

Reduced coenzyme Q₁₀ in female smokers and its association with lipid profile in a young healthy adult population

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Abstract

Introduction: Cigarette smoking has a negative effect on body reserve of antioxidants and cholesterol metabolism. Coenzyme Q₁₀ (CoQ₁₀), a potent antioxidant synthesized as part of the cholesterol pathway, is a potential biomarker for systemic oxidative stress. We aimed to investigate gender variation in plasma lipid profile and CoQ₁₀ concentrations in healthy non-smokers and in smokers.

Material and methods: The study included 55 cigarette smokers (25 females and 30 males) and 51 non-smokers (25 females and 26 males) with the age range from 21 to 45 years, and who had no history of alcohol abuse or chronic diseases such as diabetes mellitus or obesity. Coenzyme Q₁₀ plasma concentrations were measured by reverse-phase high performance liquid chromatography (HPLC) with ultraviolet detection. Fasting plasma glucose and lipid levels were determined by standard colorimetric methods.

Results: Our results showed that CoQ₁₀ concentrations were significantly decreased in smokers, especially in females, than their non-smoker counterparts. Female smokers also exhibited a significant decrease in plasma concentrations of total cholesterol (TC), HDL-C, LDL-C, and atherogenic ratios HDL-C/TC and CoQ₁₀/LDL-C than male counterparts. Plasma triglyceride concentrations were increased in smokers irrespective of gender. Plasma CoQ₁₀ was relatively more associated with TC and LDL-C in female smokers than male smokers.

Conclusions: The adverse effects of smoking on body reserve of antioxidants and cholesterol metabolism are greater in females than in males, partially as a result of decreased CoQ₁₀ plasma concentrations, HDL-C and total-cholesterol and abnormal atherogenicity indices.

Key words: coenzyme Q₁₀, cigarette smoking, total cholesterol, low density lipoprotein, high density lipoprotein, triglycerides.

Introduction

The majority of respiratory system cancers and pulmonary vascular diseases are attributable to cigarette smoking [1, 2]. Smoking is also linked to cardiovascular diseases [3], stroke [4] and fertility disorders [5]. Moreover, an increased risk of mortality from cardiovascular diseases and cancer has been associated with cigarette smoking and exposure to cigarette smoke [6-8]. Prior studies examining the relationship between all-cause mortality and cigarette smoking have identified gender differences [9]; and alteration of cholesterol metabolism [10, 11] as significant contribut-

ing factors to the increased risk of death among active smokers.

Coenzyme Q₁₀ (CoQ₁₀), a lipid-soluble compound synthesized as part of the cholesterol pathway, is an essential cofactor in the mitochondrial respiratory chain, a potent antioxidant, and a potential biomarker for systemic oxidative stress [12-14]. Deficiencies in plasma CoQ₁₀ have been reported in many patient groups, including certain cardiovascular [15], neurological [16], hematological [17], neoplastic [18], renal [19], and metabolic diseases [20]. Moreover, plasma CoQ₁₀ concentration is an independent predictor of mortality in chronic heart failure [21].

Previous studies have reported controversial effects of smoking on CoQ₁₀. Smoking has been shown to be positively associated with plasma CoQ₁₀ [22, 23], whereas other studies reported no association [24, 25] or a negative association with plasma CoQ₁₀ levels [26]. Numerous factors have been suggested to contribute to this controversy, including gender, the levels of plasma cholesterol and triglycerides [25]. Therefore, we aimed to determine changes in CoQ₁₀ concentration induced by cigarette smoking in healthy subjects and to investigate the possible association of plasma CoQ₁₀ with gender and lipid profile.

Material and methods

Reagents

Methanol, ethanol and n-propanol were HPLC grades obtained from BDH (BDH Chemicals, Poole, Dorset, UK). Pure Coenzyme Q₁₀ and benzoquinone were obtained from Sigma (St. Louis, MO, USA) and used as standard. Reagent kits for the analysis of blood glucose, total cholesterol, LDL-C, HDL-C and triglycerides were purchased from Biosystems (S.A., Costa Brava, Barcelona, Spain).

