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Effects of oral vitamin C treatment on metabolism at rest and in response to an acute exercise in patients with poorly controlled type 2 diabetes mellitus

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KEYWORDS

Ascorbic acid; Hyperglycemia; Insulin resistance; Lipid oxidation; Physical activity.

ABSTRACT

Metabolic disturbances at rest and in responses to exercise are the hallmarks of type 2 diabetes mellitus (T2DM). Although vitamin C was shown to improve resting glycemia and lipid profile in patients with T2DM, a study investigating the effects of vitamin C treatment on the metabolic responses at rest and to low-intensity exercise in poorly controlled T2DM patients has not been conducted yet. This study aimed to investigate the effects of oral vitamin C treatment on metabolism at rest and in response to an acute exercise in patients. Twenty T2DM patients were randomly participated in the following two six-week arms with a six-week washout period: either daily placebo or 1000 mg vitamin C. On the first and last day of each session, they performed 20-min low-intensity cycling. Five-min expired gas was collected before starting and finishing the exercise. Immediately before and after the exercise, venous blood samples were collected. Vitamin C decreased resting cholesterol concentration compared with placebo treatment (p-value < 0.05) without any effect after the exercise. Post-vitamin C treatment, fat oxidation rate was higher during exercise than at baseline (p-value < 0.05). Resting total cholesterol/high-density lipoprotein-cholesterol and glucose concentrations in plasma at rest and after exercise were lower at post- compared with pre-vitamin C treatment (p-value <0.05). Additionally, pre- and post-treatment, rates of oxygen consumption and carbohydrate oxidation, and energy expenditure were higher during exercise than at baseline in both treatment arms (p-value <0.05). The findings suggest that daily treatment with 1000 mg of vitamin C for six weeks reduced the resting cholesterol concentration in patients with poorly glycemic controlled T2DM. However, this effect was not observed immediately after exercise. Nonetheless, post-vitamin C treatment, fat utilization during exercise was higher than baseline. Besides, low-intensity exercise increased oxygen consumption rate, carbohydrate oxidation rate, and energy expenditure, pre- and post- both treatment arms.

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Introduction

Worldwide, type 2 diabetes mellitus (T2DM) causes high morbidity and mortality rates and has an estimated healthcare cost as high as 1.3 trillion US dollars, which is expected to increase from 2015 to 2030[1]. T2DM has been known to impair metabolism at rest and in response to exercise including insulin resistance[2,3], dyslipidemia[4], skeletal muscle mitochondrial dysfunction[5] substrate utilization[6,7], and oxygen consumption [2,5,8].

Vitamin C (ascorbic acid), has been shown to play a significant role in improving metabolic responses in patients with T2DM[2]. Vitamin C is a cofactor of enzyme that stimulates carnitine synthesis[9,10]. This results in an improved mitochondrial function and oxygen consumption, leading to increased fat oxidation[5]. Importantly, the improved fat oxidation has been shown to improve insulin sensitivity[11]. The improved insulin sensitivity is confirmed by a study conducted by Dakehale et al. (2011) who found an improved glycemic control with T2DM patients taking oral 500 mg vitamin C twice a day for 12 weeks[12]. Afkhami-Ardekani and Shojaoddiny-Ardekani (2007) confirmed the hypoglycemic effect of the same dose of oral vitamin C for 6 weeks in patients with T2DM[13]. Furthermore, vitamin C was demonstrated to improve lipid profile including cholesterol[14,15] (by facilitating the conversion of cholesterol into bile acids) and low-density lipoprotein[13]. Importantly, previous studies examining the effects of oral vitamin C on low-intensity exercise in patients with poorly controlled T2DM, defined as HbA1c>8.5% for longer than 1 year[16], have not been conducted yet. The level of glycemic control may yield different changes in metabolic responses to the exercise. However, we have insufficient information regarding the effects of vitamin C on metabolic responses at rest and in response to low-intensity exercise in the patients.

Therefore, we aimed to primarily investigate whether oral 1000 mg vitamin C can improve fat oxidation rate and subsequently improved metabolic responses at rest and in response to low-intensity exercise in patients with poorly controlled T2DM. We hypothesized that vitamin C treatment would improve the metabolic responses at both conditions in patients with T2DM.

Materials and methods

Subjects

Of the 95 T2DM patients from Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, 24 were considered eligible to participate in the study. They were informed of their role in this study both verbally and in writing before signing a consent form. The entire protocol and consent form were approved by the Human Ethics Committee of Khon Kaen University (HE561129) in accordance with the 1964 Declaration of Helsinki. The subjects were screened according to their fasting blood chemistry and completed health questionnaires and underwent physical examinations before participating in the study. Patients with the following characteristics were included in the study: patients aged 45–60 years, patients diagnosed with T2DM at least 12 months prior to the study, patients with glycated hemoglobin A1c (HbA1c) level ≥ 8.5%, patients on oral hypoglycemic drug treatment, patients with normal lipid profile or dyslipidemia with or without lipid-lowering drugs, patients with systolic blood pressure (BP) ≤ 140 or diastolic BP ≤ 90 mmHg, patients on antihypertensive drug treatment at a similar dose throughout the study to maintain a BP of 140/90 mmHg, patients who had sedentary lifestyle, patients who did not participate in any regular exercise program for at least 6 months before the study, and patients residing in Khon Kaen Province, Thailand. Subjects were excluded from the study if their treatment plan changed (Table 1).

Twenty-four subjects (considering a 20% dropout rate) were recruited in this study according to the statistical calculations, where the mean was compared to the hypothesized value based on Johnston (2006)[16] using the WinPepi program [17]. Johnston (2006)[16] reported that vitamin C can increase fat oxidation rates from 0.48 g/min to 0.68 g/min; these data were significant with the following conditions: *p*-value<0.05, α = 0.05, β = 0.2 and power = 0.80. We measured the substrate oxidation rate using oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) from the expired gas, similar to what was used by Johnston[16].

