

The Effect of Coenzyme Q10 Administration on DNA Damage in Diabetic Polyneuropathy. A Randomized Double-Blind Placebo-Controlled Phase IIa Clinical Trial

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Abstract

Aim: To evaluate the effect of Coenzyme Q10 (CoQ10) administration on DNA damage in diabetic polyneuropathy (DPN).

Methods: We performed a randomized double-blind placebo-controlled clinical trial. A single daily dose of Placebo or CoQ10 (400 mg) was given for 16 weeks in patients with DPN. Serum levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), 8-oxoguanine-DNA-N-glycosylase (hOGG1), endogenous CoQ10, and superoxide dismutase (SOD) were analyzed. We included 9 healthy control subjects (HC) to compare baseline - final levels for each group.

Results: Baseline levels of 8-OHdG in Placebo patients were 5.15 ± 0.59 , and final levels, 5.95 ± 1.21 ng/mL ($p=0.715$). Baseline levels of 8-OHdG in the CoQ10 patients had 6.60 ± 0.80 ng/mL, and final, 7.10 ± 0.74 ng/mL ($p=0.768$). Baseline levels of hOGG1 in HC were 0.39 ± 0.01 ng/mL, while in the Placebo patients was 0.42 ± 0.01 ng/mL ($p=0.027$ vs. HC) and final 0.41 ± 0.00 ng/mL ($p=0.353$ baseline-final). The CoQ10 patients had baseline levels of hOGG1, 0.43 ± 0.01 ng/mL ($p=0.01$ vs. HC), and final of 0.43 ± 0.01 ng/mL. A significant reduction of SOD activity was found in both groups. Superoxide dismutase baseline in Placebo patients was 6.29 ± 0.74 ($p=0.025$ vs. HC) and the CoQ10 patients had 5.37 ± 0.58 U/L ($p=0.006$ vs. HC). At the end of the study, tendency for SOD to increase was noted in the CoQ10 patients to 6.07 ± 0.68 U/L ($p=0.15$). Baseline levels of CoQ10 in HC were 1380.12 ± 135.51 ng/mL and the Placebo patients had 579.56 ± 67.91 ng/mL ($p<0.001$ vs. HC). The CoQ10 patients had tendency to improve levels from baseline from 999.09 ± 108.49 ng/mL to 1062.39 ± 152.10 ng/mL at the final result.

Conclusion: The administration of CoQ10 offering antioxidant benefits with tendency to augment the SOD activity and improve endogenous CoQ10 concentrations. No effect of supplementation with CoQ10 on oxidative DNA damage in DPN patients was observed.

Keywords: DNA damage, Oxidative stress, Coenzyme Q10, Antioxidants, Diabetic polyneuropathy

Introduction

Diabetes mellitus (DM) is a worldwide public health problem that currently affects over 415 million people globally [1]. DM is accompanied by micro and macrovascular complications in 60-70% of patients. These complications are the key cause of morbidity and mortality among type 2 DM patients [2]. Clinically, diabetic peripheral polyneuropathy (DPN) is the most significant diabetic complication [3] that affects ≈ 110 million people around the world [4]. Diabetic polyneuropathy is a multifactorial disease that affects the function of all organs of the body either directly or indirectly due to altered glucose metabolism that affects neural tissue, including central and peripheral nerves, through oxidative stress mechanisms [5]. The oxidative stress plays an important role in mediating DPN [6]. The reactive oxygen species (ROS) as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and nitric oxide (NO) free radicals are essential for normal physiology, but they also have the capacity to accelerate the process of aging and to mediate cellular degeneration in disease states. The ROS together produce highly active singlet oxygen, hydroxyl radicals, and peroxynitrite that can attack proteins, lipids, and nucleic acids [7]. Damage to the nucleic acids (DNA) can be measured indirectly with the 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a mutagenic, sensitive, and specific marker. Excessive ROS induce DNA damage with the

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capacity to activate the enzyme 8-oxoguanine-DNA-N-glycosylase (hOGG1) associated with DNA repair [8].