Subjects

A total of 106 healthy volunteers (56 males, and 50 females), with an age range of 21-45 years and having no history of alcohol abuse or diseases such as diabetes mellitus or obesity, volunteered to participate in the research after their consent was obtained. Volunteers were excluded from the study if they were on antioxidant or lipid-lowering medication. The research has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association; and has been approved by the Ethics Committee of the university. Subjects were grouped according to their cigarette smoking status into non-smokers ($n = 51$) and smokers ($n = 55$) groups. Smoking status was assigned according to the following criteria: (1) at least 21 years old, (2) daily smokers, (3) at least a 5-year smoking history, (4) smoke at least 15 cigarettes per day. Anthropometric measurements such as

height and weight were made following standard techniques.

Biochemical tests

Blood samples were drawn at about 9.00 am after an overnight fast into heparinized Vacutainers (BD Diagnostics, Franklin Lakes, NJ, USA). Following centrifugation for 10 min at 4°C, plasma fraction was removed and stored at -80°C till analysis.

Fasting plasma glucose levels, total cholesterol (TC), triglycerides (TG), HDL-C, total protein and albumin were determined in all the subjects. Assays were performed with a Dade Behring Dimension RxL clinical chemistry system (Dade Behring; Germany) using its own kits. LDL-cholesterol (LDL-C) was calculated using the Friedewald's formula [27]. The atherogenic ratio of TC/HDL-C as well as the atherogenic index of plasma (AIP), calculated as $\log(TG/HDL-C)$, with TG and HDL-C, were measured in molar concentrations [28].

Total plasma coenzyme Q₁₀ was measured by reverse-phase high performance liquid chromatography (HPLC) with ultraviolet detection using the method of Mosca *et al.* [29] with a slight modification. The method is based on oxidation of CoQ₁₀ in the sample by treating it with *para*-benzoquinone. Coenzyme Q₁₀ was then quantitatively extracted into 1-propanol with a fast one-step extraction procedure, after centrifugation, samples were injected directly into the HPLC apparatus [30]. Pre-oxidation of the sample ensures quantification of total CoQ₁₀ by ultraviolet (UV) detection. The separation was performed using reversed-phase Supelcosil LC18 column (Supelco, Bellefonte, PA, USA). The mobile phase was represented by ethanol-methanol (65-35%). The flow rate was 1.5 ml/min and UV detection was carried out at 275 nm. This method achieves a linear detector response for peak area measurements over the concentration range of 0.05-3.47 μM with a CV of 1.6% for samples approaching normal values (1.02 μM). Supplementation of the samples with known amounts of CoQ₁₀ yielded a quantitative recovery of 97.5-99%.

Statistical analysis

Results were expressed as mean \pm standard deviation. Statistical analysis was performed using an unpaired *t* test with *Welch's* correction. Correlation analysis was done using linear regression and Pearson's correlation coefficient. Statistical significance was defined by $p < 0.05$. All statistical tests were two-tailed. Calculations were done using SPSS for Windows (release 13.0, 2004; SPSS Inc., Chicago, IL).

Results

A total of 106 subjects (50 females and 56 males) met eligibility criteria and were grouped according to cigarette smoking habit into a non-smoking

group ($n = 51$) and a currently smoking group ($n = 55$). No significant differences in age, sex or body mass index (BMI) were observed between studied groups (Table I).