Procedures

This study was conducted at the Nutrition and Exercise Sciences Laboratory, Faculty of Medicine, Khon Kaen University. This study was a prospective, double-blind, placebo-controlled crossover study. The capsules were provided and coded by Blackmores Co. Ltd, Australia, The 1000 mg of vitamin C capsule consisted of vitamin C 1000 mg (ascorbic acid 400 mg, sodium ascorbate 350 mg and calcium ascorbate 400 mg). In addition, 1000 mg of placebo consisted of calcium hydrogen phosphate anhydrous, carnauba wax, cellulose microcrystalline, citric acid anhydrous, Daucus Carota root dry powder, starch – tapioca, talc – purified. Both the researchers and patients were blinded on the code until the study was completed. On the testing day, the subjects were asked to sleep overnight for at least 8 hours, refrain from taking in alcohol and caffeine and from performing strenuous exercise the day before the study, and arrive fasted at the laboratory at 6:30 am. On the first visit, the subjects' peak $\dot{V}O_2$ ($\dot{V}O_{2,peak}$) was measured. One week later, all subjects were randomly divided into two arms receiving 6 weeks of daily treatment of either placebo (PLA) or vitamin C with a 6-week washout period between the treatments. The supplements were administered immediately following breakfast. On the first and last day of each treatment arm, all subjects performed cycling exercise at 30% $\dot{V}O_{2,peak}$ (16.5±2 watts) for 20 min. Immediately before and after the exercise, 16-mL blood samples were obtained from the subjects' antecubital vein. Besides, before starting and finishing the exercise, 5-minute expired gas were collected and analyzed using a gas analyzer (mixing chamber system) (PowerLab 8/30 ADInstruments, Australia) to determine the $\dot{V}O_2$ and $\dot{V}CO_2$ (g/min). The $\dot{V}O_2$ and $\dot{V}CO_2$ were used to calculate the respiratory exchange ratio (RER) and carbohydrate and fat oxidation rates using the Péronnet and Massicotte equation, without considering the protein oxidation rate[18]. Venous blood samples were obtained immediately before and after exercise to determine the plasma ascorbate, glucose, insulin, and lipid concentrations.

Randomization and blinding

The allocation sequence was generated by computer-generated random numbers and kept in sequentially numbered and sealed envelopes. Predictability of a random sequence was reduced by keeping in a separate document that was unavailable to those who enrolled participants or assigned interventions.

Peak oxygen consumption ($\dot{V}O_{2,peak}$) test

The $\dot{V}O_{2,peak}$ was determined by an incremental exercise test. All subjects began with a 2-min free workload cycling (0 watt). They subsequently continued the cycling with a workload of 30–50 watts depending on their fitness status. Workloads were increased by 20–30 watts every 3 min. The test continued until the subjects presented the maximum symptoms of dyspnea (9–10) and fatigue (18–20), determined by the rating of perceived dyspnea and rating of perceived exertion scales; were unable to maintain a cycling speed of at least 60 rpm; had an increased heart rate (HR) (calculated using the following formula: HR_{max} [220 – age]); had steady or decreasing VO2; and had RER >1.15. The expired gas samples, oxygen saturation, and electrocardiogram (ECG)

were recorded throughout the test, and the dyspnea and fatigue symptoms were evaluated every 3 min and at the end of the test. During the test, room temperature was 25±1°C, and humidity was 57±7%.

Measurements

Anthropometry and body composition

Body mass and height were measured using a stadiometer (Detecto, USA); subsequently, the body mass index (BMI) was calculated by kilograms per meter squared. Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest, and hip circumference was measured at the widest point. The total body composition, lean body mass, and fat mass were measured by dual-energy X-ray absorptiometry (Lunar Prodigy whole body scanner, GE Medical Systems, USA). All scans were performed while the subjects were lying supine and wearing light indoor clothing.

Physiological measurements

Heart rate and BP were measured while the subjects were assuming a sitting position using an automatic sphygmomanometer (UA-767 Plus, UK) with the cuff wrapped around the upper arm.

Substrate utilization

From breath-by-breath expired gas ($\dot{V}O_2$, $\dot{V}CO_2$) measurements, total fat and carbohydrate oxidation rates were calculated using the Péronnet and Massicotte equation[18].

Blood chemistry

A total of 16-mL blood samples were obtained from the subjects' antecubital vein. Subsequently, blood samples were immediately separated into four tubes: an ethylenediaminetetraacetic acid tube (12 mL) to measure lipid concentrations, a sodium fluoride tube (1 mL) to measure glucose concentrations, a blood clotting tube (1 mL) to measure insulin concentrations, and a heparinized blood tube (2 mL) to measure plasma vitamin C concentrations. The heparinized blood tube was wrapped in aluminum foil and placed in an ice bath before being centrifuged. All of the tubes except the sodium fluoride tube were then centrifuged at 3000 g for 10 min at 4°C. Aliquots of plasma were frozen immediately and stored at -20°C. Blood glucose concentrations were measured by the glucose oxidase method immediately after blood collection (YSI 2300 STAT Plus™, USA, which was auto-calibrated in every five-sample test). Plasma insulin concentrations were measured with a radioimmunoassay kit (MP Biomedical, GmbH, Germany) at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University. Fasting insulin and glucose concentrations were used to calculate insulin resistance (homeostatic model assessment for insulin resistance, HOMA-IR) scores and determine ß-cell function (homeostatic model assessment of ß-cell function, HOMA-ß). Cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride concentrations were analyzed using the cholesterol oxidase-peroxidase method, the homogeneous HDL-C plus method, and the glycerol phosphate oxidase- phenol 4-aminoantipyrine peroxidase (GPO-PAP) method, respectively (Reflotron Plus, USA, which was calibrated daily). Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated using the Friedewald formula. All measurements were performed in duplicate.

Physical activity and dietary assessment

Subjects were asked to maintain their daily physical activity and dietary habits during the study period. They were asked to record their physical activity and food intake for 3 days per week: two weekdays and one weekend day. The records were used to analyze energy expenditure (EE) following the compendium of physical activities. Energy intake was analyzed using the INMUCAL program (INMUCAL software, Mahidol University, Thailand).

