Alterations of erythrocyte free radical defense system, heart tissue lipid peroxidation, and lipid concentration in streptozotocin-induced diabetic rats under coenzyme Q_{10} supplementation

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ABSTRACT

Objective: Free radicals play an important role in genesis and development of various chronic diseases and aging. Our objective is to study the effects of coenzyme Q_{10} (CoQ_{10}) supplementation on erythrocyte antioxidants, heart tissue lipid peroxidation end products and lipid concentration in different age of diabetic rats.

Methods: In this study, the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and the content of reduced glutathione (GSH) were determined in erythrocytes. The products of lipid peroxidation were determined in the heart tissues of streptozotocin-induced diabetic rats and in healthy rats at 4, 8, and 13-months of age. The above mentioned antioxidant systems of erythrocytes were also determined after supplementation of diabetic and healthy rats with CoQ_{10}. This study was carried out in King Fahad Medical Research Center, Jeddah, Kingdom of Saudi Arabia between 2000 and 2001.

Results: In erythrocytes of diabetic rats the activity of GSH-Px was significantly decreased ($p<0.001$) in all different age groups, whereas the activity of SOD was significantly increased ($p<0.001$). However, in erythrocyte of streptozotocin-induced diabetic rats, the concentration of GSH and high-density lipoprotein (HDL)-cholesterol were significantly lower than non-diabetic rats. Moreover, the concentration of heart tissue lipid peroxidation end products, and plasma glucose, cholesterol and triacylglycerol were significantly increased ($p<0.001$) in all age groups of diabetic rats. Daily supplementation with CoQ_{10} (10 mg/kg body weight, one month) after induction of diabetes to the rats resulted in the following changes: an increase in both erythrocyte GSH concentration and GSH-Px activity, and slightly increases in plasma HDL-cholesterol. However, SOD activity was significantly decreased ($p<0.05$). In addition, the levels of lipid peroxidation end products, and triacylglycerol were significantly decreased ($p<0.05$) in diabetic rats supplemented with CoQ_{10}.

Conclusion: The results of the present study indicated that CoQ_{10} supplementation helps to prevent clinical complications during the course of the disease in diabetic rats.

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Oxidative stress is an imbalance between free radicals generation and antioxidant defense system; it has been implicated in the etiology of chronic diseases such as diabetes, atherosclerosis, cancer, and neurological disorders and aging.[1-3] In some human diseases, increased oxidative stress can make an important contribution to disease pathology. Severe oxidative stress can cause cell damage and death. Diabetes mellitus is characterized by increased production of reactive oxygen species (ROS) such as superoxide anions (O_2), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2), and sharp reduction in antioxidant defense. Both type I and type II diabetes are powerful and independent risk factors for coronary artery disease (CAD), stroke, and peripheral arterial disease. Atherosclerosis accounts for virtually 80% of all deaths among diabetic patients. Prolonged exposure to hyperglycemia is now recognized as a major factor in the pathogenesis of atherosclerosis in diabetes.[4,5] Hyperglycemia may lead to increased generation of free radicals via multiple mechanisms, including non-enzymatic glycosylation of proteins and monosaccharide auto-oxidation, polyol pathway activity, indirect production of free radicals through cell damage from other causes, and reduced antioxidant reserves.[6-8] Chronic hyperglycemia also leads to the activation of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent aldose reductase, which diminishes the NADPH available for glutathione reductase; consequently, the ratio of reduced to oxidized glutathione decreases.[9-11] Experiments have shown that antioxidant enzyme activities, including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), decreased in diabetic rats.[12-14] The level of vitamin E was found to be diminished in diabetes. Moreover, glutathione (GSH), which protects hemoglobin and some thiol dependent enzymes or membrane proteins from oxidative damage decreased in diabetic rats.[8,15] Several key enzymes in ROS defence are unusually low in pancreatic islets compared with other tissues, suggesting that the islet cells are uniquely susceptible to oxidative stress-induced damages.[3,16] Gene expression and activity of several key antioxidant enzymes such as Cu, Zn-SOD, Mn-SOD, GSH-Px, and CAT are all markedly decreased compared with other tissues such as liver.[3] The gene expression levels of the cytoplasmic Cu, Zn-SOD and the mitochondrial Mn-SOD in the pancreatic islet cells were 30-40% of levels found in liver. Glutathione peroxidase expression was 15%, and CAT gene expression was not detectable in pancreatic islets. Corresponding protein and activity levels were also found to be markedly lower in the islet cells. The low levels of antioxidant enzyme gene expression may account for the exquisite sensitivity of the beta-cells to ROS and free radical-induced damage leading to beta-cells death and insulin dependent diabetes mellitus (IDDM).[14,17]

