



ORIGINAL ARTICLE

Effect of CoQ₁₀ Supplementation on Oxidative Stress and Muscle Bioenergetics in Type II Diabetes: A Pilot Study

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Abstract

Objectives: To compare oxidative stress, mitochondrial oxidative phosphorylation capacity, microvascular function, and exercise performance of type II diabetics (T2D) to that of control subjects and to determine whether Co-enzyme Q₁₀ (CoQ₁₀) supplementation has any effects on these measures.

Methods: Fourteen older adults (7 T2D patients with mild peripheral arterial disease (PAD) (69.9 ± 5.1 years) and 7 age-matched controls (67.0 ± 8.9 years) participated in the study. Each participant went through 2 week placebo followed by 2 week CoQ₁₀ supplementation trials. During each trial, participants performed plantar flexion exercise during which gastrocnemius phosphocreatine (PCr), pH, oxygen (O₂) saturation and BFindex were measured using ³¹P magnetic resonance spectroscopy (MRS) and near infrared spectroscopy (NIRS). **Results:** Comparing T2D to controls, significant differences were observed in PCr_{re} (22.3 ± 13.4 vs. 49.4 ± 36.6 mM·kg⁻¹·min⁻¹) and BFindex (5.3 ± 2.9 vs. 9.7 ± 2.9%·s⁻¹) (p = 0.03). Moreover, O₂ saturation recovery was significantly longer for T2D (70.9 ± 52.1 vs. 31.8 ± 8.4 s; p < 0.01). When comparing placebo trial to CoQ₁₀ trial, significant reduction in Malondialdehyde (MDA), after CoQ₁₀ trial, was observed in controls (1.29 ± 0.41 vs. 0.88 ± 0.35 μM, p < 0.05) but not in T2D (1.31 ± 0.19 vs. 1.47 ± 0.36 μM, p > 0.05). With the CoQ₁₀ trial, a trend of improved PCr/Pi in T2D patients at rest was seen (6.87 ± 1.2 to 8.59 ± 2.7). However, no other significant changes were observed with CoQ₁₀ supplementation for either group, including pH, PCr recovery (T2D: 22.3 ± 13.4 vs. 23.2 ± 11.4 mM·kg⁻¹·min⁻¹; CON: 49.4 ± 36.6 vs. 43.7 ± 33.3 mM·kg⁻¹·min⁻¹, p = 0.9), and BFindex (T2D: 5.3 ± 2.9 vs. 3.9 ± 2.2%·s⁻¹; CON: 9.7 ± 2.9 vs. 9.2 ± 2.4%·s⁻¹, p = 0.5).

Conclusion: Noted reduction in MDA seen in healthy, older individuals suggests a positive antioxidant effect of CoQ₁₀ supplementation. However, when PAD is present, as can be the case of T2D, two weeks of CoQ₁₀ supplementation at 200 mg·day⁻¹ may not be long enough or potent enough to produce similar effects.

Keywords

CoQ₁₀, Diabetes, Antioxidant, Metabolic function, Hemodynamics, Exercise

Abbreviations

ΔBV: Blood Volume Change; ABI: Ankle Brachial Index; AOX: Antioxidant; ATP: Adenosine Triphosphate; BFindex: Microvascular blood flow; BMI: Body Mass Index; CoQ10: Coenzyme Q10; ETC: Electron Transport Chain; H+: Proton; Hb/Mb-oxy: Hemoglobin/Myoglobin-O₂ Saturation; ipH: Intracellular pH; MDA: Malondialdehyde; NIRS: Near Infrared Spectroscopy; NO: Nitric Oxide; NOS: Nitric Oxide Synthase; MRS: Magnetic Resonance Spectroscopy; PAD: Peripheral Arterial Disease; PCr: Phosphocreatine; PCrre: PCr Resynthesis Rate; RM: Repetition Maximum; ROS: Reactive Oxygen Species; SSI: Steady State Incremental Exercise; T2D: Type 2 Diabetes; T50NIR: Half-time to Recovery; VO: Venous Occlusion

Introduction

In the United States, 30.3 million people (9.4% of the population) have diabetes, most of whom suffer from type 2 diabetes (T2D) [1]. Hyperglycemia in T2D may alter glucose metabolic pathways leading to formation of unfavorable by-products such as reactive oxygen species (ROS) [2]. Elevated ROS and its subsequent oxidati-

ve stress, due to hyperglycemia in T2D, have been proposed as a major contributing factor leading to diabetic complications [3-5] including vascular dysfunction [6-8]. Peripheral Arterial Disease (PAD) is a common finding in T2D [9] in which blood flow and subsequent O₂ supply to working muscles are impaired thereby limiting functional capacity [10,11]. Moreover, T2D associated with PAD, has been shown to increase mortality rate in this population [12].

Another factor contributing to impaired functional capacity in T2D is mitochondrial dysfunction. Studies have reported decreased oxidative phosphorylation activity [13,14], as well as mitochondrial DNA damage [15], in T2D. Moreover, defects in the capacity to metabolize glucose and fatty acids results in accumulation of fatty acids and triacylglycerol in non-adipose tissues, including skeletal muscle [16]. These accumulating fatty acids are prone to lipid peroxidation increasing ROS, a process thought to be one of the major causes of mitochondrial damage [17].

While hyperglycemia in T2D increase ROS production, it can also lead to glycation of antioxidant (AOX) enzymes thereby reducing AOX availability [18,19]. Thus, hyperglycemia upregulates ROS formation while down-regulating AOX formation leading to an even higher level of oxidative stress. For example, mitochondrial reducing equivalents, such as co-enzyme Q₁₀ (CoQ₁₀), have been found to be deficient in T2D [20,21]. This suggests that a quantitative and/or functional deficiency in CoQ₁₀ may potentially occur in T2D, further diminishing metabolic efficiency and functional capacity.