Treatment compliance

We counted the number of tablets remaining at each visit, and checked the subjects' medical records to examine treatment compliance, and phoned to follow up the subjects' treatment adherence.

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences statistics software package for Windows version 20. The differences within and between the supplement groups were

tested by analysis of variance with repeated measures. When a significant difference was observed, a post hoc analysis using the Bonferroni adjustment was performed. All differences were considered significant at p-value < 0.05. All data were expressed as the means ± SD, except when stated elsewhere.

Results

Of the 24 patients (20 females, 4 males) in the study, 20 (83% of the total patients, 16 females and 4 males) were fully compliant with the study. Three patients withdrew from the study due to a change in their treatment plans; they began receiving daily insulin injections during the washout period. Another patient had acute knee pain; thus, she could not perform the cycling exercise Figure 1. Patients' baseline anthropometrics are shown in Table 1. All subjects maintained their medication and treatment plan during 2 treatment arms. Several subjects took hypoglycemic, antihypertensive, and lipid-lowering agents, but none of them required insulin injections (Table 2). The intensity of the exercise was confirmed to be low by percent of $\dot{V}O_2$ (approximately 30% $\dot{V}O_{2,\,peak}$) and workload (27.8±9.5 %Wmax) was not different between the treatment arms (Table 1).

Table 1 Baseline anthropometric and physiological characteristics of T2DM patients

	Baseline	
	Placebo	Vitamin C
Age (yr)	53±7	53±7
M/F (n)	4/16	4/16
Body mass (kg)	61.2±2	61.1±10
Height (m)	1.6±0.1	1.6±0.1
BMI (kg/m^2)	23.9±1	23.9±2
Waist circumference (cm)	88.7±2	89.3±8
Hip circumference (cm)	98.3±6	98.2±3
W/H ratio	0.9±0.2	0.9±0.1
Body fat (%)	34.6±6	34.6±6
Fat mass (kg)	21.5±5	21.5±5
Lean body mass (kg)	21.5±5	21.5±5
Resting HR (/min)	79±1.43	81±11
SBP (mmHg)	121±2.52	121±9
DBP (mmHg)	79±1.79	81±11
$\dot{V}O_{2,\,peak}$ (mL/kg/min)	20.1±6	20.1±6
% $\dot{V}O_{2,\,peak}$	34.1±1.2	33.1±8.3
Workload (%Wmax)	27.8±9.5	27.8±9.5
Time since diagnosis (yr)	7.9±4.7	7.9±4.7
FBG (mg/dL)	194±3	227±5
HbA1c (mmol/mol)	87.7±2.8	85.9±3
HbA1c (%)	10±0.3	10±0.3
ALT (mg/dL)	32.3±28	32.3±28
Cr (mg/dL)	0.7±0.2	0.7±0.2

Note: Values are means ± SD, n = 20 (16 females, 4 males). M, males; F, females; BMI, Body mass index; W/H, Waist:Hip circumference ratio. SBP; Systolic blood pressure, DBP; Diastolic blood pressure, $\dot{V}O_{2,\,peak}$; Peak oxygen consumption, HbA1c; The glycated haemoglobin A1c, FBG; Fasting, ALT; Alanine aminotransferase, Cr, Creatinine.

Table 2 Number of the T2DM patients taking the medicines

	Placebo	**Vitamin C**
Diabetes Mellitus type 2		
Metformin (500 mg)	16	16
Glibenclamide(5 mg)	6	6
Glipizide (5 mg)	7	7
Hypertension		
Enalapril (2.5 mg)	3	3
Propranolol (10 mg)	4	4
Aspirin (300 mg)	1	1
Amlodipine (20 mg)	1	1
Hydrochlorothiazide (50 mg)	1	1
Dyslipidaemia		
Simvastatin (20 mg)	4	4
Atorvastatin (20 mg)	2	2