Table II summarizes the main differences in the measured parameters between studied groups. There were no significant differences in plasma CoQ₁₀ concentrations between male and female in the non-

Table I. Age and body mass index (BMI) of female and male individuals for all groups

Parameter	Gender	Non-smokers ($n = 51$)	Smokers ($n = 55$)	Value of p^b
Number (%)	Female ($n = 50$)	25 (49%)	25 (45%)	0.845 ^c
	Male ($n = 56$)	26 (51%)	30 (55%)	
Age [years] ^a	Female ($n = 50$)	36.3 ±12.1	34.6 ±7.5	0.553
	Male ($n = 56$)	34.3 ±12.7	35.1 ±12.7	0.815
	Value of p	0.565	0.201 ^c	
BMI [kg/m ²] ^a	Female ($n = 50$)	24.2 ±3.0	25.5 ±3.3	0.153
	Male ($n = 56$)	25.9±2.0	27.0 ±4.8	0.258
	Value of p	0.863	0.177 ^c	

^aResults are presented as mean ± SD, ^b p values are calculated by unpaired t -test with Welch's correction, ^c p value is calculated by Fisher's exact test (2-sided)

Table II. Plasma coenzyme Q10 (CoQ₁₀) and biochemical parameters in studied groups

Parameter ^a	Gender	Non-smokers ($n = 51$)	Smokers ($n = 55$)	Value of p^b
CoQ ₁₀ [μmol/l]	Female ($n = 50$)	1.32 ±0.30	0.44 ±0.20	0.0001
	Male ($n = 56$)	1.50 ±0.45	0.76 ±0.35	0.006
	Value of p	0.053	0.003	
Glucose [mmol/l]	Female ($n = 50$)	5.60 ±0.60	6.20 ±1.90	0.141
	Male ($n = 56$)	5.40 ±0.40	5.90 ±1.70	0.128
	Value of p	0.178	0.548	
Cholesterol [mmol/l]	Female ($n = 50$)	5.10 ±0.90	4.10 ±0.90	0.005
	Male ($n = 56$)	4.90 ±0.80	4.70 ±1.13	0.44
	Value of p	0.40	0.034	
HDL-C [mmol/l]	Female ($n = 50$)	1.39 ±0.31	0.90 ±0.12	0.0001
	Male ($n = 56$)	1.29 ±0.29	1.03 ±0.26	0.02
	Value of p	0.07	0.001	
LDL-C [mmol/l]	Female ($n = 50$)	2.95 ±0.33	2.02 ±0.46	0.001
	Male ($n = 56$)	3.03 ±0.41	3.11 ±0.61	0.563
	Value of p	0.48	0.001	
Triglycerides [mmol/l]	Female ($n = 50$)	1.69 ±0.54	2.02 ±0.44	0.003
	Male ($n = 56$)	1.78 ±0.42	2.26 ±0.51	0.001
	Value of p	0.164	0.105	
AIP	Female ($n = 50$)	0.09 ±0.05	0.35 ±0.13	0.001
	Male ($n = 56$)	0.13 ±0.09	0.34 ±0.11	0.001
	Value of p	0.07	0.961	
TC/HDL-C	Female ($n = 50$)	3.67 ±1.10	4.56 ±1.53	0.026
	Male ($n = 56$)	3.89 ±1.09	4.56 ±1.44	0.056
	Value of p	0.523	0.967	
CoQ ₁₀ /LDL-C [μmol/mmol]	Female ($n = 50$)	0.44 ±0.14	0.19 ±0.08	0.001
	Male ($n = 56$)	0.46 ±0.16	0.29 ±0.12	0.001
	Value of p	0.45	0.001	

AIP – The atherogenic index of plasma, defined as $\log(TG/HDL-C)$, ^aresults are presented as mean ± SD, ^b p values are calculated by unpaired t test with Welch's correction

smoking group. In contrast, female smokers showed significantly ($p < 0.003$) lower plasma CoQ₁₀ levels than male smokers. In addition, plasma CoQ₁₀ concentrations were significantly lower in male ($p < 0.006$) and female ($p < 0.0001$) smokers compared with their non-smoking counterparts. No significant differences were found between the studied groups regarding fasting blood glucose levels.