CoQ₁₀ is a potent antioxidant [22,23] and a component of the ETC which has been shown to improve mitochondrial [24,25] and vascular function [26,27]. Furthermore, CoQ₁₀ has shown to reduce fasting plasma glucose in diabetes [23]. Thus, supplemental CoQ₁₀ has the potential to improve functional capacity of T2D and possibly reduce the progression of disease. In the present study, we examined and compared these parameters of T2D patients to that of control subjects and how short-term dietary CoQ₁₀ supplementation may alter these measures. We hypothesized that; 1) T2D patients would exhibit impaired mitochondrial and microvascular functions, and exercise performance when compared to age-matched control subjects and 2) T2D patients would show significant improvements in these measures after CoQ₁₀ supplementation.

Methods

Subjects

Fourteen older individuals were recruited to participate in the study (7 T2D patients with a mild case of PAD (Fontain class IIa) (Age: 69.9 ± 5.1 yrs) and 7 age, gender and activity matched non-diabetic individuals (Age: 67.0 ± 8.9 yrs). All subjects were non-smokers and en-

gaged in no more than one planned physical activity per week. All T2D patients were previously diagnosed with T2D (fasting glucose ≥ 126 mg/dL) and in good glycemic control. The study protocol was approved by the Institutional Review Board at the University of Pennsylvania (IRB#: 803931). Informed consent was obtained from each subject before enrollment of the study.

Prior to the study, each subject's laboratory and physical examination information, including vital signs, height, weight, diet, activity habits, medication, ankle brachial index (ABI), and blood work were obtained. Once enrolled in the study, the same measures and questionnaire were obtained during each visit to monitor and record any changes during the course of the study. All study subjects were encouraged to stay on the same diet, medication, and activity level throughout the study.

Experimental procedure

This study used a non-randomized, single blinded, cross-over design, where all T2D patients and control subjects received 2 weeks of placebo treatment first, followed by 2 weeks of CoQ₁₀ treatment at 200 mg·day⁻¹ (100 mg capsule, twice a day). The treatment order was not randomized due to a long wash out period of CoQ₁₀. An oral, over-the-counter CoQ₁₀ dietary supplement was used (Swanson Health Products, Inc., Fargo, ND). To account for a possible order effect, an initial baseline trial was conducted before the placebo trial.

Each subject was asked to report three times; 1) Baseline (visit 1); 2) 2 weeks post placebo (visit 2); and 3) 2 weeks post CoQ₁₀ supplement (visit 3). Identical exercise testing was performed during each visit. Blood samples were drawn from the antecubital vein before and after exercise in all trials to measure changes in Malondialdehyde (MDA) level to estimate oxidative stress status.

Exercise protocol

All exercise protocols were performed in a 2 Tesla, 75 cm bore MRS magnet. Co-registration of ³¹P-MRS and NIRS (Cogniscope; NIM, Inc.) was performed to observe dynamic changes of phosphate (³¹P) metabolites, intracellular pH (ipH) and total hemoglobin/myoglobin-O₂ saturation (Hb/Mb-oxy) in the medial gastrocnemius at rest, during and after exercise. For exercise protocol, dynamic plantar flexion in supine position was performed, using workloads relative to pre-determined one repetition maximum (RM) (Figure 1). For T2D group, the leg that the patient was having the most pain was used throughout the study.

12 s Dynamic exercise: After obtaining resting measures, each subject performed a 12-s dynamic plantar flexion exercise (~ 2 contraction/s) at 40% of 1RM. All subjects stopped exercising after 12-s or when medial gastrocnemius muscle PCr level declined to 50% of the resting PCr, whichever came first [28]. From the MRS

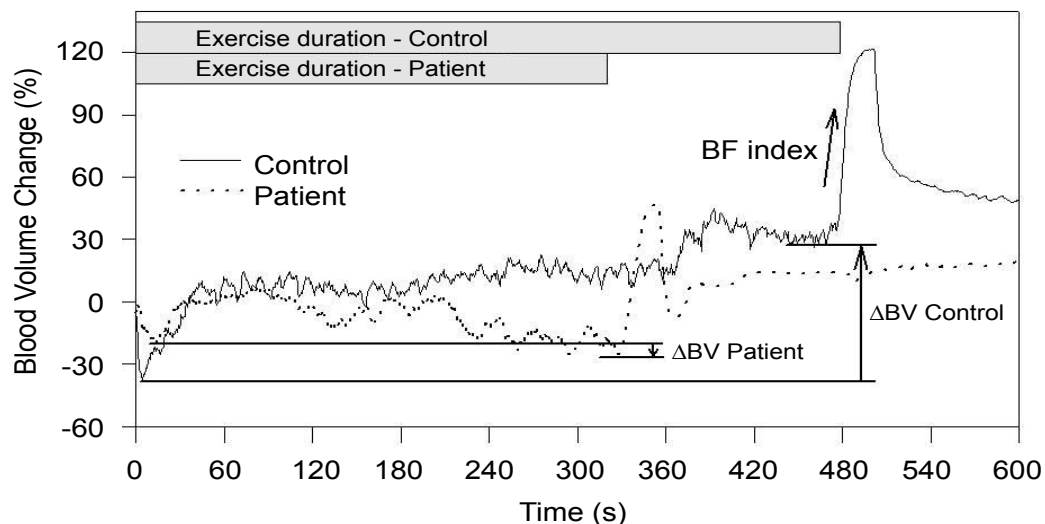


Figure 1: Typical changes in blood volume during SSI exercise in a control subject (solid line) and a T2D patient (dotted line).