Coenzyme Q10 (CoQ10) is a well-known antioxidant with bio-energetic and anti-inflammatory effects [9]. Deficiency of CoQ10 may occur in DM patients as a consequence of an increase in the cytosolic redox potential that over-delivers electrons into the mitochondrial transport system and uncouples the production of ATP [10]. Previous studies have reported that supplementation with CoQ10 reduced glycated hemoglobin (HbA1c) significantly in overweight and obese patients with type 2 DM [11]. Experimental studies show that early, long-term treatment with CoQ10 prevents nerve conduction impairment and several sensory symptoms of DPN, and maintains dorsal root ganglia (DRG) neuron levels of phospholipase C (PLC β 3), which is a key molecule in pain processing [12]. The administration of CoQ10 to a group of neuropathic diabetic patients apparently has beneficial effects on total antioxidant capacity (TAC) and high-sensitivity C reactive protein (hs-CRP) [13].

To date there is no evidence of the effect of CoQ10 on the oxidative DNA damage in DPN. The aim of the current study was to evaluate the effect of CoQ10 on oxidative DNA damage in DPN.

Materials and methods

Study design

A randomized double-blind placebo-controlled phase Ila clinical trial was performed in type 2 DM patients with DPN. We made a parallel assignment of subjects by means of a computer-based blocking method of randomization and a 1:1 allocation.

Study population

Patients were recruited from primary care clinics by open invitation from the general population, with or without insurance. Inclusion criteria were: male or female subjects, between 18-80 years old, with type2DM diagnosed according to criteria of the American Diabetes Association [14] with glycated hemoglobin (HbA1c) \leq 10%, the DPN in stage 1 or 2. Patients signed the informed consent. Subjects were excluded if they had: any antioxidant therapy in the previous 3 months, known adverse reactions to CoQ10, hepatic or kidney failure, pregnancy, breastfeeding, and/or other neuropathic etiology different from DPN. The patients were eliminated if there was lack of adherence to treatment (<80% of pill intake), acute complication of DM, and/or evidence of severe impairment which resulted in inability to continue treatment. Sample size was calculated based on the determination of biomarkers resulting in 20 patients per group. For purposes of comparison a group of 9 healthy control subjects (HC) from the general population were recruited from preventive medicine at the same primary care clinics.

Study procedure

All patients were blinded and instructed to take a daily, night time, oral dose of physically similar Placebo or CoQ10 (400 mg). The treatment was provided every 4 weeks for 16 weeks, in a dark vial with 30 pills without the knowledge of the other researchers. Codes were assigned to ensure patient confidentiality. The first visit was to determine if subjects were eligible for randomization: an informed consent was explained to each person until they fully understood the study characteristics, then a clinical directed

history was collected and a physical examination was performed. Blood samples were obtained by venipuncture for complete blood analysis and biomarkers. On a second visit, biochemical analyses were interpreted to complete selection criteria and patients were allocated to one of the aforementioned groups. Every subject was given a diary where they could mark the date and schedule of pill ingestion. They were also taught to annotate any drug-related adverse reactions. Every 4 weeks gathered the information provided by patients through their diaries.

The blood sample was taken for biosafety analysis (Complete blood count, creatine phosphokinase (CPK), and hepatic and kidney tests), pill count was carried out, and a new vial was provided. On the last visit a blood sample analysis that included biomarkers in addition to the rest of the procedure, was performed. Regular physicians assessed metabolic parameters and health status, and the researchers established ongoing communication with them by email or telephone; and practitioners were asked if any new treatments were implemented during follow-up.

Biochemical analysis

The blood samples were collected in two separate tubes: one with 0.1% of ethylenediaminetetraacetic acid (EDTA), and other dry. The plasma and serum were separated by centrifugation at 2,000 rpm for 10 minutes at room temperature. The samples were stored at -80°C degrees until use. All of the technical readings of optical density were made with the Synergy HT (BIOTEK) microplate reader.

8-hydroxy-2'-deoxyguanosine

For evaluations of the 8-OHdG we followed the method suggested by the manufacturer of the ELISA kit (No. ab10124 Abcam, Cambridge, United Kingdom). The plasma sample, the EIA buffer, the standards, and the 8-OHdG - AChE tracer were added to all the wells except the blank. Then, the monoclonal antibody 8-OHdG was added and the plate and incubated for 18 h at 4°C. Afterwards, the plate was washed with buffer for the recommended times and 200 μ L of Ellman's reactive were added to each well. The optical density was read at 405 nm. The values are expressed in ng/mL.