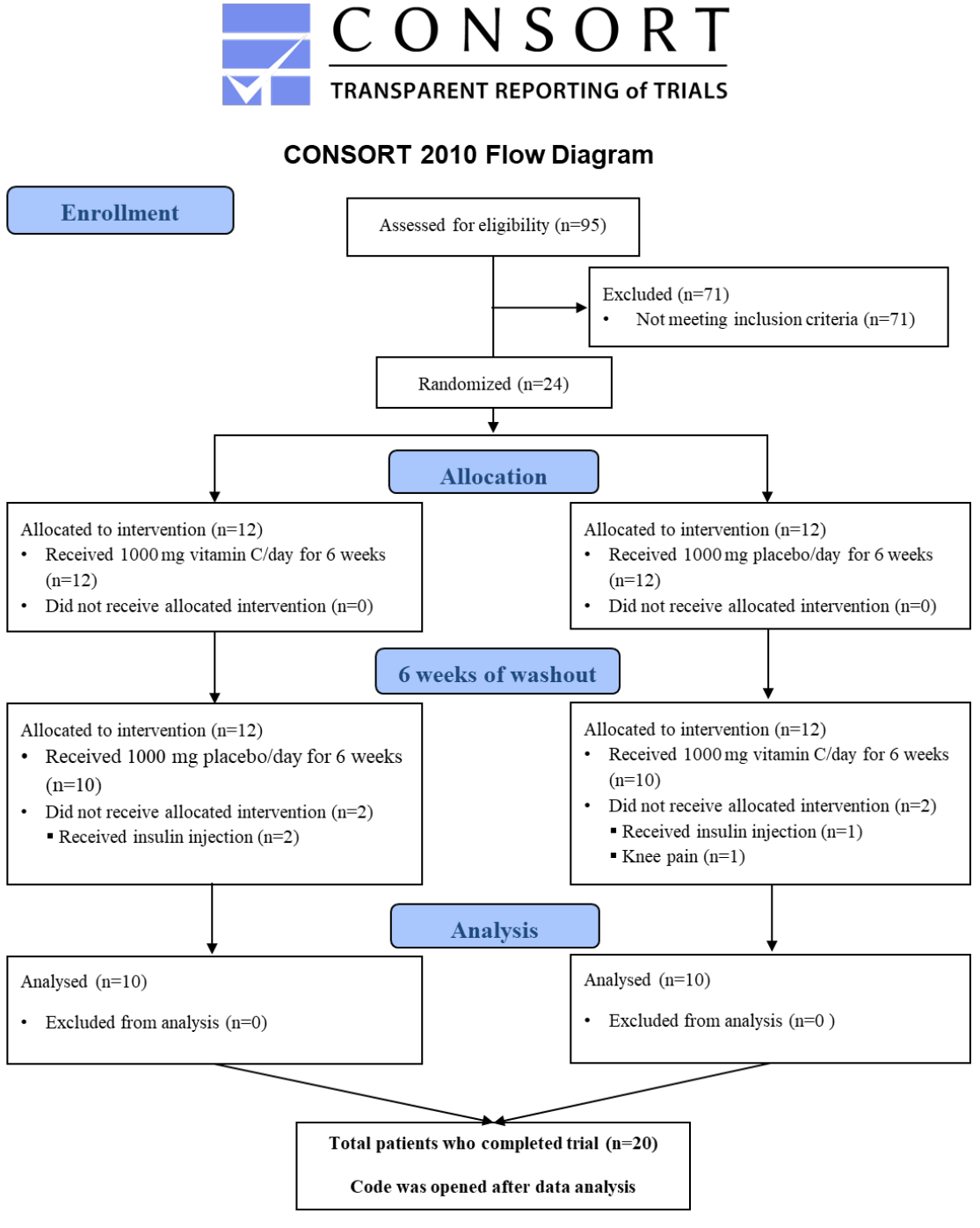

Figure 1 Consort flow chart of patient enrollment

Daily energy intake and energy expenditure

There were no significant differences in the amount or composition of dietary and energy intake and energy expenditure (EE) the week before starting and finishing the treatment between the vitamin C and PLA arms (Supplementary Table 1).

Effect of vitamin C on substrate utilization

All patients had more carbohydrate than fat oxidation rate both at rest and during exercise in both treatment arms.

At rest

Pre-treatment, the fat oxidation rate (Figure 2A) and contribution to total energy expenditure (TEE) Figure 3. were significantly lower than the carbohydrate oxidation rate Figure 2B, with similar values for PLA (oxidation rate, fat [0.02±0.01 g/min, 0.21 ± 0.1 mg/kg/min] vs carbohydrate [0.20±0.1 g/min, 3.36±2.4 mg/kg/min]; contribution to TEE, carbohydrate [87.5 ± 5.1%] vs fat [12.5 ± 5.1%]) and vitamin C treatment (fat [fat 0.02±0.01 g/min, 0.30 ± 0.1 mg/kg/min] vs carbohydrate [0.22±0.1 g/min, 3.56 ± 2.4 mg/kg/min]; fat [16.4 ± 4.9%] vs carbohydrate [83.6 ± 4.9%]) (all were *p*-value<0.05) Figure 2A and 2B, and Figure 3. Post-treatment, the oxidation rates and contributions of carbohydrate and fat had not changed from their pre-treatment values.

During exercise

Interestingly, post-treatment, vitamin C caused a significantly higher fat oxidation rate Figure 2A (0.05±0.01 g/min, 0.59 ± 0.1 mg/kg/min) and contribution to TEE (13.4 ± 5.5%) (*p*-value<0.05) during exercise than at baseline, but these did not change after PLA treatment Figure 3. The fat oxidation rate and contribution to TEE were still lower than the carbohydrate oxidation rate and contribution to TEE (fat [0.05±0.01 g/min, 0.6 ± 0.1 mg/kg/min] vs carbohydrate [0.64±0.3 g/min, 11.2 ± 7.8 mg/kg/min] and fat [13.4 ± 5.5%] vs carbohydrate [86.6 ± 5.5%]) (all were *p*-value<0.05) Figure 2 and Figure 3.

Pre-treatment, during a single bout of low-intensity exercise, the carbohydrate oxidation rate Figure 2B. and contribution to TEE were significantly higher compared to their rates at rest for PLA (0.64±0.3 g/min, 11.3 ± 6.9 mg/kg/min, 93.8±3%) and vitamin C treatments (0.65±0.3 g/min, 10.7 ± 6.1 mg/kg/min, 87.6 ± 4.3%) (all were *p*-value<0.05) Figure 2B and Figure 3. Similarly, post-treatment, carbohydrate oxidation rate and contribution to TEE were significantly higher compared to their rates at rest for PLA (during exercise, 0.56±0.2 g/min, 9.9 ± 6.6 mg/kg/min, 93.8±3%) and vitamin C (during exercise, 0.64±0.3 g/min, 11.2 ± 7.8 mg/kg/min, 87.6 ± 4.3 %) treatments (all were *p*-value < 0.05) Figure 2B and Figure 3. However, there were no significant differences in the carbohydrate oxidation rate and contribution to TEE during exercise between the treatment arms.