Coenzyme Q_{10} (CoQ_{10}), also known as ubiquinone is a naturally occurring compound and is synthesized in all body tissues. The essential role of CoQ_{10} in biological energy transudation is well established, and acts as an electron carrier of the respiratory chain in the mitochondria.[11,16] Reduced CoQ_{10} ubiquinol acts as an antioxidant and decreases the action of free radicals.[18,19] Not only CoQ_{10} acts to remove oxygen free radicals but can also reduce tocopheryl radicals and semi-dihydroascorbate back to tocopherol and ascorbate. Coenzyme Q_{10} then acts as a master antioxidant as it can be reduced by metabolic supply of NADH or NADPH in the cell to form the hydroquinone.[20-22] Coenzyme Q_{10} appears to be involved in the coordinated regulation between oxidative stress and antioxidant capacity of heart tissue. When the heart is subjected to oxidative stress in various pathogenic conditions,[23,24] the amount of CoQ_{10} is decreased, which triggers a signal for increased CoQ_{10} synthesis. It has been reported that in patients with cardiac disease such as chronic heart failure, the myocardium becomes deficient in CoQ_{10} and CoQ_{10} reductase.[25] Coenzyme Q_{10} level is also reduced in other cardiovascular diseases such as cardiomyopathy.[18,25] Coenzyme Q_{10} can protect human low-density lipoprotein (LDL) from lipid peroxidation, suggesting its role in atherosclerosis.[26,27] In the present investigation, we aim to study the effect of CoQ_{10} supplementation on the activity of antioxidant enzymes and lipid peroxidation in diabetic rats induced with streptozotocin.

Methods. Male Wistar rats (200-500 g) were obtained from King Fahad Medical Research Center, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia (KSA). The animals were housed individually in a controlled environment in which the temperature was maintained at 24 ± 1^{0}C with lighting for 12 hours each day. Rats were kept on a standard laboratory diet (Grain Soils and Flourmills Organization, Jeddah, [KSA]) and water ad libitum with free access to water. Animals were divided into 2 main groups: 1) Normal rats = a total of 60 normal healthy rats were further divided into 2 sub-groups: a) 30 rats fed only on normal diet were divided into 3 sub-groups according to their ages (4, 8 and 13 months old) and b) 30 rats fed on normal diet supplemented with CoQ_{10} (10 mg/kg body weight/daily) for one month were divided into 3 sub-groups according to their ages (4, 8 and 13 months old). 2) Diabetic rats - a total of 60 rats were made diabetic by a single intra-peritoneal (IP) injection of STZ (55 mg/ kg body weight) dissolved in 50 mM citrate buffer, pH 4.5, immediately before

use. Rats showing positive glycosuria (>400 mg/100 ml, Dextrostix reagent strips) after the induction of diabetes were used. The diabetic rats were further divided into 2 sub-groups after 1, 3 and 5 months of diabetes induction: a) 30 rats, fed on only normal diet, were divided into 3 sub-groups according to their ages (4, 8, and 13 months old) and b) 30 rats, fed on normal diet supplemented with CoQ_{10} (10 mg/kg body weight/daily) for one month, were also divided into 3 sub-groups according to their ages (4, 8, and 13 months old).