Human-8-oxoguanine-DNA-glycosylase

Repair of the oxidative damage to DNA was determined through the use of the commercial kit (Human-8-oxoguanine-DNA-glycosylase MBS702793 MyBiosource®, San Diego, CA, USA). The manufacturer's instructions were followed and the reactive and samples were prepared for the indicated dilutions. 100 μ L of plasma and standards were added to the wells and the plate was incubated at 37°C. Then the biotinylated antibody was added and incubated under the same conditions. The corresponding washings were done and the HRP-avidin was added, followed by the substrate, and the stop solution at the corresponding times. The optical density was read at 450 nm and the results were ng/mL.

Superoxide Dismutase

Regarding SOD, the kit manufacturer's instructions were followed (No.706002, Cayman Chemical Company®, USA) for the detection of O $_2$ $^{\cdot -}$ generated by the xanthine oxidase and hypoxanthine enzymes through the reaction of tetrazolium salts.

The serum samples were diluted 1:5 in sample buffer; 200 μ L of the radicals' detector (diluted 1:400) was placed, and 10 μ L of the sample were added. After slow agitation, 20 μ L of xanthine oxidase were added to the wells. The microplate was incubated 20 min at room temperature and the absorbency at optical density was read at a wavelength of 440 nm. Levels are reported in U/mL.

Coenzyme Q10

To determine the plasma concentrations of CoQ10 an immuno-enzymatic method was performed following the kit manufacturer's instructions (Human CoQ10 No. CSB- E14081h, Cusabio[®] Biotech Co., Ltd, China). The sample was diluted 1:100 and 50 μ L of the sample or standard was placed in each well. Then, 50 μ L of HRP- conjugate 1X was added, it was agitated for 60 seconds and then incubated for 40 min at 37°C. Washings were made with the buffer for the corresponding times. The substrate was added to each well and the plate was incubated for 20 min at 37°C. Then, 50 μ L of the stop solution were added with gentle agitation, and the optical density was measured at 450 nm. The results are expressed as ng/mL.

Statistical analysis

Categorical variables are presented as frequencies and percentages and were analyzed with Chi² or Fisher's exact test. Continuous variables are expressed as mean \pm standard or error deviation of the mean. The Wilcoxon test was used for intra-group comparisons and Mann-Whitney test for comparisons between groups. Results were interpreted as a two-sided value of $p \leq 0.05$ considered significant, with a 95% confidence interval.

Ethical considerations

The study was performed in accordance with the Helsinki statements provided at the 64th General Assembly in Fortaleza, Brazil (October, 2013), and in agreement with national and international laws. Codes were assigned to ensure patient confidentiality and an informed consent was read and signed before entering the trial. The protocol was reviewed and approved by the local Research and Ethics Committee of the University of Guadalajara, (Registration CEI/044/2014) and given the international clinical trial number NCT02129231 (ClinicalTrials.gov).

Results

Demographic and metabolic characteristics

The demographic characteristics were similar. Females predominated in both groups: 70% in the Placebo patients and 62.5% in the CoQ10 patients ($p=0.108$). The average age was 54.7 ± 9.6 years in the Placebo and 58.8 ± 9.2 in the CoQ10 ($p=0.204$). Weight was similar in both groups with 73.7 ± 11.4 kg in the Placebo patients vs. 73.8 ± 8.6 kg in the CoQ10 patients ($p=0.802$). The history of the type 2 DM between the groups were similar with 10.5 ± 8.3 years in the Placebo vs. 9.7 ± 6.2 years in the CoQ10 patients. Smoking was positive in 38% of the Placebo and 42% in the CoQ10 patients ($p=0.710$). The characteristics of height, body mass index (BMI), and arterial pressure were homogenous between groups.

Metabolic parameters and liver and kidney safety

The baseline evaluations of the metabolic parameters of glucose, HbA1c, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl-transferase (GGT), total cholesterol (TC), low density cholesterol (LDL-c), high density cholesterol (HDL), triglycerides (TG), and total bilirubin (TB), direct and indirect, were similar to baseline values. The glucose concentration in the final results decreased significantly in both groups: Placebo baseline levels were 186.40 ± 14.48 mg/dL and final 150.2 ± 11.2 mg/dL ($p=0.004$, Wilcoxon test); and, the baseline glucose in the CoQ10 was 159.50 ± 11.05 mg/dL and final 138.1 ± 11.0 mg/dL ($p=0.020$, Wilcoxon test), without difference between the groups ($p=0.319$). The LDL-c in Placebo patients decrease from 126.68 ± 8.76 mg/dL baseline to final 109.6 ± 7.8 mg/dL ($p=0.01$). A reduction in the concentration of TB was observed in the final results in the CoQ10 from 0.77 ± 0.06 mg/dL to 0.63 ± 0.08 mg/dL ($p=0.027$); and in the indirect bilirubin from 0.65 ± 0.05 mg/dL to 0.49 ± 0.06 mg/dL ($p=0.012$).