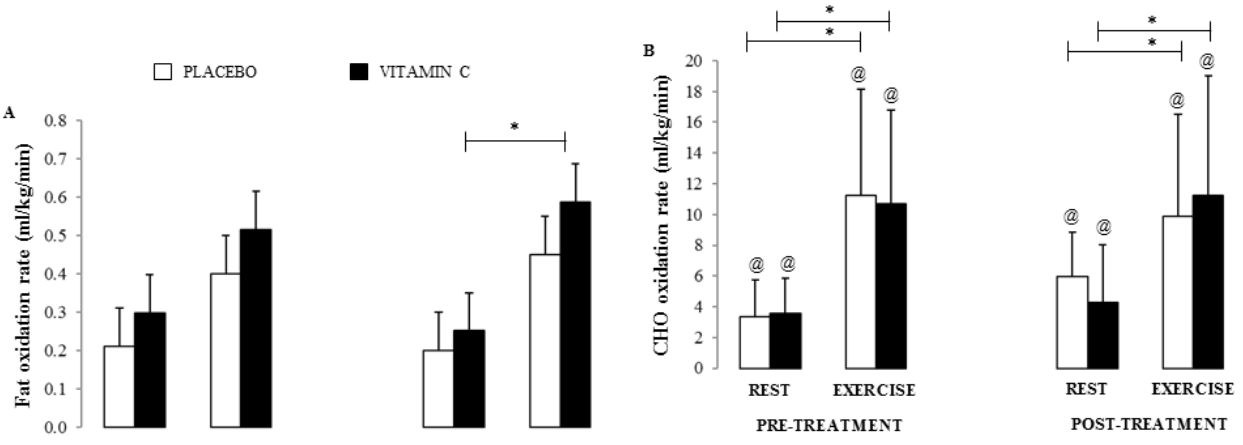

Figure 2 Substrate oxidation rate at rest and during low-intensity exercise pre- and post-treatment. (A) Fat oxidation rate (ml/kg/min). (B) Carbohydrate oxidation rate (ml/kg/min). Data are expressed as mean ± SD. (n=20; 16 females, 4 males). *Significantly different from rest (baseline) in the same treatment arm (p-value < 0.05), @Significantly different from fat oxidation rate in the same treatment arm (p-value<0.05).

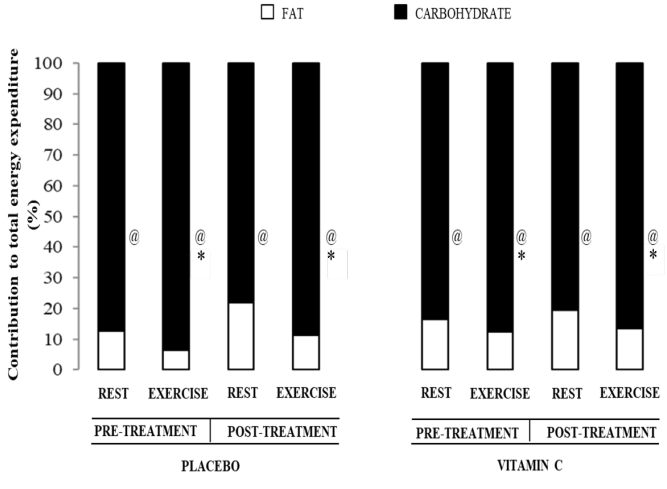

Figure 3 Contribution of substrate to total energy expenditure (%) at rest and during low-intensity exercise, pre- and post-treatment. Data are expressed as mean (n=20; 16 females, 4 males). *Significantly different from rest (baseline) in the same treatment arm (p-value<0.05), @Significantly different from fat oxidation rate in the same treatment arm (p-value<0.05).

Effect of vitamin C on circulating substrate and insulin concentrations

At rest

Cholesterol concentrations were significantly lower in post-treatment concentration compared to the PLA arm (-12.6%, p-value<0.05) (Table 3).

Post-vitamin C treatment, the TC/HDL ratio significantly lower (-14%, p-value<0.05) compared to the pre-vitamin C treatment concentration with no change in PLA treatment (Table 3).

On the contrary, post-vitamin C treatment, blood glucose concentrations significantly decreased at rest (-17.5%, *p*-value<0.05), whereas the blood glucose concentrations did not change after receiving PLA treatment (Table 3). However, after 6 weeks of vitamin C treatment, there were no changes in other plasma lipid concentrations, insulin resistance, or beta cell function at rest in either arm (Table 3).

After exercise

In both pre- and post-vitamin C treatments, fasting blood glucose concentrations after exercise were significantly lower compared to at baseline (-5.8%, pre-treatment; -10.5%, post-treatment; *p*-value<0.05), whereas the blood glucose concentrations did not change after receiving PLA treatment (Table 3). However, post-treatment, there were no changes in plasma lipid concentrations, insulin concentrations, or beta cell function immediately after exercise in either group (Table 3).

Table 3 Blood chemistry parameters at rest and during low-intensity exercise before and after treatment of T2DM patients

	Placebo				Vitamin C			
	Pre		Post		Pre		Post	
	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
Blood glucose (mg/dL)	193.8±3	184.2±3	190.5±4	179.9±3	226.6±5	213.3±4*	186.9±2$^{\$}$	167.1±2$^{* \$}$
Cholesterol (mg/dL)	197.8±2.3	201.3±2.2	205.4±1.8	206.3±2.0	207.1±2.3	205.2.2±2	181.0±8.3$^{\$ \#}$	203.4±1.6
TG (mg/dL)	183.0±5.4	177.8±5.0	169.5±4.2	162.5±3.6	162±2.8	163±2.9	177.43±3.8	172.11±3.7
HDL (mg/dL)	44.5±0.4	45.8±0.4	44.6±0.5	45.3±0.4	43.7±0.47	45.4±0.47	46.6±0.5	46.7±0.6
LDL (mg/dL)	128.1±2.2	131.3±2.1	136.3±1.8	140.4±2.0	136.2.1±4	133.9±2.3	127.2±1.2	130.1±1.4
LDL/HDL ratio	2.9±0.2	2.9±0.2	3.2±0.2	3.2±0.3	3.2±0.3	3.1±0.3	3.0±0.3	3.0±0.3
TC/HDL ratio	4.5±0.3	4.5±0.3	4.8±0.3	4.7±0.3	4.9±0.3	4.7±0.3	4.2±0.4$^{\$}$	4.7±0.3
Serum insulin (μIU/mL)	13.8±0.2	18.3±0.8	17.1±0.4	17.2±0.4	14.1±0.3	14.6±0.4	15.8±0.3	14.0±0.2
HOMA-IR	6.59±0.87	-	8.16±1.35	-	8.05±1.2	-	7.34±1.01	-
HOMA-β	47.34±7.8	-	63.84±10.4	-	42.5±6.6	-	48.14±4.9	-
EE (kcal/min)	0.85±0.5	2.88±1**	0.97±0.6	2.54±0.8**	0.99±0.6	2.82±1.5**	1.04±0.8	2.75±1.6**
VO2 (L/min)	0.16±0.1	0.50±0.1**	0.19±0.1	0.47±0.1**	0.16±0.1	0.44±0.1**	0.18±0.1	0.47±0.1**

Note: Values are mean ± SE, n = 20 (16 females, 4 males). Pre-, pre-treatment; Post-, post- treatment; TG, Triglycerides; HDL, high density lipoprotein; LDL, Low density lipoprotein HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-β, homeostatic model assessment of β-cell function; EE, energy expenditure; VO_2, oxygen consumption. ***** Significantly different from rest in the same treatment arm (*p*-value<0.05), ****** Significantly different from rest (baseline) in the same treatment arm (*p*-value<0.001), $^{\$}$ Significantly different from before treatment in the same treatment arm (*p*-value<0.05), $^{\#}$ Significantly different from PLA treatment arm in the same condition (*p*-value<0.05).