Regarding plasma cholesterol; female smokers showed significantly decreased plasma concentrations of total cholesterol compared with female non-smokers ($p < 0.005$) and male smokers ($p < 0.03$). Regarding HDL-C, male smokers showed significantly ($p < 0.02$) lower HDL-C than non-smoker males, whereas no significant differences were found in HDL-C concentrations between male and female non-smokers. Female smokers showed the lowest concentrations of plasma HDL-C (0.9 ± 0.12 mmol/l), which was significantly lower ($p < 0.001$) than non-smoker females. No gender differences were found in LDL-C concentrations between non-smoker groups, however in smoker groups, females showed significantly lower LDL-C than males ($p < 0.001$). Female smokers showed also significantly decreased LDL-C than non-smoker females ($p < 0.001$). Men showed higher triglycerides than women both in the non-smoking group and in the smoker group, but this trend did not reach statistical significance, whereas smokers in both in male and female groups showed significantly increased triglycerides compared with their non-smoker counterparts ($p < 0.001$ and 0.003 , respectively).

Table II also shows the average values of the atherogenic indices AIP, TC/HDL-C, and CoQ₁₀/LDL-C. All indices showed significant differences between smokers and non-smokers irrespective of sex, but CoQ₁₀/LDL-C was the only index differing significantly between female and male smokers ($p < 0.001$).

Spearman's correlation analyses of data are presented in Table III. Plasma CoQ₁₀ concentrations were significantly correlated with TC and LDL-C only in female smokers. No correlations were observed between CoQ₁₀ and age, BMI, glucose, HDL-C, or triglycerides.

Discussion

Cigarette smoking has a negative impact on oxidant defense systems not only through the production of reactive oxygen species (ROS) in smoke but also through the consumption of the body reserve of antioxidants [31-33]. In addition to these data, our results show that smoking has a negative impact on the plasma levels of the antioxidant CoQ₁₀. These results support the assumption that smoking induced-oxidative stress may be the result of, and/or the cause of, decreased plasma CoQ₁₀ [33].

The impact of smoking on plasma CoQ₁₀ was significantly more marked in female than male smok-

Table III. The correlation between plasma coenzyme Q₁₀ (CoQ₁₀) and biochemical parameters in studied groups

Parameter ^a	Group	Non-smokers (n = 51)	Smokers (n = 55)
Age [years]	Female (n = 50)	0.196	0.399
	Male (n = 56)	-0.22	0.106
BMI [kg/m ²]	Female (n = 50)	0.400	0.477
	Male (n = 56)	0.321	0.326
Glucose [mmol/l]	Female (n = 50)	0.107	0.342
	Male (n = 56)	0.024	0.333
Cholesterol [mmol/l]	Female (n = 50)	0.258	0.630***
	Male (n = 56)	0.397*	0.301
HDL-C [mmol/l]	Female (n = 50)	-0.266	0.093
	Male (n = 56)	0.090	0.209
LDL-C [mmol/l]	Female (n = 50)	0.199	0.577**
	Male (n = 56)	0.426*	0.031
Triglycerides [mmol/l]	Female (n = 50)	0.477**	-0.182
	Male (n = 56)	-0.030	0.297
AIP	Female (n = 50)	0.238	0.334
	Male (n = 56)	0.182	0.271
TC/HDL-C	Female (n = 50)	0.158	0.397
	Male (n = 56)	0.224	0.309
CoQ ₁₀ /LDL-C [μmol/mmol]	Female (n = 50)	0.274	0.596***
	Male (n = 56)	0.146	0.466**

^aResults are presented as a correlation coefficient, *significant at $p < 0.05$, **significant at $p < 0.01$, ***significant at $p < 0.001$

ers ($p < 0.003$). The same trend has been reported in previous studies which demonstrated that CoQ₁₀ is affected by gender [25, 34]. This finding may provide an additional factor for the higher vulnerability to the detrimental effects of cigarette smoking in female compared with male smokers. Previous studies have emphasized that females may respond differently to tobacco-specific carcinogens based on several factors, including that females having higher DNA adduct levels, an increased CYP1A1 expression, a decreased DNA repair capacity and an increased incidence of K-ras gene mutations [35-38].