Oxidative DNA damage and DNA repair

A tendency for levels of the marker 8-OHdG to increase was observed in both groups after the intervention. Baseline levels of the marker of oxidative DNA damage in the Placebo patients were similar from baseline 5.15 ± 0.59 ng/mL to final 5.95 ± 1.21 ng/mL ($p=0.72$). The baseline 8-OHdG in the CoQ10 patients was 6.60 ± 0.80 ng/mL and higher at the final result 7.10 ± 0.74 ng/mL ($p=0.77$).

Baseline levels of the hOGG1 DNA repair enzyme in Placebo patients were 0.42 ± 0.01 ng/mL ($p=0.03$) vs. HC (0.39 ± 0.01 ng/mL) and the final results 0.41 ± 0.01 ng/mL ($p=0.35$). Baseline levels of the hOGG1 enzyme in the CoQ10 patients were 0.43 ± 0.01 ng/mL ($p=0.01$ vs. HC) similar to the final result with 0.43 ± 0.01 ng/mL ($p=0.83$) (Table 1).

Superoxide dismutase

Levels of SOD in HC were 10.22 ± 0.93 U/mL. The activity of the SOD was significantly diminished in patients with DPN. Baseline levels in Placebo patients had 6.29 ± 0.74 U/mL ($p=0.03$ vs. HC) and the final result was similar 6.85 ± 0.97 U/mL ($p=0.21$). Baseline SOD in CoQ10 patients had 5.37 ± 0.58 U/mL ($p=0.01$ vs. HC) and the final result was 6.07 ± 0.68 U/mL ($p=0.15$) with slight increase.

Coenzyme Q10

The concentration of endogenous CoQ10 found in HC was 1380.12 ± 135.51 ng/mL. Baseline concentration of endogenous CoQ10 in the Placebo patients was 579.56 ± 67.91 ng/mL ($p<0.001$ vs. HC) and the CoQ10 patients had 999.09 ± 108.49 ($p=0.011$ vs. HC). After the intervention both groups demonstrated a tendency for increased concentrations of CoQ10. The Placebo patients obtained CoQ10 levels of 771.94 ± 76.49 ng/mL ($p=0.11$) and the CoQ10 patients achieved levels of 1062.39 ± 152.10 ng/mL ($p=0.97$) (Table 1).

Discussion

The role of CoQ10 deficiency in the development of DM microvascular complications has been previously demonstrated, and the antioxidant effects on mitochondria makes CoQ10 a good strategy for adjuvant therapy in patients with DPN [15].

Even though, the modulation of metabolic markers by CoQ10 in DM has not been fully established [16], in experimental studies

	Oxidative DNA damage									
	8-OH-2dG ng/mL					hOGG1 ng/mL				
	Baseline	<i>p</i> U-M	Final	<i>p</i> U-M	<i>p</i> WXC	Baseline	<i>p</i> U-M	Final	<i>p</i> U-M	<i>p</i> WXC
Healthy control	4.73 ± 0.34					0.39 ± 0.01				
Placebo	5.15 ± 0.59	0.63	5.95 ± 1.21	0.95	0.72	0.42 ± 0.01	0.03*	0.41 ± 0.00	0.05*	0.35
CoQ10	6.60 ± 0.80	0.14	7.10 ± 0.74	0.02*	0.77	0.43 ± 0.01	0.01*	0.43 ± 0.01	0.03*	0.83
	Endogenous Antioxidants									
	SOD U/mL					CoQ10 ng/mL				
	Baseline	<i>p</i> U-M	Final	<i>p</i> U-M	<i>p</i> WXC	Baseline	<i>p</i> U-M	Final	<i>p</i> U-M	<i>p</i> WXC
Healthy control	10.22 ± 0.93					1380.12 ± 135.51				
Placebo	6.29 ± 0.74	0.03*	6.85 ± 0.97	0.05*	0.21	579.56 ± 67.91	<0.001*	771.94 ± 76.46	0.001*	0.11
CoQ10	5.37 ± 0.58	0.01*	6.07 ± 0.68	0.01*	0.15	999.09 ± 108.49	0.011*	1062.39 ± 152.10	0.012*	0.97