Effect of vitamin C on $\dot{V}O_2$ and energy expenditure

At rest

There were no significant differences in the values of $\dot{V}O_2$ and EE at rest pre- and post-treatment in both arms (Table 3).

During exercise

At pre- and post-treatment in both PLA and vitamin C arms, $\dot{V}O_2$ and EE during low-intensity exercise were significantly higher than at rest (pre-treatment [PLA, 150%; vitamin C, 100%], post-treatment [PLA, 150%; vitamin C, 150%], all *p*-value 0.001) without any differences between arms (Table 3).

Discussion

To the best of our knowledge, this study is the first showing that the oral vitamin C treatment at 1000 mg/day for 6 weeks decreased plasma TC at rest in patients with poorly controlled T2DM. Moreover, we found that post-vitamin C treatment showed increased fat oxidation rate during low-intensity exercise, decreased resting plasma TC/HDL-C, and decreased plasma glucose concentration at rest and immediately after the exercise. Furthermore, pre- and post-treatment, rates of oxygen consumption and carbohydrate oxidation, and EE were increased from baseline for both treatment arms. Nonetheless, any significant effects of vitamin C treatment on other metabolic variables at both conditions were not observed.

We primarily hypothesized that oral 1000-mg vitamin C for 6 weeks can improve fat oxidation rate and subsequently improved other metabolic parameters at rest and during a single bout of low-intensity exercise in patients with T2DM. However, the hypocholesterolemic effect at rest is the only outcome that supports our hypothesis. This effect was supported by the previous studies in patients with T2DM using 1000–2000 mg of vitamin C[13,19,20], but other studies using 50–500 mg of vitamin C[13,21] did not reveal a similar effect. The high dose in the former may contribute to the hypocholesterolemic effect. However, Afkhami-Ardekani and Shojaoddiny-Ardekani did not observe the hypocholesterolemic effect following the patients' oral intake of 1000 mg of vitamin C for 6 weeks, which is possibly due to the difference in the research design used. Afkhami-Ardekani and Shojaoddiny-Ardekani used a double-blind, placebo-controlled, noncrossover design, which may have been influenced by the confounding covariates and has less statistical efficiency than the crossover design. This non-crossover design may weaken the result.

Regarding the important lipid indicators, that is, TC and TC/HDL-C ratio, which are positively associated with cardiovascular disease [22,23], the 12.6% reduction in TC concentration after vitamin C treatment shown in this study is potentially significant in reducing cardiovascular risk[22]. Regarding the report of Law et al. (1994), the reductions of 26.1 mg/dL cholesterol in this study can reduce the incidence of ischemic heart disease by 60.8% at the age of 40 years, and reducing to 21.4% at 80 years[24]. The hypocholesterolemic effects can be explained by both direct and indirect mechanisms. These include increased cholesterol absorption, increased bile acid synthesis[25] (by activating the cytochrome P450-dependent enzyme cholesterol-7-apha-hydroxylase)[25], decreased hepatic lipoprotein secretion, and increased apolipoprotein B or E receptor activity and cholesterol content; these actions are associated with the increased clearance of cholesterol from the blood.

It is noted that after vitamin C treatment, the hypocholesterolemic effect was not observed when the patients performed the exercise. The reason why TC increased after the exercise remains unknown. No study explored the effect of low-intensity exercise on TC. Although there are previous studies found exercise could increase TC it is strenuous exercise[26,27]. However, even low physical activity such as a change in posture from lying to standing can increase TC concentration[28]. In response to the standing up, total plasma TC concentrations have been shown to be related to plasma noradrenaline concentrations[26]. The standing up is known to increase sympathetic nervous system activity[26]. Thus, the change in sympathetic nervous system activity may influence short-term changes in plasma total cholesterol levels, possibly due to haemodynamic changes.

Therefore, low-intensity exercise in this study which is higher activity than standing up, is likely to increase total plasma cholesterol concentrations. This result may attenuate the hypercholesteremic effect after the exercise in this study.

The increased fat oxidation rate during exercise in the vitamin C treatment arm in this study may possibly be due to the increased plasma ascorbic acid due to vitamin C intake (Supplementary Figure 1). Vitamin C is a cofactor of two enzymes in carnitine biosynthesis, namely, ε-N-trimethyl-L-lysine hydroxylase and γ-butyrobetaine hydroxylase[9,10]. The increased vitamin C intake may thus increase the levels of carnitine, leading to increased carnitine palmitoyltransferase, which transports fatty acids into the mitochondrial matrix and increases fat oxidation in the skeletal muscle. However, either the dose or duration of vitamin C treatment in this study may not be sufficient to cause the significant difference in all variables between the arms, except the resting plasma cholesterol and ascorbic acid (unpublished data). Further studies assessing either the higher dose or longer duration of oral vitamin C treatment may provide significant data. Additionally, the higher oxygen consumption, carbohydrate oxidation rates, and EE pre- and post-treatment during low-intensity exercise compared with baseline (at rest) in both treatment arms are not surprised. This finding is consistent with the study by Ghanassia and colleagues (2006) who showed that the lipid oxidation rate during exercise in patients with T2DM shifted toward carbohydrates as the predominant source [3]. Furthermore, in Thai subjects, the Thai diet, which has carbohydrates as the main component, is considered another factor that increases the carbohydrate oxidation rate at rest and during exercise in this study. This has been shown in observations made previously in non-diabetes Thai subjects[29]. Additionally, we have shown that low-intensity exercise increased oxygen consumption, resulting in increased EE. This result is considered beneficial when planning for weight reduction strategies. Thus, the data provide a beneficial and sufficient knowledge regarding exercise training for patients with T2DM.