All the chemicals were of analytical reagent grade and were obtained from BDH chemicals (Poole, United Kingdom). Coenzyme-Q10 was obtained from PharmaAssure, United States of America. Streptozotocin (N [methylnitrozocarbamoyl]-D-glucosamine) was obtained from Sigma chemical company, Poole, United Kingdom. Kits for glucose and lipid determinations were obtained from BioMerieux, Laboratory Reagents and Instruments (Marcy-L, Etoile, France) according to standarized assay methods.[28-31] Kits for lipid peroxidation, GSH, and antioxidant enzymes determinations were purchased from CN Biosciences, Ireland.

Collection and separation of samples. After the animals had fasted overnight, blood was collected by cardiac puncture under light ether anesthesia in tubes containing ethylenediaminetetraacetic acid (EDTA). Plasma and erythrocytes were separated by centrifugation to determine the lipid profiles and antioxidants then erythrocytes were washed with 0.9% sodium chloride solution to remove white blood cells. Plasma and erythrocytes were stored at -20°C until analysis. Also the hearts were quickly removed and washed with ice-cold normal saline (0.9% NaCl) containing 1 mM EDTA as an anticoagulant to remove any red blood cells and clots from the tissues. The hearts were then blotted on absorbent paper and weighed. Hearts were placed in liquid nitrogen and then stored at -80^{0}C until analyzed.

Determination of antioxidants. The concentration of GSH in hemolysates was measured according to the method of Barhoumi et al,[32] and Chaudierej et al.[33] The first step leads to the formation of substitution products (thioethers) between a proprietary reagent (7-trifluoromethyl -4-thioquinolone) and all mercaptans (RSH) which are present in the sample. The second step is a ß–elimination reaction mediated by 30% NaOH which specifically transforms the substitution product obtained with GSH into a chromophoric thioneine with a maximal absorbance at 400 nm. Antioxidant enzymes were measured in hemolysates. The activity of GSH-Px was determined with t-butyl hydroperoxide at 37°C; this was carried out according to the method of Leopold.[34] One unit of GSH-Px activity is defined as 1µmol reduced ß-nicotinamide adenine dinucleotide phosphate oxidized/minute. Superoxide dismutase activity was determined according to the method of Nebot et al.[35] The assay is based on the SOD-mediated increase in the rate of autooxidation of 5, 6, 6 a, 11b-tetrahydro-3,9,10-trihydroxybenzoflurene in aqueous alkaline solution. This auto-oxidation yields a chromophore with a maximal absorbance wavelength of 525 nm.

Assay of heart tissue lipid peroxidation end products. Lipid peroxidation end products assay was carried out according to the method of Janero,[36] Esterbauer and Cheeseman,[37] and Lapenna and Cuccurullo.[38] The principle of lipid peroxidation assay depends on the reaction of 2 molecules of N-methyl–2-phenylindole with one molecule of Malondialdehyde (MDA) and 4-hydroxynonenals at 45^{0}C yields a stable chromophore with maximal absorbance at 586 nm.

Results. The general characteristics of control and STZ-induced diabetic rats at different ages are shown in **Table 1**. The body weights and heart weights of STZ-induced diabetic rats were significantly reduced (p<0.001) at different ages when compared with control rats (**Table 1**). The concentrations of plasma glucose were significantly increased (p<0.001) in the diabetic animals at different age groups (**Table 2**). However, the supplementation of CoQ_{10} to these animals has not shown any significant effect on the concentration of plasma glucose.