Mean ± SEM, U-M: Mann-Whitney's *U* test, WXC: Wilcoxon test. **p* value vs. healthy control

Table 1: Oxidants And Antioxidants. An increase in the repairer enzyme of oxidative DNA damage is observed (hOGG1) in DPN vs. healthy controls. The concentrations of endogenous antioxidants, SOD and CoQ10 were found diminished. The intervention with CoQ10 demonstrates a tendency to elevate concentrations of antioxidants without showing significant changes vs. the Placebo group in the modification of DNA damage and repair.

of DPN, the administration of CoQ10 for six months in rats demonstrated no effect on blood glucose levels. However, clinical trials demonstrate that the consumption of 75 mg/day of CoQ10 for 8 weeks in DM patients reduces glucose levels approximately 20% [17], and the administration of 200 mg/day for 12 weeks in DM significantly reduces levels of HbA1c [18]. In this study the administration of 400 mg/day of CoQ10 showed a significant reduction of glucose levels without modification of the HbA1c. Nonetheless, the Placebo patients showed significant reduction in glucose levels, which could be explained by life-style modification during the development of the study. In experimental studies of liver damage, the administration of CoQ10 for 10 days did not show modifications to bilirubin levels [1] but, in clinical studies of a healthy population, the administration of CoQ10 prior to physical activity was able to decrease levels of TB [19], coinciding with our study which presented a decrease the TB and indirect bilirubin in the CoQ10 patients; a phenomenon that can be attributed to its participation in the mitochondrial respiratory chain on proportioning better oxygenation to the tissues, as well as its anti-inflammatory effect.

The oxidative damage generated by ROS in the macromolecules is controlled by a spectrum of enzymatic and non-enzymatic antioxidants; including the enzymatic SOD, which is an enzyme of intracellular defense that works as a frontline against free radicals, with activity that is relatively greater in the peripheral nerves [20]. It has been demonstrated that levels of SOD in erythrocytes are diminished in DPN compared to healthy controls, similar to our results on finding lesser SOD activity in DPN, which indicates the important role of this enzyme in the protection of damage to the tissues. The effect of CoQ10 on SOD has been previously evaluated in coronary artery disease where SOD activity was increased after the administration of 300 mg/day for 12 weeks [21] Results of the present study demonstrate a tendency for SOD activity to improve after the administration of CoQ10.

Coenzyme Q10 is found among the most common non-enzymatic, endogenous antioxidants. Low levels and reduced activity of CoQ10 has been reported in DM patients [10]. Regarding CoQ10 levels and the microvascular complications of DM, there is a report where diminished CoQ10 was found in diabetic retinopathy [22] and, it seems, the behavior of CoQ10 in

DPN is similar based on the results of this study where we found decreased levels of CoQ10 compared to HC. The bio-energetic, anti-inflammatory, and protector effects of the CoQ10 suggests it is an attractive alternative for the management of DPN [23]. In experimental studies of DPN, the administration of CoQ10 has shown improvement in the nerve conduction velocity to sensorial stimuli as well as diminishing the thermic hyperalgesia and mechanical allodynia; effects which can be attributed to its antioxidant activity [24]. There are few reports of clinical studies on the use of CoQ10 in DPN [25] and on the alteration of endogenous concentrations of CoQ10 after its administration. In coronary disease elevation in the levels of plasma CoQ10 was found after administration of 300 mg/day for 12 weeks. In DM the administration of CoQ10 200 mg/day for 12 weeks augmented by nearly three times the endogenous levels, without achieving significant changes to endothelial function [26]. Results here demonstrate a tendency for levels of endogenous CoQ10 to increase. A more prolonged treatment and a greater number of patients might permit the observation of significant changes in the markers of oxidative stress.

Oxidative stress is considered the main mechanism for the development of DM complications, and 8-OHdG is one of the most abundant products of oxidative DNA damage. The evaluation of 8-OHdG is one of the most abundant products of oxidative DNA damage, the evaluation of the 8-OHdG emerges as a biomarker sensitive to oxidative damage, especially for patients in early stages of the microvascular complications of DM [27] Previous studies have reported elevation of the 8-OHdG in patients with diabetic retinopathy [28] as well as a correlation with its urinary excretion to predict the development of diabetic nephropathy [29]. We did not find significantly increased levels of 8-OHdG marker in DPN with respect to HC, however, the higher levels of the hOOG1 enzyme were found in DPN vs. HC, which could suggest that the elevation of the enzyme occurred in response to the oxidative damage and that concentrations of the 8-OHdG were compensating.