Three groups of drugs were used in this study: hypoglycemic, antihypertensive, and lipid-lowering drugs. All of them improve blood glucose, insulin, and lipid concentrations and BP via their mechanisms of action. However, all patients in our study maintained their medication programs, including their medications' doses, types, and frequencies, suggesting that the drugs did not affect our results. Besides, we are aware of a potential food-drug interaction between oral vitamin C and hypoglycemic and anti-dyslipidemic drugs. Fortunately, there has been no report on the interaction in this study. Therefore, food-drug interactions may not affect our results.

This study has several limitations. First, glucose and free fatty acid kinetics were not measured, such as by muscle biopsies and stable isotope tracers. Therefore, a further study investigating both kinetics will disclose important pathophysiological effect of oral vitamin C in patients with T2DM. Second, we investigated Thai patients[17] who have different substrate utilization patterns from the western population[3]. Thus, generalizing our results with other populations is not possible. Third, our study was conducted for 6 weeks only. Finally, this study has a female-to-male ratio of 4:1. Since sex had an effect on substrate utilization[30], we cannot conclude our results to male patients.

Conclusion

The findings suggest that a daily treatment with 1000 mg vitamin C for 6 weeks decreased the resting plasma cholesterol concentrations in patients with poorly controlled T2DM. However, this effect was not observed during exercise. The mechanisms attributed to the hypocholesterolemic effects of vitamin C in patients with T2DM remain to be determined. Vitamin C treatment has no effect on substrate oxidation rate and other circulating substrates at rest and during exercise. Additionally, pre- and post-treatment, oxygen consumption, carbohydrate oxidation rate, and EE were higher during exercise than at rest without any effect from oral vitamin C treatment.

The hypocholesterolemic change in patients with poor controlled T2DM in this study seems to be alternative beneficial effect of the oral vitamin C on reducing the risk of cardiovascular complications. Elevated cholesterol is a strong risk factor for cardiovascular disease.

Take home messages

- Daily treatment with 1000 mg of vitamin C for six weeks reduced the resting cholesterol concentration in T2DM patients with poor control.
- Post-vitamin C treatment, fat utilization during exercise was higher than baseline.
- Low-intensity exercise increased carbohydrate oxidation rate in T2DM patients.

Conflicts of interest

The authors declare no conflict of interest.

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References

1. Bommer C, Sagalova V, Heesemann E, Manne-Goehler J, Atun R, Barnighausen T, et al. Global Economic Burden of Diabetes in Adults: Projections From 2015 to 2030. Diabetes Care 2018; 41: 963-70.
2. Sanguanwong S, Tangvarasittichai O, Sengsuk C, Tangvarasittichai S. Oral Supplementation of Vitamin C Reduced Lipid Peroxidation and Insulin Resistance in Patients with Type 2 Diabetes Mellitus. Int J Toxicol Pharmacol Res 2016; 8: 114-9.
3. Ghanassia E, Brun JF, Fedou C, Raynaud E, Mercier J. Substrate oxidation during exercise: type 2 diabetes is associated with a decrease in lipid oxidation and an earlier shift towards carbohydrate utilization. Diabetes Metab 2006; 32: 604-10.
4. Battisti WP, Palmisano J, Keane WE. Dyslipidemia in patients with type 2 diabetes. relationships between lipids, kidney disease and cardiovascular disease. Clin Chem Lab Med 2003; 41: 1174-81.
5. Kulkarni SS, Salehzadeh F, Fritz T, Zierath JR, Krook A, Osler ME. Mitochondrial regulators of fatty acid metabolism reflect metabolic dysfunction in type 2 diabetes mellitus. Metabolism 2012; 61: 175-85.
6. Taskinen MR, Bogardus C, Kennedy A, Howard BV. Multiple disturbances of free fatty acid metabolism in noninsulin-dependent diabetes. Effect of oral hypoglycemic therapy. J Clin Invest 1985; 76: 637-44.
7. Suk MH, Moon YJ, Park SW, Park CY, Shin YA. Maximal Fat Oxidation Rate during Exercise in Korean Women with Type 2 Diabetes Mellitus. Diabetes Metab J 2015; 39: 328-34.
8. Wahl MP, Scalzo RL, Regensteiner JG, Reusch JEB. Mechanisms of Aerobic Exercise Impairment in Diabetes: A Narrative Review. Front Endocrinol (Lausanne) 2018; 9(181).
9. Hulse JD, Ellis SR, Henderson LM. Carnitine biosynthesis. beta-Hydroxylation of trimethyllysine by an alpha-ketoglutarate-dependent mitochondrial dioxygenase. J Biol Chem 1978; 253: 1654-9.
10. Rebouche CJ, Paulson DJ. Carnitine metabolism and function in humans. Annu Rev Nutr 1986; 6: 41-66.
11. Daniele G, Xiong J, Solis-Herrera C, Merovci A, Eldor R, Tripathy D, et al. Dapagliflozin Enhances Fat Oxidation and Ketone Production in Patients With Type 2 Diabetes. Diabetes Care 2016; 39: 2036-41.