Table 3 shows the effect of CoQ_{10} supplementation on plasma lipid concentrations of normal and STZ-induced diabetic rats in different age groups. The concentrations of plasma total cholesterol and triacylglycerol were significantly increased (p<0.001) in the diabetic animals as compared with the controls throughout different age groups, whereas the concentration of plasma HDL-cholesterol was significantly decreased (p<0.001 at 4 months, p<0.05 at 8 months, p<0.005 at 13 months) in the diabetic animals. However, the supplementation of CoQ_{10} to these animals has not shown any significant effect on the concentration of plasma total cholesterol, but the concentration of plasma triacylglycerol was significantly decreased (p<0.05) in control at 4 and 8 months and at all age groups of diabetic rats supplemented with CoQ_{10} (**Table 3**). The plasma concentration of HDL-cholesterol was significantly increased (p<0.05) in rats supplemented with CoQ_{10} (**Table 3**). The concentrations of erythrocyte reduced GSH was significantly decreased (p<0.005 at 4 and 13 months; and p<0.001 at 8 months) in diabetic rats as compared with normal controls at different age groups (**Table 4**). These concentrations were significantly increased (p<0.02) with the supplementation of CoQ_{10} at 13 months of normal rats and all age groups of the diabetic rats (**Table 4**).

Table 1 - Body weight and heart weight of control and streptozotocin-induced diabetic rats.

Age (months)	Body weight		Heart weight	
	Control	Diabetic	Control	Diabetic
4	320.4 ± 3.5	292.0 ± 4.4†	0.88 ± 0.03	0.71 ± 0.04*
8	372.5 ± 7.2	340.5 ± 5.3†	1.02 ± 0.01	0.83 ± 0.03†
13	449.5 ± 8.9	402.1 ± 3.4†	1.23 ± 0.05	0.94 ± 0.03†

p values are reported as mean ± SE, N= 8 to 10 rats, **p*<0.005, †*p*<0.001 as compared with control (student's t-test).

Table 2 - Effects of Coenzyme Q_{10} supplementation on plasma glucose concentration of control and streptozotocin-induced diabetic rats.

Plasma glucose (mmol/L)	Normal		Diabetic	
	Without CoQ10	With CoQ10	Without CoQ10	With CoQ10
Age (months)				
4	84.5 ± 3.1	84.6 ± 3.7	228.2 ± 3.4*	219.3 ± 3.5
8	88.5 ± 3.6	87.5 ± 2.9	228.9 ± 3.2*	220.9 ± 2.5
13	93.0 ± 3.2	92.9 ± 2.8	441.2 ± 3.2*	433.6 ± 2.1

Values are reported as mean ± SE, N= 8 to 10 rats, **p*<0.001 (student's t-test)

Table 4 shows that the activity of erythrocyte GSH-Px was significantly decreased (*p*<0.001, 30%) in diabetic rats as compared with the normal controls at the different ages. However, with the supplementation of CoQ_{10}; the enzyme activities were non-significantly affected in the normal rats at all age groups, whereas the enzyme activities were significantly increased (*p*<0.01 at 4 and 8 months; and *p*<0.005 at 13 months) in the diabetic rats. The activities of erythrocyte SOD were significantly increased (*p*<0.001 at 4 and 8 months, 30%; and *p*<0.01 at 13 months of age, 20%) in STZ-induced diabetic rats when compared with, normal control rats at different ages. Supplementation of CoQ_{10} has only slight increase effect on the enzyme activities of the normal control group. This increase was non-significant at 8 and 13 months, but significant (*p*<0.05) at 4 months. In contrast, the enzyme activities were significantly decreased (*p*<0.05 at 4 and 8 months; *p*<0.001 at 13 months) with the supplementation of CoQ_{10} to the diabetic animals. The concentrations of heart tissue lipid peroxidation end products were significantly increased (*p*<0.001, 50-60%) in diabetic rats as compared with normal controls at the different age groups. However, when CoQ_{10} was supplemented, the concentrations of lipid peroxidation end products of the heart tissue were significantly reduced (*p*<0.05).

Discussion. Diseases of the kidney, liver pancreas and heart may contribute in the development of secondary hyperlipidemia, atherosclerosis or oxidative stress. Therefore, diabetes mellitus is associated with secondary hyperlipidemia, atherosclerosis or oxidative stress.[39]

Table 3 - Effects of Coenzyme Q_{10} supplementation on plasma lipid concentration for normal and diabetic rats.