Additional factors have been suggested to contribute to interindividual susceptibility to smoking-related morbidity and mortality including BMI [39], age [40], and total cholesterol [41]. Therefore, we studied these parameters in relation to plasma CoQ₁₀ concentrations of healthy smokers and non-smokers. In agreement with Wolters and Hahn study [42], no significant correlation was found between CoQ₁₀ levels and BMI or age. However, results of the present study show that smoking is associated with abnormal lipid profile, and that these abnormalities were more significantly evident

in female than male smokers. Female smokers exhibited significantly lower TC, LDL-C, and HDL-C values than both female non-smokers ($p < 0.005$; $p < 0.024$; $p < 0.0001$ respectively), and male smokers ($p < 0.03$; $p < 0.042$; $p < 0.028$ respectively). These findings are in agreement with a previous study in women aged less than 50 years [43]. These findings are in agreement with the Munster Heart Study (PROCAM) [44] and the Framingham Study [45] which reported decreased TC and LDL-C concentrations in smokers but not in non-smokers. These studies have also found evidence that total mortality in middle-aged men is increased at low TC and LDL-C concentrations in smokers but not in non-smokers [44, 45].

The relationship between serum TC level and mortality rate was found U-shaped as a result of a U-shaped association with mortality from infectious diseases, a negative association with mortality from cancer and alcohol-related diseases, and a positive association with mortality from cardiovascular diseases and diabetes/nephropathy. Accumulated evidence suggests that a low cholesterol level may be a metabolic consequence of cancer rather than a precursor [46]. Moreover, increasing evidence now suggests that low cholesterol levels are associated with not only cancer, but also adverse effects on the brain causing neuropsychiatric syndromes [47] and haemorrhagic stroke [48]. In the present study, results showed a significant positive correlation between CoQ₁₀ plasma concentrations and TC and LDL-C (both at $p < 0.001$) only in female smokers. This relationship suggests that those subjects with lower TC and LDL-C, as in the situation of female smokers, are more likely to have lower CoQ₁₀, and therefore could be more prone to cancer, infectious and brain diseases later in their life.

Moreover, these data are in agreement with earlier studies demonstrating a close connection between CoQ₁₀ and plasma lipids, as about 60% of plasma CoQ₁₀ is associated with LDL-C, and that CoQ₁₀ is mainly associated with plasma lipids rather than with cellular components [49, 50].

Numerous studies have indicated that LDL-C is associated with atherosclerosis plaque initiation and vulnerability to rupture, while cigarette smoking is more associated with plaque progression [51]. The atherogenic index of plasma (AIP) that has been suggested as a marker of plasma atherogenicity [28], was found in the present study to be significantly higher in male and female smokers ($p < 0.001$). We further tested whether this trend is valid for other proposed atherogenic indices TC/HDL-C and TG/HDL-C [52, 53]. Both indices were found significantly higher in smokers than non-smokers irrespective of sex.

Studies have proposed the ratio CoQ₁₀/LDL-C as a sensitive marker for development of athero-

sclerotic changes and coronary artery disease [50, 54, 55]. In our study, this ratio was significantly lower in smokers compared with non-smokers; however it was significantly lower in female smokers compared with male smokers suggesting that female smokers may be at increased risk of developing atherosclerosis. In addition, this finding may provide an additional clarification for previous findings of a much stronger association between smoking and coronary heart disease in women than men [51], which was attributable to arteriolar differences [51, 56].

In conclusion, this study suggests that the adverse effects of smoking are greater in females than in males, partially as a result of decreased CoQ₁₀ plasma concentrations, HDL-C and total-cholesterol and abnormal atherogenicity indices. There is clearly a need for further large-scale studies to be designed in such a way that they are sensitive to a range of factors that contribute to gender difference in susceptibility to smoking.

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