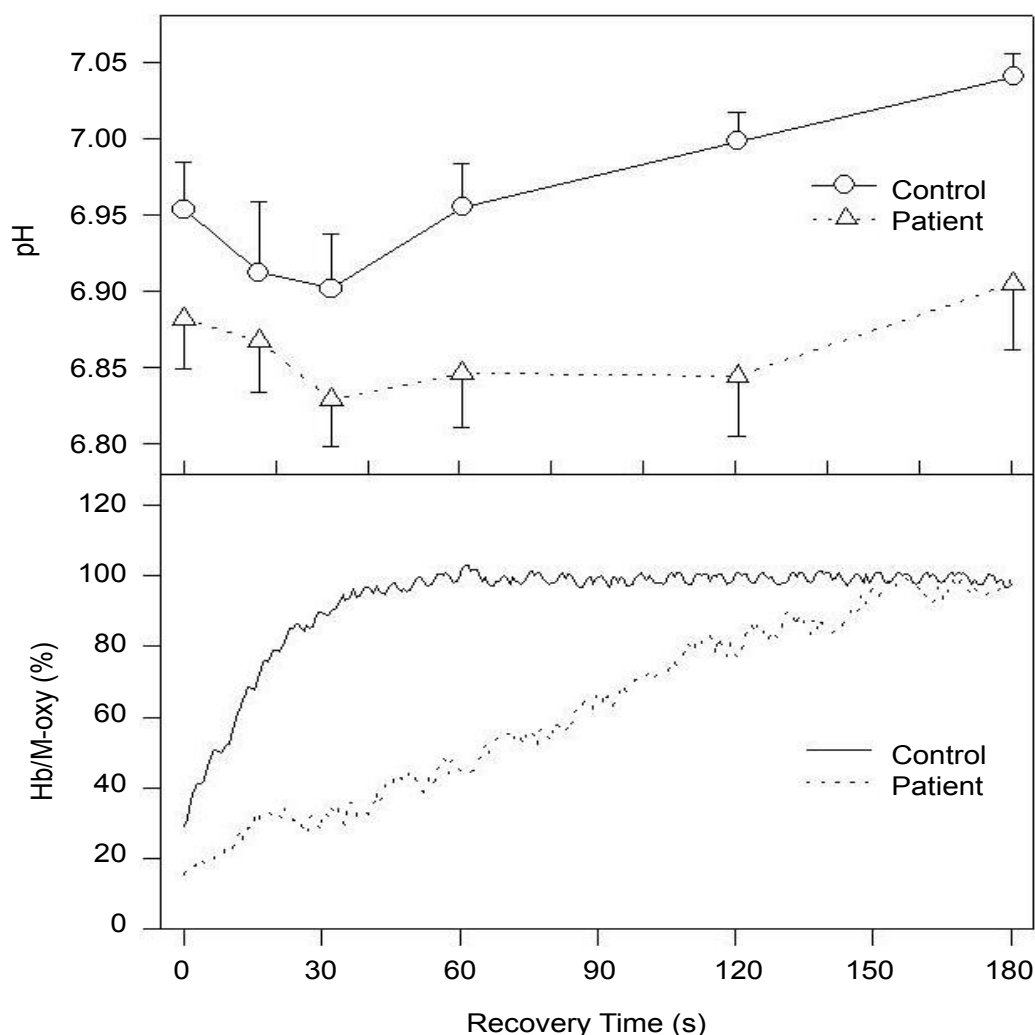


Figure 2: Typical changes in ipH (circle and triangle; top panel) and Hb/Mb-oxy (solid and dotted lines; bottom panel) after SSI exercise in a control subject (circle and solid line) and a T2D patient (triangle and dotted line).

spectra, post-exercise PCr, ipH, and PCr re-synthesis rate (PCr_{re}) were measured as a function of mitochondrial oxidative phosphorylation capacity. From NIR spectra, Hb/Mb-Oxy during exercise was measured as a function of O_2 utilization. Half-time to recovery (T_{50NIR}),

which is the time from cessation of exercise to 50% of resting Hb/Mb-oxy, was measured as a function of O_2 delivery. Recovery delay was measured from cessation of exercise to the start of Hb/Mb-oxy recovery.

Steady State Incremental Exercise (SSI): Each

subject also performed SSI by pushing down the pedal against a set resistance at a rate of 30 plantar flexions/min with a 1-s work/rest cycle (Figure 2). Starting workload was set at 30% of 1 RM and increased by 10% every two minutes. Exercise was stopped when PCr level declined to < 50% of the resting PCr. Exercise capacity was determined by the time it took for each subject to deplete 50% of resting PCr. From NIR spectra, blood volume change (ΔBV) during exercise and initial rate of total Hb change (ΔtHB_{rate}) immediately after exercise were used to determine BFindex. From MRS spectra, post-exercise PCr, ipH, and ipH recovery were measured.

Data analysis

All data were analyzed using a commercially available statistical software tool pack (Sigma Stat). A two-way ANOVA (Analysis of Variance) with placebo vs. CoQ₁₀ trials and T2D vs. control subjects as factors were conducted on all variables. Significance level (α) was set to 0.05. To examine relationships between MRS and NIRS metabolic measures, a pearson product-moment correlation was used.

Results

T2D patients vs. Control subjects

Resting response: T2D and control subjects' resting variables are summarized in Table 1. There were no significant differences in anthropometric, heart rate (HR) or blood pressure (BP) measures. T2D patients' ABI for both right and left were below 1 (range = 0.61 ~ 0.95) and were significantly lower than the control subjects ($p = 0.001$). T2D patients' resting ³¹P spectra revealed significantly smaller PCr ($p = 0.04$), PCr/Pi ($p = 0.01$), and larger Pi ($p = 0.04$) (Table 1).

