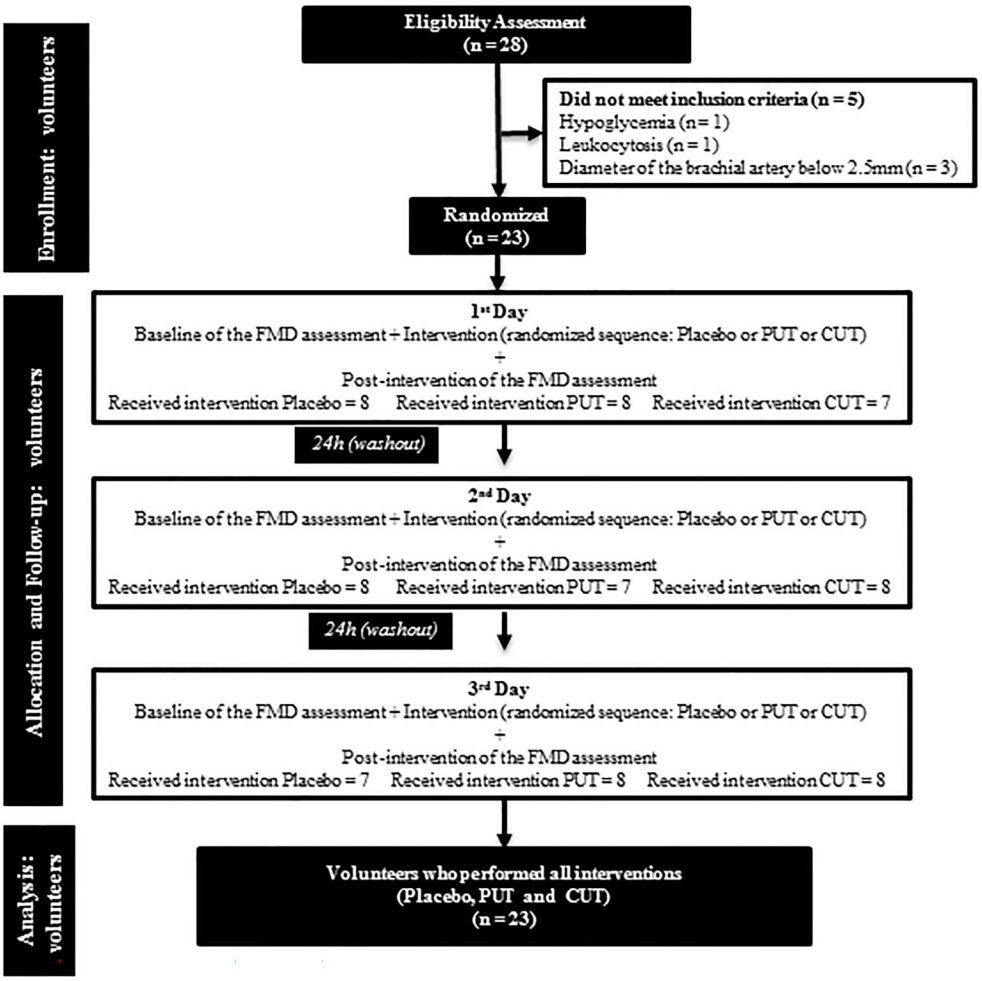

Figure 1. Flowchart of subjects allocated in the study. FMD: flow-mediated dilation; PUT: pulsed ultrasound therapy; CUT: continuous ultrasound therapy.

Endothelial function measurements

Flow-mediated dilation (FMD) was measured using high-resolution vascular ultrasound (Logiq P6, GE Healthcare, GE Ultrasound Korea Ltda., South Korea) according to guidelines (21,22) to evaluate arterial endothelium-dependent vasodilation. Briefly, changes in brachial artery diameter until 60 s of reactive hyperemia, after deflation of a cuff placed around the upper arm and inflated to 50 mmHg above the systolic blood pressure for 5 min, were compared with a baseline measurement. A pulsed-wave Doppler velocity signal was measured to evaluate basal blood flow and flow immediately after cuff release obtained no later than 15 s after cuff deflation (assessed using Doppler bean-vessel angle $\leqslant 60°$). The increased diameter after a sublingual nitroglycerin spray (0.4 mg) was used as a measurement of endothelium-independent vasodilation. The vessel diameter responses to reactive hyperemia and nitroglycerin are reported as the percent change relative to the diameter immediately before cuff inflation and to the diameter immediately before drug administration (%FMD = [(hyperemia maximum diameter – baseline pre-cuff diameter) / (baseline pre-cuff diameter)] $\times$ 100) and before drug administration (%NMD = [(nitroglycerin maximum diameter – baseline pre-cuff diameter) / (baseline pre-cuff diameter)] $\times$ 100) (16–18,21,22).