Studies that evaluate the effect of CoQ10 on the 8-OHdG marker and the hOOG1 in humans, are limited. In young, healthy females the administration of 100 mg/day of CoQ10 in its reduced form demonstrated a protector effect against oxidative damage on reducing urinary levels of the 8-OHdG [30], contrary

to that reported in smokers where the consumption of 30 mg/3 times daily of CoQ10 for 2 months did not effectuate changes in levels of 8-OHdG [31]. The effect of CoQ10 on the DNA restorative enzyme (hOGG1) has been evaluated in postprandial oxidative stress, where the administration of 200 mg/day was compared with different diets, resulting in the Mediterranean diet + CoQ10 reducing the expression of genes that code for proteins related to DNA repair dependent on p53, suggesting that oxidative damage existed in a diet enriched with CoQ10 [32]. In the present study, CoQ10 did not result in significant changes in the levels of 8-OHdG and hOGG1 during the study period. Likely, prolonging the administration period as well as considering the reduced form of CoQ10 would permit obtaining a greater clinical significance.

In conclusion, the administration of CoQ10 offers antioxidant benefits with a tendency to augment SOD activity and improve endogenous CoQ10 concentrations. We did not find any effect of supplementation with CoQ10 on oxidative DNA damage in DPN patients. The short length of CoQ10 administration is the most important limitation of our study.