Exercise response: End exercise PCr ($p = 0.745$), PCr depletion rate at the onset of exercise and end exercise ipH were not significantly different between T2D and control subjects for either 12 s ($p = 0.634$) or SSI ($p = 0.165$) exercise (Figure 3). ipH remained above 6.75 (range = 6.85 to 6.96) for all groups during all exercise tests, indicating that exercises were performed under relatively aerobic conditions as designed. 12-s end exercise Hb/Mb-oxy (%) was not significantly different in T2D when compared to control subjects (Table 1 and Figure 3) ($p = 0.547$). During SSI exercise,

Table 1: Metabolic and hemodynamic responses at rest, during and after exercise in T2D patients and control subjects.

	T2D (n = 8)	Control (n = 7)	P
Resting			
PCr (mM)	37.87 ± 3.24	41.09 ± 2.14	0.04
Pi (mM)	5.97 ± 0.99	4.78 ± 1.06	0.04
PCr/Pi	6.52 ± 1.30	8.93 ± 1.81	0.01
Cr (mM)	5.68 ± 0.49	6.16 ± 0.32	0.04
ADP (μM)	7.78 ± 0.29	7.67 ± 0.72	0.678
pH	7.02 ± 0.02	7.02 ± 0.05	0.880
Exercise & Recovery			
SSI Ex. Duration (s)	321.00 ± 116.77	420.00 ± 105.48	0.03
NIRS			
End Hb/Mb-oxy (%)	24.88 ± 6.91	30.39 ± 13.82	0.547
T ₅₀ (s)	77.35 ± 55.21	30.80 ± 8.60	0.05
Recovery Delay (s)	32.41 ± 16.53	14.18 ± 7.02	0.02
ΔBV (%)	-5.12 ± 55.87	65.90 ± 22.73	0.008
BFindex (%/s)	4.98 ± 2.17	10.11 ± 3.90	0.01
MRS			
End PCr (%)	46.31 ± 16.81	49.40 ± 17.60	0.745
dPCr/dt (%/s)	-4.00 ± 1.80	-3.73 ± 1.79	0.786
PCr _{re} (mM/kg/min)	22.31 ± 13.35	49.37 ± 36.62	0.015
Tc (s)	67.21 ± 69.51	35.59 ± 23.65	0.119
pH End ex. (12-s)	6.979 ± 0.047	7.006 ± 0.034	0.634
pH End ex. (SS)	6.893 ± 0.084	6.954 ± 0.083	0.165
pH 1 min post (12-s)	6.910 ± 0.043	7.007 ± 0.057	0.002
pH 1 min post (ss)	6.851 ± 0.086	6.955 ± 0.076	0.028

Note: PCr: Phosphocreatine; Pi: Inorganic phosphate; PCr/Pi: Ratio of PCr to Pi concentration; ipH: intracellular pH; T_{50NIR}: Time to 50% of Hemoglobin/Myoglobin-reoxygenation from cessation of exercise; ΔBV : Blood volume change, BFindex: Blood flow index; End PCr: End exercise PCr; dPCr/dt: Change in PCr over change in time; PCr_{re}: Phosphocreatine resynthesis rate; Tc: Time constant; 12-s: 12-s all out exercise; ss: steady state incremental exercise. Data are expressed in mean ± SD.