Brachial artery diameter measurements were performed offline by two evaluators using a semiautomatic quantitative analysis system after the proceedings. The second evaluator (F.S.P.) performing measurements was blinded to the data obtained by the first evaluator (M.H.). The differences between evaluators (mean vessel diameter) that were larger than 0.01 mm were repeated. The intra-observer and inter-observer coefficients of variation (CVs) for brachial artery diameter were 0.46 and 0.88%, respectively. All data were

measured twice, and final values are reported as means (21,22).

Physical and biochemical measurements

Anthropometric variables were measured, and blood samples were collected in the fasting state (8 h) on the first day of evaluations. Systemic arterial pressure and fasting glycemia were verified at the beginning and end of each intervention day. Accutrend test strips for Accutrend® Plus glucometer (ROCHE, Brazil) were utilized for glycemic control.

The hemogram blood tests (erythrogram and leukogram) were automatically processed (ABX kits, Horiba Diagnóstica, Brazil) and analyzed by microscopy. Cholesterol, triglycerides, high-density lipoprotein cholesterol (HDLc), glucose, and urea were measured using commercial kits from LAB TEST (Brazil) and analyzed in LAB MAX 240® (Japan) equipment. The low-density lipoprotein cholesterol (LDLc) was calculated by Friedewald's formula. Fibrinogen was examined by the equipment START (Diagnóstica Stago, France) using LAB TEST commercial tests. Ultra-sensitive C-reactive protein was evaluated by nephelometry (Nephelometer Beckman Coulter, model Image using reagents from the lab, CCRP, IMMAGE, USA). Glucose levels were measured by the Trinder assay (calorimetry) in the LAB MAX 240® equipment. Insulin was assessed by the chemiluminescence method using the Immulite® equipment (Diagnostic Products Corporation, USA). Insulin resistance was assessed by the homeostasis model assessment of insulin resistance (HOMA-IR) (25). Glycosylated hemoglobin (HbA_{1c}) was determined by the enzymatic method using the LAB MAX 240® equipment.

Data analysis

Data are reported as means ± SD. The distribution of variables was tested by the Shapiro-Wilk normality test. The analysis of variance for repeated measures (ANOVA), followed by Bonferroni *post hoc* test, was applied. Pearson's correlation coefficient was calculated to show the correlation of ultrasound therapeutic effects with brachial %FMD and the reproducibility of ultrasound therapeutic effects. Variations between and within groups are reported as mean difference (MD) and 95% confidence interval (95%CI). Additionally, effect size differences between baseline assessments and interventions were calculated using Cohen's *d* and reported by the following criteria: trivial <0.2, small 0.2–0.49, moderate 0.5–0.79, and large >0.8. A value of $P<0.05$ was considered statistically significant.

Results

Characteristics of patients

For the initial evaluation, twenty-eight patients were enrolled, but based on the eligibility criteria, five patients were excluded: one with hypoglycemia, one with leukocytosis, and three with a brachial artery diameter below 2.5 mm.

The sample comprised twenty-three patients (n=7 men, 30%) with a previous diagnosis of 12.5 (±8.1) years of T2DM. Table 1 shows the physical, laboratory, and metabolic characteristics of patients. Nine (39%) were over 60 years of age, only three (13%) were eutrophic (BMI 18 to 25 kg/m^2), and six patients had elevated blood pressure (above 120/80 mmHg) on the evaluation days. Hematocrit, platelets, and total leukocytes and their fractions (data not shown) were all within normal limits, but one patient had anemia. Fasting plasma glucose was below 110 mg/dL in only five patients. HOMA-IR was above expected values in nine patients and glycated hemoglobin was above reference values (>7%) in eight patients. Lipid profile was within recommended limits in twelve (52%) patients. Lipid fractions were elevated in seven patients for total cholesterol (>200 mg/dL), six patients for triglycerides (>200 mg/dL), ten for HDLc (<45 mg/dL), and twelve for LDLc (>130 mg/dL). Renal function was measured by urea (31.5 ± 7.5 mg/dL) and creatinine (0.8 ± 0.2 mg/dL), and hepatic function by glutamic pyruvic transaminase (30.9 ± 18.5 U/L). Results for glutamic oxaloacetic transaminase (25.8 ± 5.2 U/L) and alkaline phosphatase (74 ± 20.8 U/L) were normal for the population. Fourteen patients (60%) had a high value of C-reactive protein (>3 mg/dL), while fibrinogen had remained within reference values for all samples.