Conflicts of interest

The authors have no conflicts of interest to report

References

1. International Diabetes Federation. IDF Diabetes Atlas. 7th ed (2015).
2. Ini-Isabee W, Lee S, Khalaf K, Jelinek HF, Khandoker AH, Alsafar H. Identifying common genetic risk factors of diabetic neuropathies. *Front Endocrinol (Lausanne)*. 2015;6:88.
3. Zenker J, Ziegler D, Chrast R. Novel pathogenic pathways in diabetic neuropathy. *Trends Neurosci*. 2013;36(8):439-449.
4. Matuszewski W, Bandurska E, Wiatr D, et al. Diabetic neuropathy. *Polish Ann Med*. 2013;20:154-159.
5. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001;414:813-820.
6. Oyenihi AB, Ayeleso AO, Mukwevho E, Masola B. Antioxidant strategies in the management of diabetic neuropathy. *Bio Med Res Int*. 2015.
7. Vincent AM, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocr Rev*. 2004;25(4):612-628.
8. Cooke MS, Olinski R, Evans MD. Does measurement of oxidative damage to DNA have clinical significance? *Clin Chim Acta*. 2006;365(1-2):30-49.
9. Villalba JM, Parrado C, Santos-Gonzalez M, Alcain FJ. Therapeutic use of coenzyme Q10 and coenzyme Q10-related compounds and formulations. *Expert Opin Investig Drugs*. 2010;19(4):535-554.
10. Chew GT, Watts GF. Coenzyme Q10 and diabetic endotheliopathy: oxidative stress and the 'recoupling hypothesis'. *QJM*. 2004;97(8):537-548.
11. Mehrdadi P, Kolahdouz Mohammadi R, Alipoor E, Eshraghian MR, Esteghamati A, Hosseinzadeh-Attar MJ. The effect of coenzyme Q10 supplementation on circulating levels of novel adipokine adiponin/CTR12 in overweight and obese patients with type 2 diabetes. *Exp Clin Endocrinol Diabetes*. 2017;125(3):156-162.
12. Tie-Jun SS, Ming-Dong Z, Hugo Z, et al. Coenzyme Q10 prevents peripheral neuropathy and attenuates neuron loss in the db/db mouse, a type 2 diabetes model. *Proc Natl Acad Sci USA*. 2013;110(2):690-695.
13. Akbari-Fakhrabadi M, Zeinali-Ghotrom A, Mozaffari-Khosravi H, Hadi-Nodoushan H, Nadjarzadeh A. Effect of coenzyme Q10 on oxidative stress, glycemic control and inflammation in diabetic neuropathy: A double blind randomized clinical trial. *Int J Vitam Nutr Res*. 2014;84(5-6):252-260.
14. American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care*. 2016;39(1):13-22.
15. Ates O, Bilen H, Keles S, et al. Plasma coenzyme Q10 levels in type 2 diabetic patients with retinopathy. *Int J Ophthalmol*. 2013;6(5):675-679.
16. Moradi M, Haghghatdoost F, Feizi A, Larijani B, Azadbakht L. Effect of coenzyme Q10 supplementation on diabetes biomarkers: A systematic review and meta-analysis of randomized controlled clinical trials. *Arch Iran Med*. 2016;19(8):588-596.
17. Mohammed NK, Al-Sabbagh M, AL-Jezaeri K. Role of L-carnitine and coenzyme Q10 as adjuvant therapy in patients with type 2 diabetes mellitus. *Eur J Clin Nutr*. 2014;2(5):82-86.
18. Ashkani ES, Esmaeilzadeh E, Bagheri F, Emami Y, Farjam M. The Effect of Co-enzyme Q10 on acute liver damage in rats, a biochemical and pathological study. *Hepat Mon*. 2013;13(8):e13685.
19. Diaz C, Guisado R, Kajarabille N, et al. Coenzyme Q10 supplementation ameliorates inflammatory signaling and oxidative stress associated with strenuous exercise. *Eur J Nutr*. 2012;51(7):791-799.
20. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. *Sultan Qaboos Univ Med J*. 2012;12(1):5-18.
21. Lee BJ, Tseng YF, Yen CH, Lin PT. Effects of coenzyme Q10 supplementation. (300 mg/day) on antioxidation and anti-inflammation in coronary artery disease patients during statins therapy: a randomized, placebo-controlled trial. *Nutr J*. 2013;12(1):142.
22. Ates O, Bilen H, Keles S, et al. Plasma coenzyme Q10 levels in type 2 diabetic patients with retinopathy. *Int J Ophthalmol*. 2013;6(5):675-679.
23. Villalba JM, Parrado C, Santos-Gonzalez M, Alcain FJ. Therapeutic use of coenzyme Q10 and coenzyme Q10-related compounds and formulations. *Expert Opin Investig Drugs*. 2010;19(4):535-554.
24. Zhang YP, Eber A, Yuan Y, et al. Prophylactic and antinociceptive effects of coenzyme Q10 on diabetic neuropathic pain in a mouse model of type 1 diabetes. *Anesthesiology*. 2013;118(4): 945-954.
25. Prashanth J, Jesudoss-Prabhakaran AC. The beneficial effect of Coenzyme Q in diabetic neuropathy: An overview. *International Journal of Nutrition, Pharmacology Neurological Diseases*. 2012;2:80-83.
26. Lim SC, Lekshminarayanan R, Goh SK, et al. The effect of coenzyme Q10 on microcirculatory endothelial function of subjects with type 2 diabetes mellitus. *Atherosclerosis*. 2008;196:966-969.
27. Nishikawa T, Sasahara T, Kiritoshi S, et al. Evaluation of urinary 8-hydroxydeoxyguanosine as a novel biomarker of macrovascular complications in type 2 diabetes. *Diabetes Care*. 2003;26:1507-1512.
28. Dong QY, Cui Y, Chen L, Song J, Sun L. Urinary 8-hydroxydeoxyguanosine levels in diabetic retinopathy patients. *Eur J Ophthalmol*. 2008;18(1):94-98.
29. Xu GW, Yao QH, Weng QF, Su BL, Zhang X, Xiong JH. Study of urinary 8-hydroxydeoxyguanosine as a biomarker of oxidative DNA damage in diabetic nephropathy patients. *J Pharm Biomed Anal*. 2004;36(1):101-104.
30. Ito K, Watanabe C, Nakamura A, Oikawa-Tada S, Murata M. Reduced coenzyme Q10 decreases urinary 8-Oxo-7,8-Dihydro-2'-Deoxyguanosine concentrations in healthy young female subjects. *J Med Food*. 2015;8(8):835-840.
31. Prieme H, Loft S, Nyssonson K, Salonen JT, Poulsen HE. No effect of supplementation with vitamin E, ascorbic acid, or coenzyme Q10 on oxidative DNA damage estimated by 1 -deoxyguanosine excretion in smokers. *Am J Clin Nutr*. 1997;65:503-507.
32. Gutierrez-Mariscal FM, Yubero-Serrano EM, Rangel-Zuniga OA, et al. Postprandial activation of p53-dependent DNA repair is modified by Mediterranean diet supplemented with coenzyme Q10 in elderly subjects. *J Gerontol A Biol Sci Med Sci*. 2014;69(7):886-893.