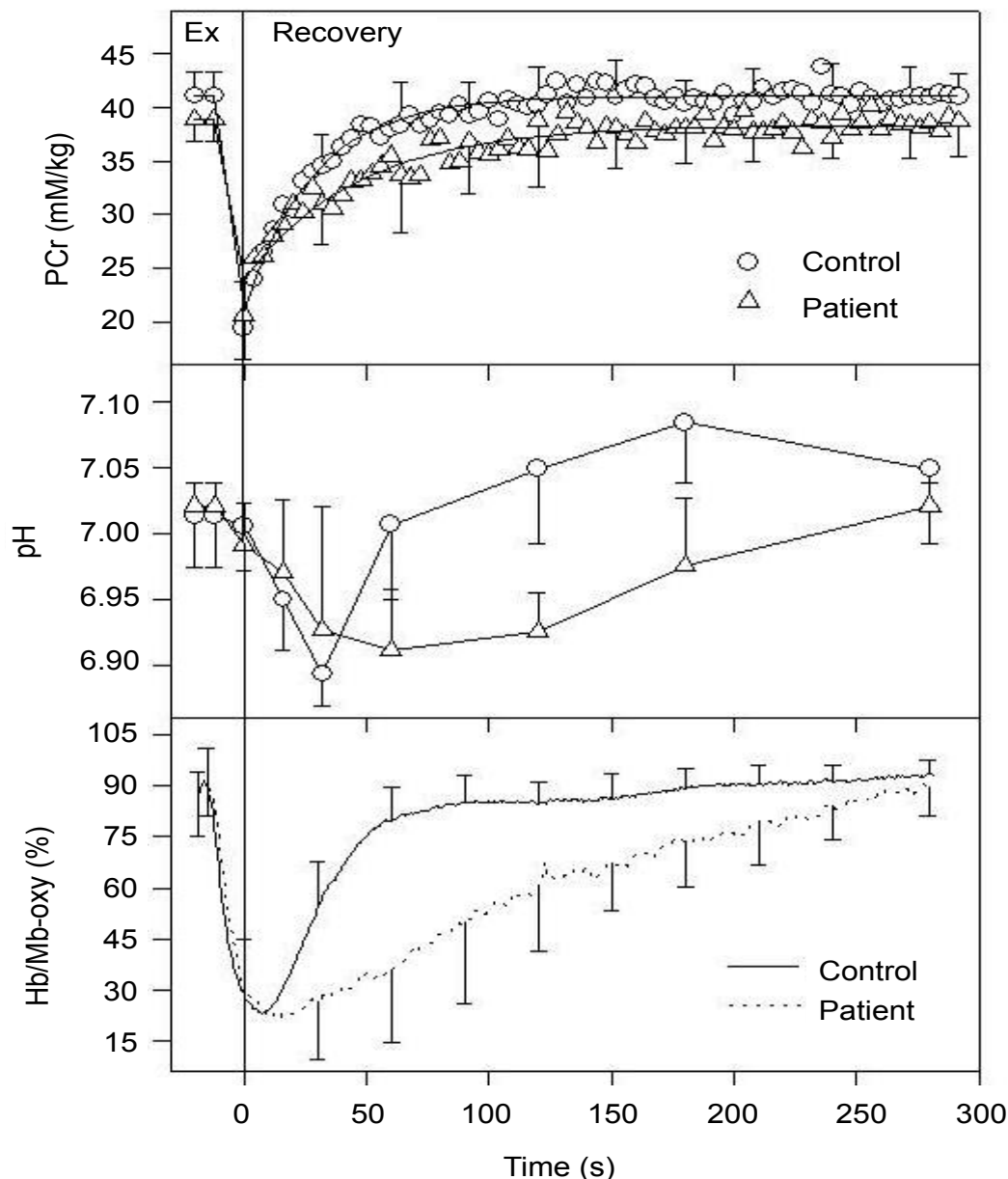


Figure 3: Changes in PCr (top panel), ipH (middle panel) and Hb/Mb-oxy (bottom panel) at rest, during and after 12 s exercise in control subjects (circle and solid line) and T2D patients (triangle and dotted line).

T2D reached 50% of resting PCr significantly earlier than control subjects resulting in shorter exercise duration ($p = 0.03$) (Table 1). A significantly larger increase of ΔBV was observed in control subjects whereas in T2D, after an initial increase, ΔBV either stayed the same or decreased ($p = 0.008$) (Figure 1).

Recovery response: PCr_{re} and Tc after 12-s exercise was significantly less in T2D when compared to control subjects ($p = 0.02$) (Figure 3 and Table 1). When PCr_{re} and Tc were correlated to NIR measures, strong and significant relationships were shown between PCr_{re} and T_{50NIR} ($r = -0.46$; $p = 0.01$), PCr_{re} and recovery delay ($r = -0.46$; $p = 0.01$), PCr_{re} and ΔBV ($r = 0.60$; $p < 0.001$). ipH recovery was also significantly slower in T2D for both 12-s ($p = 0.002$) and SSI ($p = 0.03$) exercises (Figure 2 and Figure 3). T_{50NIR} in O_2 saturation was significantly slower in T2D when compared to control subjects ($p = 0.05$) (Figure 2, Figure 3 and Table 1). Moreover, there

was a significant delay in HbO_2 recovery in T2D ($p = 0.02$) (Table 1).

Effect of CoQ₁₀

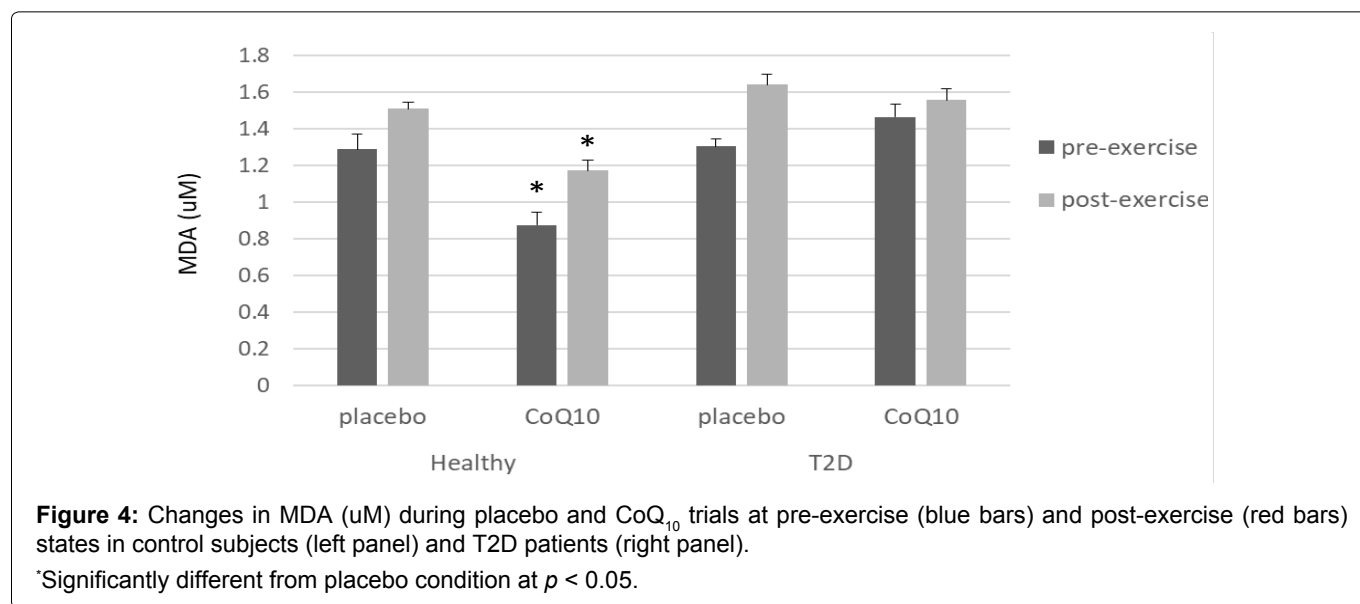
Resting response: A significant reduction in MDA, after CoQ₁₀ trial, was observed in control subjects (1.29 ± 0.41 vs. 0.88 ± 0.35 μM , $p < 0.05$) but not in T2D (1.31 ± 0.19 vs. 1.47 ± 0.36 μM , $p > 0.05$) (Figure 4). With CoQ₁₀ supplementation, reduced Pi and elevated PCr/Pi were seen in T2D to a point where significant differences seen, prior to CoQ₁₀, between the groups were diminished. However, these changes were not statistically significant ($p > 0.05$) (Table 2).