12. Dakhale GN, Chaudhari HV, Shrivastava M. Supplementation of vitamin C reduces blood glucose and improves glycosylated hemoglobin in type 2 diabetes mellitus: a randomized, double-blind study. Adv Pharmacol Sci 2011; 2011:1-5.
13. Afkhami-Ardekani M, Shojaoddiny-Ardekani A. Effect of vitamin C on blood glucose, serum lipids & serum insulin in type 2 diabetes patients. Indian J Med Res 2007; 126: 471-4.
14. Odumosu A, Wilson CW. Hypocholesterolemic effects of vitamin C, clofibrate and diosgenin in male guinea-pigs [proceedings]. Br J Pharmacol 1979; 67: 456P-7P.
15. Ginter E, Zdichynec B, Holzerova O, Ticha E, Kobza R, Koziakova M, et al. Hypocholesterolemic effect of ascorbic acid in maturity-onset diabetes mellitus. Int J Vitam Nutr Res 1978; 48: 368-73.
16. Glycemic Management in Type 2 Diabetes, Available at: https://www.aace.com/disease-state-resources/diabetes/depth-information/glycemic-management-type-2-diabetes. (Accessed 5 December 2019), 2019.
17. Abramson JH. WINPEPI updated: computer programs for epidemiologists, and their teaching potential. Epidemiol Perspect Innov 2011; 8: 1.
18. Peronnet F, Massicotte D. Table of nonprotein respiratory quotient: an update. Can J Sport Sci 1991; 16: 23-9.
19. Eriksson J, Kohvakka A. Magnesium and ascorbic acid supplementation in diabetes mellitus. Ann Nutr Metab 1995; 39: 217-23.
20. Paolisso G, Balbi V, Volpe C, Varricchio G, Gambardella A, Saccomanno F, et al. Metabolic benefits deriving from chronic vitamin C supplementation in aged non-insulin dependent diabetics. J Am Coll Nutr 1995; 14: 387-92.
21. Kim MK, Sasaki S, Sasazuki S, Okubo S, Hayashi M, Tsugane S. Long-term vitamin C supplementation has no markedly favourable effect on serum lipids in middle-aged Japanese subjects. Br J Nutr 2004; 91: 81-90.
22. Virani SS, Wang D, Woodard LD, Chitwood SS, Landrum CR, Zieve FJ, et al. Non-high-density lipoprotein cholesterol reporting and goal attainment in primary care. J Clin Lipidol 2012; 6: 545-52.
23. Lloyd-Jones DM, Wilson PW, Larson MG, Beiser A, Leip EP, D'Agostino RB, et al. Framingham risk score and prediction of lifetime risk for coronary heart disease. Am J Cardiol 2004; 94: 20-4.
24. Law MR, Wald NJ, Thompson SG. By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischaemic heart disease? BMJ 1994; 308: 367-72.
25. Simon JA. Vitamin C and cardiovascular disease: a review. J Am Coll Nutr 1992; 11: 107-25.
26. Krum H, Conway EL, Howes LG. Acute effects of exercise on plasma lipids, noradrenaline levels and plasma volume. Clin Exp Pharmacol Physiol 1991; 18: 697-701.
27. Hagan RD, Upton SJ, Avakian EV, Grundy S. Increases in serum lipid and lipoprotein levels with movement from the supine to standing position in adult men and women. Prev Med 1986; 15: 18-27.
28. Hegsted DM, Nicolosi RJ. Individual variation in serum cholesterol levels. Proc Natl Acad Sci U S A 1987; 84: 6259-61.
29. Janyacharoen T, Auvichayapat P, Tsintzas K, Macdonald IA, Leelayuwat N. Effect of gender on fuel utilization during exercise at different intensities in untrained Thai individuals. Eur J Appl Physiol. 2009; 107: 645.
30. Tarnopolsky MA. Gender Differences in Substrate Metabolism During Endurance Exercise. Can J Appl Physiol 2000; 25: 312-27.

Supplementary

Table 1 Dietary composition, total EI and EE before and after treatment of T2D patients

	Placebo		Vitamin C	
	Before	**After**	**Before**	**After**
CHO (g/day)	241.0±13	261.4±13	228.1±13	210.7±23
Protein (g/day)	86.8±4	95.4±9	73.4±2	67.9±5
Fat (g/day)	55.2±4	62.3±2	78.1±2	79.3±5
Vitamin A (mg/day)	259.6±20	221.8±10	545.4±53	278.6±23
Vitamin C (mg/day)	341.3±21	407.9±18	235.9±17	308.2±20
Vitamin E (mg/day)	17.8±1.2	16.9±7.6	15.7±1.2	16.7±0.9
Dietary fiber (g/day)	188.8±8.2	193.6±6.3	184.5±12	135.2±8
Total EI (kcal/day)	1,808±47	1,988±64	1,909±68	1,828±82
Total EE (kcal/day)	1,492±38	1,500±33	1,495±38	1,490±38

Note: Values are means ± SE, n = 20 (14 females, 4 males). EI, Energy intake; EE, Energy expenditure; T2D, Type 2 diabetes mellitus; CHO, Carbohydrate.

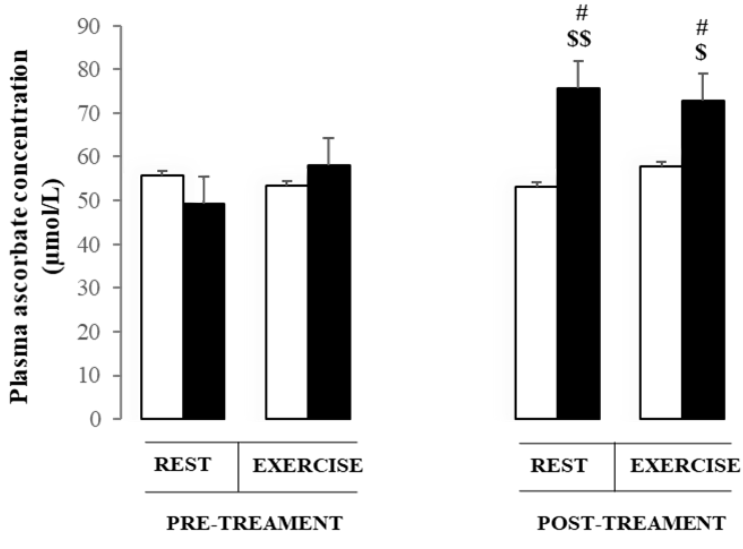

Figure 1 Plasma ascorbate concentration at rest and immediately after exercise pre- and post-treatment. Data are expressed as mean ± SD (n=20; 16 females, 4 males). $^{\$}$Significantly different from before treatment in the same treatment arm (p-value<0.05), $^{\$\$}$Significantly different from before treatment in the same treatment arm (p-value<0.01), $^{\#}$Significantly different from PLA treatment arm in the same condition (p-value<0.05).