Sub-heading	Normal		Diabetic	
	Without CoQ10	With CoQ10	Without CoQ10	With CoQ10
Total cholesterol (mmol/L)				
4 months	1.93 ± 0.08	1.88 ± 0.12	2.42 ± 0.11‡	2.42 ± 0.04
8 months	2.12 ± 0.09	2.11 ± 0.12	2.51 ± 0.04‡	2.47 ± 0.05
13 months	2.38 ± 0.04	2.31 ± 0.04	2.70 ± 0.05‡	2.60 ± 0.06
Triacylglycerol (mmol/L)				
4 months	1.54 ± 0.03	1.46 ± 0.02*	2.66 ± 0.04‡	2.51 ± 0.08*
8 months	1.59 ± 0.04	1.51 ± 0.02*	2.74 ± 0.05‡	2.53 ± 0.04†
13 months	1.71 ± 0.04	1.63 ± 0.05	3.10 ±0.13‡	2.72 ± 0.05*
HDL-cholesterol (mmol/L)				
4 months	0.95 ± 0.03	1.04 ± 0.03*	0.76 ±0.04‡	0.84 ± 0.04
8 months	0.87 ± 0.02	0.93 ± 0.02*	0.75 ± 0.02*	0.83 ± 0.04*
13 months	0.86 ± 0.03	0.94 ± 0.03*	0.73 ±0.03†	0.78 ± 0.06

Values are reported as mean ± SE, N= 8 to10 rats, **p*<0.05, †*p*<0.005, ‡*p*<0.001 (student's t-test)

Oxidative damage by ROS appears to be involved in the pathogenesis of diabetic complications.[7,40] In this study, the significant decrease in body and tissue weights as well as the significant elevation of plasma glucose concentration in STZ-induced diabetic rats are typical and consistent with other reports.[41] The present results show that the plasma lipid concentrations of the diabetic animals are significantly affected throughout the different age groups (elevation of total cholesterol and triacylglycerol and reduction in HDL-cholesterol) which are in agreement with other results on animals and humans.[42,43] However, supplementation of CoQ_{10} to diabetic rats resulted in a significant increase of HDL-cholesterol, without affecting total cholesterol in the plasma, but the triacylglycerol concentration was significantly decreased. The concentration of heart tissue lipid peroxidation end products and the activity of erythrocyte SOD are significantly increased in the diabetic rats when compared with normal. This increase in tissue lipid peroxidation end products of the diabetic animals may indicate that oxidative cell damage has already occurred which also coincide with the work of Uzel et al[44] and Ustinova et al.[45] The increased erythrocyte Cu, Zn-SOD activity also supports the hypothesis of radical-mediated injury in this disease.[46] In addition, the highest SOD activity in red blood cells obtained at the onset of diabetes may be interpreted as compensatory activation mechanism due to increased superoxide radical generation. However, the heart tissue concentration of lipid peroxidation end products and the erythrocyte SOD activity are significantly reduced with CoQ_{10} supplementation to diabetic rats at the different age groups. These results agree with the results of Maulik[47] who observed a significant reduction of hearts lipid peroxidation products in heart tissues of normal rats under CoQ_{10}. The present study shows that diabetes induced by streptozotocin resulted in a significant decrease in the concentration of erythrocyte reduced GSH and erythrocyte GSH-Px activity either in young or old rats. Chronic hyperglycemia leads to the activation of the NADPH dependent aldose reductase, which diminishes the NADPH available for GSH reductase; consequently, the ratio of reduced to oxidized GSH decreases.[48] Glutathione, ubiquitous tripeptide, is involved in numerous cellular functions, including DNA and protein synthesis, amino acid transport, enzyme activation, protection of cells from harmful effects of radiation and free radicals and reactive oxygen intermediates.[13] The low GSH-Px activity obtained in this study could be directly explained by the low GSH concentration found in diabetic rats, since GSH is a substrate and a co-factor of this enzyme. As such, low GSH-Px activity may produce oxidative stress propensity. Moreover, pancreatic beta-cells are particularly sensitive to cytotoxic damage caused by free

Table 4 - Effect of Coenzyme Q10 supplementation on erythrocyte antioxidants and heart lipid peroxidation end product of normal and diabetic rats.