Exercise response: Similar to resting responses, post exercise MDA was significantly lower, after CoQ₁₀ trial, when compared to placebo trial in controls (1.17 ± 0.28 vs. 1.51 ± 0.17 μM , $p < 0.05$) but not in T2D (1.64 ± 0.29 vs. 1.56 ± 0.31 μM , $p > 0.05$)

Table 2: Metabolic and hemodynamic responses at rest, during and after exercise in T2D patients and control subjects during placebo and CoQ10 conditions.

Group	T2D (n = 7)		Control (n = 7)		P	P	P
Trial	Placebo	CoQ ₁₀	Placebo	CoQ ₁₀	Group	Tx	Inter
Resting							
PCr (mM)	39.12 ± 1.98	38.93 ± 3.66	41.09 ± 2.13	39.82 ± 1.76	0.459	0.637	0.358
Pi (mM)	5.84 ± 1.07	4.99 ± 1.75	4.78 ± 1.06	4.61 ± 0.95	0.237	0.518	0.695
ipH	7.02 ± 0.02	7.00 ± 0.02	7.01 ± 0.04	7.02 ± 0.01	0.329	0.319	0.218
Exercise							
SSI Ex. Duration (s)	312.8 ± 117.6	321.7 ± 135.5	408.9 ± 103.5	424.6 ± 88.3	0.030	0.720	1.00
NIRS							
T ₅₀	70.93 ± 52.13	69.10 ± 33.72	31.83 ± 8.36	32.43 ± 10.02	0.004	0.960	0.920
BFindex (%/s)	5.33 ± 2.88	3.99 ± 2.23	9.74 ± 2.92	9.22 ± 2.42	0.010	0.469	0.736
MRS							
End PCr (mM)	45.58 ± 17.24	42.13 ± 4.68	49.4 ± 17.60	51.76 ± 14.57	0.562	0.632	0.980
PCr _{re} (mM·kg·min ⁻¹)	22.31 ± 13.35	23.20 ± 11.39	49.37 ± 36.61	43.74 ± 33.28	0.034	0.905	0.960
Tc (s)	67.21 ± 69.51	53.61 ± 22.55	35.59 ± 23.65	32.54 ± 15.18	0.014	0.802	0.770
pH End ex. (12-s)	6.979 ± 0.074	6.96 ± 0.036	7.002 ± 0.036	7.004 ± 0.049	0.094	0.663	0.586
pH 1 min post ex. (12-s)	6.886 ± 0.064	6.915 ± 0.063	6.972 ± 0.038	6.988 ± 0.059	0.001	0.311	0.793

Note: PCr: Phosphocreatine; Pi: Inorganic phosphate; ipH: intracellular pH; T_{50NIRS}: Time to 50% of Hemoglobin/Myoglobin-reoxygenation from cessation of exercise; BFindex: Blood flow index; End PCr: End exercise PCr; PCr_{re}: Phosphocreatine resynthesis rate; Tc: Time constant; 12-s: 12-s all out exercise; SSI: steady state incremental exercise. Data are expressed in mean ± SD.



(Figure 4). However, improvements in exercise duration after CoQ₁₀ supplementation was not statistically different for either group (Table 2). Further, PCr changes at the onset of exercise, end exercise ipH, or ΔBV during SSI exercise was not significantly altered for either group with CoQ₁₀.

Recovery response: PCr_{re} after 12-s exercise, was not significantly different for either group under CoQ₁₀ trial (Table 2 and Figure 5). Slight improvement in ipH recovery was seen in T2D after CoQ10 trial (6.89 vs. 6.92). However, these changes were not statistically significant ($p > 0.05$). Hb/Mb-oxy during recovery was not significantly different with CoQ₁₀ treatment for ei-

ther T2D or control group ($p = 0.960$) (Table 2 and Figure 5). Moreover, no significant CoQ₁₀ treatment effect ($p = 0.469$) was detected in BFindex for either group.

Discussion

T2D Patients vs. Control subjects

Resting response: This study found a significantly lower PCr, PCr/Pi and higher Pi in T2D when compared to control subjects. Studies have reported abnormal resting Pi metabolites and/or ratios in different diseases. For example, lower PCr/Pi has been reported in mitochondrial [29] and COPD patients [30], while higher Pi/PCr has been associated with T2D