Twenty patients (87%) used oral hypoglycemic drugs and metformin was the most used drug (13 patients). Seven patients used metformin-associated with other pharmaceuticals (glimepiride: 5; gliclazide: 1; glibenclamide: 1) and insulin was used by five of them. Only three did glycemic control by diet and lifestyle orientation. Acetylsalicylic acid was used by five patients, and eight were using simvastatin. Drug control for systemic arterial pressure was used by seventeen patients. Three patients used blocker receptor AT1, 10 used blocker receptor AT1 associated with a diuretic, and three of them used a beta-blocker. Two patients used angiotensin-converting-enzyme inhibitor with beta-blocker, one used angiotensin-converting-enzyme inhibitor and beta-blocker, and one used calcium channel blockers associated with blocker receptor AT1 with a diuretic.

Endothelial function

The results of brachial artery measurements after therapeutic ultrasound are shown in Table 2. The variables of the basal assessments are shown as an average of the three measurements (3 days). Basal diameter (P=0.523), baseline blood flow (P=0.815), and hyperemic blood flow (P=0.244) were not different on the days of interventions. Endothelial-independent vasodilation evaluated by diameter (P=0.771) and dilation percentage (P=0.642) after nitroglycerin were similar after Placebo, PUT, and CUT

Table 1. Clinical and fasting metabolic characteristics of patients.

Characteristic	Patients with type 2 diabetes (n=23)
Age (years)	55.6 ± 9.1
Body mass index (kg/m^2)	28.6 ± 3.3
Waist/hip	0.96 ± 0.11
Systolic blood pressure (mmHg)	131.1 ± 12.5
Diastolic blood pressure (mmHg)	86.6 ± 6.7
Hematocrit (mL/%)	40.1 ± 3.1
Erythrocytes ($\times 10^5$/mm^3)	4.6 ± 0.4
Hemoglobin (g/dL)	13.3 ± 1.1
Platelets ($\times 10^3$/mm^3)	283 ± 67
Total leukocytes ($\times 10^3$/mm^3)	6714 ± 1590
Plasma glucose (mg/dL)	141.0 ± 48.2
Insulin (μU/mL)	10.8 ± 8.0
HOMA-IR	4.0 ± 3.6
Glycated hemoglobin (%)	6.4 ± 1.6
Total cholesterol (mg/dL)	175.4 ± 38
Triglycerides (mg/dL)	129.0 ± 65.5
HDLc (mg/dL)	44.9 ± 9.4
LDLc (mg/dL)	106.5 ± 41.0
C-reactive protein (mg/L)	4.1 ± 2.9
Fibrinogen (mg/dL)	282.2 ± 43.1

Data are reported as means ± SD. HDLc: High-density lipoprotein cholesterol; LDLc: Low-density lipoprotein cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance.

Table 2. Results of ultrasound measurements of the brachial artery.

Endothelial function	Basal	Interventions			P
		Placebo	PUT	CUT	
Baseline diameter (mm)	3.79 ± 0.48	3.81 ± 0.46	3.78 ± 0.49	3.78 ± 0.47	0.523
Baseline flow (mL/min)	226 ± 118	218 ± 108	230 ± 96	231 ± 127	0.815
Hyperemia diameter (mm)	4.06 ± 0.51	4.07 ± 0.50	4.12 ± 0.53	4.13 ± 0.51	0.028
Hyperemic flow (mL/min)	240 ± 87	267 ± 104	234 ± 97	263 ± 96	0.244
FMD (%)	7.22 ± 3.35	7.21 ± 3.38	9.29 ± 2.50*†	9.46 ± 3.23*†	< 0.001
NMD diameter (mm)	4.52 ± 0.6	4.55 ± 0.6	4.53 ± 0.6	4.56 ± 0.6	0.771
NMD (%)	22.9 ± 3.2	23.1 ± 3.7	23.8 ± 2.1	23.8 ± 2.8	0.642

Data are reported as means ± SD (95% confidence interval) for endothelial function (n=23, 7 men). Basal: average of three baseline measurements; PUT: pulsed ultrasound therapy; CUT: continuous ultrasound therapy; FMD: flow-mediated vasodilation; NMD: nitroglycerin-mediated vasodilation. *P < 0.05 *vs* baseline measures, †P < 0.05 *vs* Placebo (ANOVA).

application. The diameter after hyperemia showed an apparent difference between interventions (P=0.028), but these results were not confirmed by the Bonferroni *post hoc* test (P > 0.05) and the respective 95%CI (Table 3).