Sub-heading	Normal		Diabetic	
	Without CoQ10	With CoQ10	Without CoQ10	With CoQ10
Reduced glutathione (mmol/L)				
4 months	1.20 ± 0.03	1.23 ± 0.03	1.02 ± 0.04†	1.13 ± 0.02*
8 months	1.14 ± 0.03	1.15 ± 4.0	0.90 ± 5.0‡	1.08 ± 0.03*
13 months	1.07 ± 0.03	1.15 ± 0.02*	0.83 ± 0.06†	1.07 ± 0.05‡
Glutathione peroxidase (m-units/ml)				
4 months	275.0 ± 9.1	295.9 ± 10.1	193.5 ± 10.1‡	222.2 ± 6.1*
8 months	271.7 ± 8.9	294.3 ± 9.6	192.7 ± 7.6‡	222.0 ± 4.6*
13 months	269.8 ± 7.9	290.2 ± 11.0	189.4 ± 7.4‡	220.6 ± 5.6†
Superoxide dismutase (units/ml)				
4 months	117.6 ± 2.8	126.3 ± 3.4*	152.8 ± 3.7†	141.3 ± 4.3*
8 months	120.1 ± 2.9	126.2 ± 3.1	153.5 ± 3.6†	141.6 ± 3.3*
13 months	128.4 ± 2.9	135.4 ± 3.8	157.4 ± 3.3*	142.9 ± 2.8†
Lipid peroxidation end products (µmol/g)				
4 months	1.9 ± 1	1.4 ± 1†	3.2 ± 2‡	2.6 ± 1
8 months	2.0 ± 1	1.5 ± 1*	3.2 ± 2‡	2.7 ± 2*
13 months	2.4 ± 1	1.8 ± 2†	3.6 ± 2‡	2.9 ± 1*

Values are reported as mean ± SE, N= 8 to 10 rats, *p<0.05, †p<0.005, ‡p<0.001 (student's t-test).

radicals, and activity of antioxidant enzymes such as GSH-Px in these cells are low.[49] These results agree with the results of Mukherjee et al,[50] who observed a significant reduction of GSH contents after 15 days, and reduction of GSH-Px activity after 3 weeks in liver, kidney, brain and blood of streptozotocin-treated rats. In contrast, Scaleczky et al[46] found an increase in GSH-Px activity in diabetic rats. The possible disarrangement between high enzymatic SOD activity and low GSH-Px activity in erythrocytes of diabetic rats are probably because GSH-Px removes H_2O_2 produced by superoxide dismutase catalyzed reaction and an imbalance between the 2 enzymes may occur. The GSH levels and GSH-Px activity of erythrocytes obtained from diabetic rats were significantly increased with the supplementation of CoQ_{10} at different age groups stages as presented in this study. These alterations in lipoproteins, lipid peroxidation end products, and GSH concentrations as well as in SOD and GSH-Px activities with CoQ_{10} supplementation's to aging and diabetic rats are most likely because of the antioxidative effects of this substance. Coenzyme Q_{10}, ubiquinol-$_{10}$, is the most abundant form of ubiquinone or ubiquinol in the diet. This lipid soluble antioxidant functions as a component of electron transport systems; it also protects vitamin E by reducing alpha-tocopheryl radicals. In addition, it prevents in-vitro LDL oxidation.[47,49,50] Furthermore, the reduced form of CoQ_{10} has been shown to act as an antioxidant against free radical-mediated oxidation in membranes and lipoproteins.[18] The results of the experimental animal studies cannot be extrapolated directly to human disease. This finding has raised questions on the etiologic role of free radicals in human diabetes.

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