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For further information about this article or if you need reprints, please contact:

Khalid S. Al-Numair
Department of Food Science
and Nutrition,
College of Food Science
and Agriculture,
King Saud University,
P.O. Box 2460,
Riyadh 11451,
Saudi Arabia

Tel: 00966-1-4675885

Serum Lycopene, β -Carotene and α -Tocopherol Levels and Oxidative Stress in Healthy Active Saudi Male Cigarette Smokers

Khalid S. Al-Numair

This study was designed to evaluate the effect of cigarette smoking on hypertension and the serum levels of lycopene, β -carotene and α -tocopherols in relation to the concentration of oxidative marker malondialdehyde (MAD). In addition, serum levels of low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) and their specific apolipoproteins B and A1 (Apo B and A1), respectively, were evaluated in cigarette smokers. Two hundred healthy men (100 smokers and 100 non-smokers) aged between 30 and 50, from Jeddah, the second largest city in Saudi Arabia, volunteered to participate in this study. The mean systolic and diastolic blood pressure values were found to be significantly ($p < 0.05$) higher for smokers than for non-smokers. The serum concentrations of lycopene and β -carotene were significantly ($p < 0.05$) lower in cigarette smokers than in non-smokers whereas a slight decrease (not significant) in serum α -tocopherol was observed in smokers. In the same respect, there was a significant ($p < 0.05$) increase in the oxidative marker (MAD) in smokers. So, the Pearson's correlation coefficient for the serum lycopene and β -carotene levels and the serum malondialdehyde concentration in smokers were significantly inversely ($p < 0.05$) higher than that of non-smokers, whereas no significant correlation between serum α -tocopherol level and MAD concentration was observed in both smokers and non-smokers. Accordingly, smoking was shown to significantly ($p < 0.05$) increase LDL-C and its specific apo B, but significantly ($p < 0.05$) decrease HDL-C and specific apo A1. This makes the risk of chronic diseases and death higher in smokers. The obtained results indicate that lycopene and β -carotene are the most potential antioxidant, while α -tocopherol play a secondary role in the cigarette smoke free radical scavenge.

Key words: Cigarette smoke, hypertension, lycopene, β -carotene, α -tocopherol, lipid profile, malondialdehyde

INTRODUCTION

The oxidant-antioxidant system is balanced in healthy conditions (Irshad and Chaudhuri, 2002). Prooxidants and antioxidant maintain a ratio and shift in this ratio towards prooxidants gives rise to oxidative stress (Suwamalert *et al.*, 2007). It has been demonstrated that smoking may cause an increase in oxidative stress and an imbalance in antioxidant nutrient intake and status (Faruque *et al.*, 1995; Mezzetti *et al.*, 1995; Ross *et al.*, 1995; Ma *et al.*, 2000; Wei *et al.*, 2001). Potential explanations for the elevated levels of oxidative stress biomarkers in a population of smokers include both increased Reactive Oxygen Species (ROS) production from smoke exposure as well as an impaired antioxidant defense system. While cigarette smokers often have lower blood levels of antioxidants compared to non-smokers, it remains unclear whether this occurs primarily as a function of decreased dietary intake of antioxidant-rich food (Marangon *et al.*, 1998; Birkett, 1999; Ma *et al.*, 2000; Palaniappan *et al.*, 2001) or depletion of antioxidant circulation through exposure to chronic smoke (Alberg, 2000).

Several studies indicate that cigarette smoking lowers plasma β -carotenes, vitamin C and vitamin E levels and increases oxidative damage in smokers (Banerjee *et al.*, 1998; Durak *et al.*, 2000; Lykkesfeldt *et al.*, 2000; Codandabany 2000; Zhou *et al.*, 2000). Beta-carotene functions as an efficient singlet oxygen quencher and as a radical trapping antioxidant at low oxygen pressure to reduce the extent of nuclear damage and to inhibit lipid peroxidation induced by enzymatic sources of oxyradicals such as xanthine oxidase systems (Palozza and Krinsky, 1992). Tocopherol is the principal lipid soluble chain-breaking antioxidant in plasma and in the membrane of this tissues and acts as a predominant antioxidant in the LDL particle by trapping peroxy free radicals (Baker *et al.*, 1999).

Lycopene and α -tocopherol have been shown to be associated with a lower risk of chronic diseases such as cardiovascular disease and cancers (Tapiero *et al.*, 2004). The levels of these serum antioxidants may be turned down in smokers because they may be used to quench the free radicals (Trobs *et al.*, 2002; Bruno and Traber, 2005). Frohlich *et al.* (2006) mention that lycopene may be more effective to scavenge free radical in smokers than α -tocopherol because the metabolite of lycopene may have different biological activities than α -tocopherol. Lycopene has 2 and 10 times more singlet oxygen quenching activity than β -carotene and α -tocopherol, respectively (Heber and Lu, 2002).

Cigarette smoke is a potent exogenous source of free radicals and it has been shown to be associated with a higher risk of chronic diseases such as cancer and cardiovascular diseases (Dautzenberg, 2004; Nordquist *et al.*, 2004). Malondialdehyde (MAD) is one of the final products of lipid peroxidation process in cells. An increase in free radicals causes an overproduction of MAD, which is commonly known as a marker of oxidative stress (Del Rio *et al.*, 2005). Al-Numair (2006) found that water-pipe smoking decreases plasma HDL-C and its apo A1. However, it increases LDL-cholesterol, apo B, triglyceride and malondialdehyde levels. Accordingly, the present study was carried out to investigate serum lycopene, α -tocopherol and β -carotene levels in healthy Saudi male smokers and to determine the correlation between these antioxidants and the level of oxidative marker (MAD). The influence of cigarette smoking on lipids profile and apo A and apo B were also determined.