Endothelial-dependent vasodilation after therapeutic ultrasound is shown in Table 2. The PUT increased FMD by 1.77% in relation to its basal measure (95%CI: 0.33 to 3.19; P < 0.01) and by 2.08% in relation to Placebo intervention (95%CI: 0.65 to 3.51; P < 0.01). The CUT also increased FMD by 2.01% in relation to its basal measure (95%CI: 0.58 to 3.45; P < 0.001) and by 2.32% in relation to Placebo intervention (95%CI: 0.89 to 3.74; P < 0.001). Effect size analysis of the %FMD suggested that the PUT (*d*=0.65) and the CUT (*d*=0.65) had moderate effects compared to the Placebo intervention. The basal %FMD and that of Placebo intervention were similar (DM: 0.31 95%CI: –1.11 to 1.73; P > 0.05), and different types of waves (continuous and pulsed) showed similar results for %FMD (MD for CUT vs PUT=0.24, 95%CI: –1.18 to 1.67; P > 0.05).

Baseline measures of %FMD were correlated with Placebo (*r*=0.739, 95%CI: 0.472 to 0.883, P < 0.001;

Table 3. Mean difference (MD) and their respective 95% confidence intervals (95%CI) of hyperemia diameter (mm) between interventions.

Interventions	MD	95%CI
Basal *vs* Placebo	–0.004	–0.077 to 0.067
Basal *vs* PUT	–0.054	–0.126 to 0.018
Basal *vs* CUT	–0.066	–0.138 to 0.006
Placebo *vs* PUT	–0.049	–0.122 to 0.022
Placebo *vs* CUT	–0.061	–0.133 to 0.011
PUT *vs* CUT	–0.011	–0.084 to 0.060

Endothelial function (n=23, 7 men). Basal: average of the three baseline measurements; PUT: pulsed ultrasound therapy; CUT: continuous ultrasound therapy.

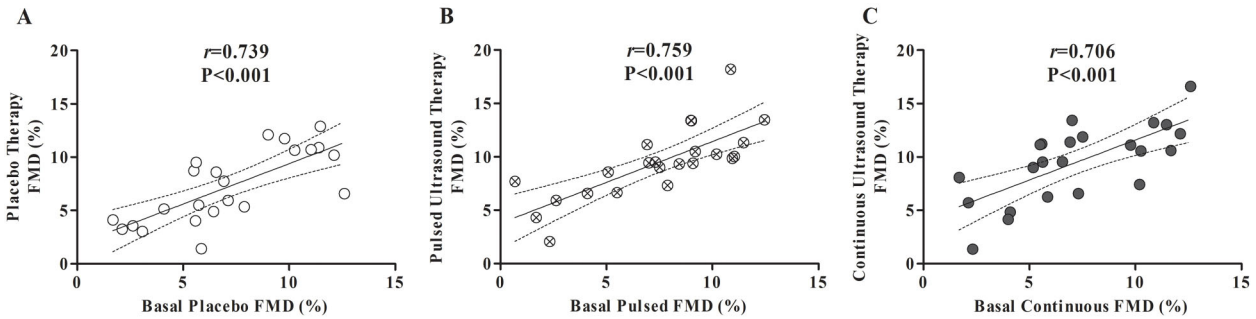

Figure 2. Correlation of therapeutic ultrasound wave forms with endothelial function. **A**: basal percent flow-mediated dilation (%FMD) after Placebo therapy; **B**: basal %FMD after pulsed ultrasound therapy; **C**: basal %FMD after continuous ultrasound therapy.

Figure 2A), PUT (r=0.759, 95%CI: 0.504 to 0.892; $P<0.001$; Figure 2B), and CUT (r=0.706, 95%CI: 0.415 to 0.866, $P<0.001$; Figure 2C) interventions. No adverse effects were reported by patients during the data collection period.

Discussion

The results of the present study demonstrated that continuous and pulsed waveforms of the 1 MHz LITUS improved endothelium-dependent vasodilation (%FMD) in T2DM patients. Patients with T2DM have endothelial dysfunction (4,6,9) due to the elevated presence of oxygen reactive species (ROS), especially $O_2\cdot^-$, which has a high affinity for nitric oxide (NO), resulting in decreased NO bioavailability and producing peroxynitrite ($ONOO^-$) (8). Peroxynitrite can cause damage by readily reacting with biological molecules, creating a vicious cycle in which more ROS are produced instead of NO (23). Besides the overproduction of $O_2\cdot^-$ (4,8), hyperglycemia causes the accumulation of glycolytic metabolites because of the inhibition of glycolytic enzymes. This leads to increased consumption of NADPH that is required for the regeneration of reduced glutathione (main intracellular antioxidant), enhancing oxidative stress and causing endothelial dysfunction (4). Endothelial dysfunction causes microcirculatory dysfunction and end-organ hypoperfusion, blood pressure increases, and activation of coagulation factors as well as platelets (23). Chronically, these activations favor the development of atherosclerosis and coronary artery disease in patients with T2DM (7,8).