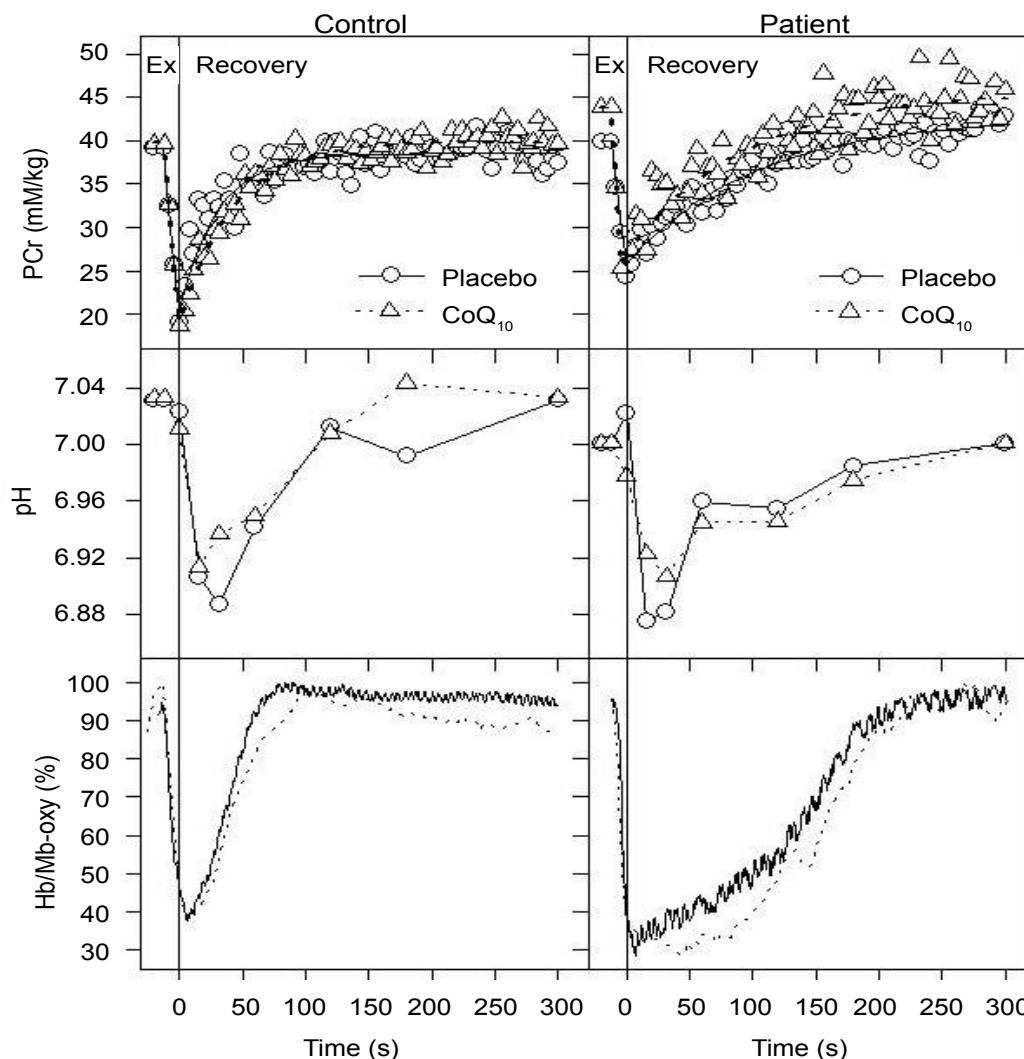


Figure 5: Changes in PCr (top panel), ipH (middle panel), Hb/Mb-oxy (bottom panel) during and after 12-s exercise in control subjects (left panels) and T2D patients (right panels) during placebo (circle and solid line) and CoQ₁₀ trials (triangle, dotted line).

[31,32]. Under the condition of constant pH, PCr/Pi is interpreted as phosphorylation potential, one of the main activators in cellular respiration [33,34]. Thus, lower resting PCr/Pi observed in T2D suggests a state of constant metabolic stress even at rest and a reduced phosphorylation potential. Under ischemic conditions, this reduced phosphorylation potential results not only in over delivered electrons (e^-) and subsequent over production of ROS but also accumulation of other metabolites, such as H^+ , Pi, and ADP, which negatively impact cellular respiration and further stress the mitochondrial respiratory system.

Exercise and recovery responses: One of the research questions in this study was whether T2D patients have impaired skeletal muscle blood flow, mitochondrial function and exercise performance when compared to control subjects. To compare groups, this study used the same relative workloads for all subjects to keep the metabolic rate equivalent. During this state, PCr_{re} was significantly less in T2D suggesting compromised mitochondrial oxidative phosphorylation capacity. Delayed PCr_{re} can occur when mitochondria is not functioning

properly. Delayed PCr_{re} can also occur when there is an insufficient O₂ supply to the mitochondria as evidenced by a significant relationship between O₂ recovery and PCr recovery ($p < 0.01$) seen in this study. These observations are in agreement with other studies reporting delayed PCr recovery after exercise under ischemic condition [35,36]. Insufficient O₂ supply to the mitochondria impairs e^- flow in the ETC and, as a result, ATP production is reduced. Furthermore, insufficient O₂ delivery while the mitochondria is fully activated during exercise can lead to overflow of e^- within the mitochondria increasing redox potential that favors ROS production.

In this study, a reduction in capillary blood flow (as indicated by lower ΔBV and BFindex) was observed in T2D during exercise. This is in agreement with other studies reporting reduced skeletal muscle blood flow in response to exercise in T2D [37,38]. A lower ΔBV and BFindex found in T2D indicates reduced O₂ supply to the muscle. When there is insufficient O₂ supply, while the demand is high, a mismatch in the O₂ flux can occur, impairing mitochondrial oxidative phosphorylation capacity. This phenomenon was clearly demonstrated where

PCr recovery was delayed in T2D and it was significantly related to T_{50NIR} and ΔBV .