The endothelial function of T2DM patients improved after the application of pulsed and continuous waveforms of 1 MHz therapeutic ultrasound. Endothelial cell cultures of human umbilical vein exposed to different LITUS intensities (27 kHz, 0.001 to 0.5 W/cm^2, 10 min) and wave types showed an increase in endothelial nitric oxide synthase (eNOS) activity and NO production that lasted 30 min, with higher effectiveness of pulsed waveform (10% duty cycle) (24). In the present research, there was an improvement in endothelium-dependent vasodilation but without differences between wave types. Experimentally, various intensities (490 kHz, 0.21, 0.35, 0.48 W/cm$^{2\ SPTA}$, 10 min) of continuous waveform caused a gradual increase in NO production, suggesting that this is an intensity-dependent action (25). In humans, the pulsed wave type (29 kHz, 30% duty cycle, 1.4 W/cm$^{2\ SATA}$) enhanced the endothelium-dependent vasodilation in the

brachial artery, starting within 2 min of application and lasting 21 min (15). Randomized clinical trials in healthy subjects using the same parameters of this study indicate that different wave types enhance endothelium vasodilation (16–18) and improve brachial artery vasodilation during 20 min and these effects are not caused by increasing prostacyclin (PGI_2) (16). The present study was in agreement with those findings and demonstrated that effects on endothelial cells were independent of the frequency of the ultrasound head.

The proposed mechanisms for LITUS effects on endothelial function are heat, strength, and pressure of mechanical waves (13). Thermal effect is due to the absorption of ultrasonic waves by the tissue, especially in continuous waveforms. Previous clinical studies with different wave types did not determine changes in the cutaneous temperature (15,17), and a continuous waveform (490 kHz, 0.48 $W/cm^{2\ SPTA}$, 10 min) increased the temperature of the adductor muscle of rats only at 0.8°C, which did not interfere in increasing NO production (25). Thus, the results of the present study were possibly due to the mechanical effects since pressure waves displace a large amount of fluids, which cause shear stress on endothelial cells (12). The flow of forces stimulates oscillations of stable microbubbles (12), called acoustic streaming or microstreaming (12,13), that also cause shear stress over vascular endothelium (13). The shear stress is converted to specific cellular signals that increase NO production (7,8,13). In addition, LITUS wave pressure may create temporary pores in the membrane, change the intercellular permeability, and rearrange the fibers of the cytoskeleton (26), which increase the permeability to Ca^{2+} ions, cause eNOS to uncouple and, consequently, form NO (27). The association of these mechanisms results in an improvement of endothelium-dependent vasodilation in T2DM patients, as was shown in the present study.

The results of this study demonstrated an improvement in the FMD by about 2.1% for the different LITUS waveforms. A meta-analysis (35 studies, with 17,280 participants) demonstrated that the 1% increase in FMD predicted 12% (95%CI: 9 to 16%) of the risk of cardiovascular events (28). These results are corroborated by another meta-analysis (32 studies, 15,191 individuals), where the reduction in the risk of cardiovascular events and all-cause mortality was predicted at 10% (95%CI: 8 to 12) (29). However, the clinical relevance of the %FMD is still under investigation (30). In another meta-analysis (10 studies, 377 patients with T2DM) that evaluated the effects of exercise training (durations $\geqslant$8 weeks) demonstrated an overall improvement in FMD by 1.77% (95%CI: 0.94–2.59%) (31). The results of exercise training are similar to those of the present study, and although the LITUS can be applied every day (11), the effect lasts only for a short time (16).

There were limitations of this study: i) the absence of prostacyclin and endothelium-derived hyperpolarizing inhibition factor; and ii) the technique evaluated the endothelial function of the brachial artery, and therefore care must be taken in extrapolating these results to other blood vessels. Nevertheless, this study was the first to evaluate the effect of LITUS on endothelial function in T2DM patients using flow-mediated dilation techniques, which is the gold standard to measure this outcome.

In conclusion, the present study showed that the pulsed and continuous LITUS waveforms (1 MHz) improved endothelium-dependent vasodilation (%FMD) of T2DM patients. Further studies should investigate the effects of therapeutic ultrasound for the management of local vascular complications in patients with diabetes. This therapeutic resource is a non-pharmacological, non-invasive, low cost, and easy-to-use tool for the improvement of endothelial function of T2DM patients.

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