Intracellular pH response: Exercise capacity of T2D was significantly lower than control subjects where T2D patients reached PCr/Pi of 1 (a state of anaerobic threshold), earlier than control subjects. Moreover, T2D patients' ipH during exercise was lower and recovery was significantly delayed in T2D (Figure 4 and Figure 5). The extent of H^+ generation depends on work intensity and ATP hydrolysis via anaerobic glycolysis. Moreover, the amount of H^+ present in the cell depends on the ability of H^+ efflux. In this study, exercise was stopped at the same metabolic state (anaerobic threshold) for all subjects. Therefore, lower intramuscular pH and delayed pH recovery in T2D reflects the reduced ability of H^+ to be released from the cytosol to the extracellular space rather than increased ATP hydrolysis. Impaired blood flow (O_2 delivery) observed in T2D appears to be the primary factor for the reduced clearance capacity of H^+ . It has been suggested that the relationship between exercise-induced PCr/Pi and acidosis is assumed to be a specific parameter for muscle ischemia [39,40]. At similar PCr/Pi, increased acidosis suggests delayed clearance of lactate or H^+ due to reduced tissue perfusion as evidenced by reduced ΔBV and BFindex. Furthermore, ipH kinetics can affect PCr recovery. Thus delayed PCr recovery, associated with reduced O_2 delivery, can be further delayed by prolonged acidic environment.

Effect of CoQ₁₀

Resting response: After CoQ₁₀ trial, a significant reduction in MDA was observed in healthy, older control subjects, suggesting CoQ₁₀ may have beneficial antioxidant effects in reducing oxidative stress. This is in agreement with other studies, which have reported a positive antioxidant role of CoQ₁₀ lowering MDA level [23,41-43]. One study reported reduction of MDA with CoQ₁₀ supplementation in children with autism spectrum disorder [42]. Another study reported similar findings in patients with multiple sclerosis [43]. Aging is often associated with elevated oxidative stress [44], causing age related tissue damage. Furthermore, reduced level of CoQ has been shown as a result of the aging process [45], making the older individuals even more prone to oxidative damage. Thus, supplementing with CoQ₁₀ can potentially benefit this population by normalizing CoQ₁₀ and also scavenging ROS, thereby reducing oxidative stress and delaying the aging process.

Interestingly, MDA level was not significantly altered with CoQ₁₀ in T2D patient group. Although studies have suggested the beneficial antioxidant effect of CoQ₁₀, clinical trials involving particularly at high-risk patients, such as T2D, are less remarkable [46]. Lack of significant changes seen in T2D patients in this study may partly be due to the dosage or the duration not potent or long enough for this population, who

may have undergone significant cellular modifications with chronic exposure to oxidative stress. For example, damaged mitochondria of type II diabetes can lead to not only deficient in CoQ [20,21] but also over production of e^- and subsequent ROS, creating a greater oxidative stress environment than healthy, older control subjects. The given dose or duration of CoQ₁₀ in current study may have not been sufficient enough to scavenge the over delivered e^- and/or reduce ROS present within the T2D mitochondria, which may be responsible for the lack of reduction in MDA level seen in this population.

Metabolic response: After the CoQ₁₀ treatment, T2D group's PCr and PCr/Pi were elevated to the values similar to the control subjects. However, these differences were not significant. Normalization of PCr/Pi ratio with CoQ₁₀ has been seen in patients with mitochondrial disease [47] and Friedreich's Ataxia [48] CoQ₁₀ can improve the metabolic status by better handling of the e^- within ETC, where CoQ₁₀ can scavenge over delivered e^- reducing ROS concentration and also enhancing e^- transport as a co-factor to improve ATP production [22,49].

Exercise and recovery responses: Similar to the resting response, a significant reduction in MDA, with CoQ₁₀ supplementation, was observed in control subjects although improvements in functional capacity or other measures were not as obvious. This is contrary to our original hypothesis that CoQ₁₀ would improve functional capacity of T2D patients. Lack of improvements seen in this study may be due to multiple factors. First and foremost, two weeks of CoQ₁₀ supplementation at 200 mg·day⁻¹ may have not been long enough or potent enough for those already having undergone significant modification of vascular tone with chronic exposure to oxidative stress. For the study, a short term but a maximum dose at safe level of 200 mg·day⁻¹ (typical dosage 100 mg·day⁻¹) was chosen to see the acute effect of the supplementation. Although some studies have reported positive effects of CoQ₁₀ supplementation with a similar duration and dosage [50,51], results from CoQ₁₀ studies involving human subjects, particularly patient populations, are rather inconclusive [52]. Solid evidence for the benefit of short-term CoQ₁₀ supplementation on T2D with PAD has yet to be established.

Another possible factor may be due to CoQ₁₀ having a greater effect on the mitochondria than microvasculature. As described earlier, PCr_{re} can be altered significantly by O_2 delivery as evidenced by current findings of strong and significant relationships between PCr_{re} and hemodynamic measures. This phenomenon is in agreement with other studies reporting the influence of O_2 delivery on mitochondrial phosphorylation capacity. Thus, while some improvements in mitochondrial oxidative phosphorylation capacity may have been pre-

sent, without sufficient O₂ delivery in mitochondria, no discernable improvements in PCr_{re} can be observed.

Limitations

Small sample size of the patient group as well as the control group decreases the statistical power, which makes it challenging to detect the small changes and draw a generalization of the population.

Conclusion

In conclusion, CoQ₁₀ supplementation exhibited a positive effect in lowering oxidative stress status, shown by reduced MDA, in healthy older individuals. Moreover, CoQ₁₀ supplementation appeared to have marginal effect on mitochondrial oxidative phosphorylation potential of T2D patients at rest without significantly affecting microvascular measures or functional capacity during exercise. Lack of significant microvascular changes after two weeks of CoQ₁₀ supplementation in T2D is most likely the reason for the lack of functional improvement in T2D.

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Statement of Equal Authors' Contribution

All authors have read the manuscript, assisted in all aspects of the research study as well as the writing of the manuscript, and have agreed to the publication of this manuscript.

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