MATERIALS AND METHODS

Selection of subjects: The study was conducted in 2007-2008, in which two hundred Saudi males ranging in age from 30-50 years from Jeddah, the second largest city in Saudi Arabia, volunteered to participate in this study. One hundred of them never smoked and the other hundred had smoked at least 10 cigarettes per day for at least one year. The study did not include women because smoking is not a norm among women in the Saudi society. None of the volunteers had any history of cardiovascular, endocrine or gastrointestinal disorders and none was receiving medication or taking any nutritional supplement. The procedures of the study were approved by a research committee and a written consent was signed by all volunteers after careful explanation of the purpose and procedures of the study.

Demographic and smoking habits information: Each subject was interviewed and asked to provide demographic and smoking habit information. The demographics included age, marital status (married and not married) and education status (low-illiterate or elementary, medium -intermediate or secondary and high-college or higher). Smoking habits included smoking period (years) and number of cigarettes smoked daily.

Anthropometric measurements and blood pressure: Anthropometric measurements (weight and height) and blood pressure were taken by well-trained staff. All anthropometric measurements were taken with the participant wearing light clothing, standing relaxed and looking straight ahead, with arms at the sides, feet

together and with weight equally distributed over both legs (Gibson, 2005). The weighing scale was zeroed before and after every measurement and standardized with a certified weight every day. Weight measurements were taken using the Clinical Detecto Balance-Beam scale (Detecto scale Inc., Brooklyn, NY). Body Mass Index (BMI) was calculated using weight (in kilograms) divided by height (in meters squared). Blood pressure (mm Hg) was measured on the same arm with a standard cuff while the participant was sitting and in a relaxed position. Three separate measurements were taken and the average was recorded.

Collection of blood and laboratory methods: Subjects were asked not to smoke 12 h before sampling to exclude the effects of acute smoking on the blood parameters studied. Two overnight fasting blood samples were collected from all subjects. The first blood samples were centrifuged at 3000 g for 10 min at room temperature and then serum was analyzed for serum lipids and malondialdehyde. The second blood sample was centrifuged at 2000 g for 15 min at 4°C, then the serum samples were stored at -80°C until analyzed for serum lycopene, β-carotene and α-tocopherol. Hemolyzed samples were excluded from analysis.

Serum concentrations of LDL-C and HDL-C determinations were performed by standard procedures with the Cobas Integra analyzer (Roche Diagnostic Systems, Switzerland) (Chon *et al.*, 1988; Hino *et al.*, 1996). Apo A1 and Apo B were determined using the Immunoturbidimetric assay method (Rifai and King, 1986). Serum concentrations of malondialdehyde as oxidative stress marker were determined colorimetrically using the malondialdehyde Assay Kit according to the method of Ohkawa *et al.* (1979) which is based on the reaction between malondialdehyde and thiobarbituric acid.

α-tocopherol, lycopene and β-carotene are fat-soluble compounds. They were extracted from serum samples and measured by reverse-phase High Performance Liquid Chromatography (HPLC) and a spectrophotometric detector using a modification of the Thumham *et al.* (1988). Samples were protected from photo degradation by extraction under dimmed natural light, excluding direct sun and fluorescent light all times. UV detector was set at a wavelength of 450 and 292 nm for detecting carotenoids (β-carotene and lycopene) and α-tocopherol, respectively. All samples were analyzed in duplicate.

Data analysis: Data analysis was performed using the Statistical Package for the Social Sciences, version 11.0 (SPSS) computer software. Descriptive statistics were

adapted to display data in means±SD and percentages. The statistical method of t-test was used to compare the mean values obtained between the smoker and non-smoker groups. Chi-square statistical test was used to compare marital and education status of study subjects by group. Differences and correlations were considered significant whenever the p-value was (p<0.05).

RESULTS

General characteristics: The mean age, body weight, height and Body Mass Index of the subjects were statistically similar between smokers and non-smokers. No significant differences in marital and education status were identified between smokers and non-smokers. Diastolic and systolic blood pressure was significantly (p<0.05) higher in smokers than in non-smokers. The mean smoking period of male smokers was approximately 13 years. The mean number of cigarettes smoked by male smokers was about 18 per day (Table 1).

Serum concentrations of lipid profile and oxidative stress marker: Serum concentrations of HDL-C and Apo A1 were significantly (p<0.05) lower in smokers than in non-smokers. However, LDL-C and Apo B were significantly (p<0.05) higher in smokers than in non-smokers. Malondialdehyde as oxidative stress marker was significantly (p<0.05) higher in smokers than in non-smokers (Table 2).

Serum concentrations of α-tocopherol, lycopene and β-carotene: The lycopene and β-carotene serum concentration was significantly (p<0.05) lower in smokers than in non-smokers whereas α-tocopherol was

Table 1: General characteristics of male active smokers and non-smokers†

Parameters	Active smokers (n = 100)	Non-smokers (n = 100)
Age (year)	39.51±8.51 ^a	40.53±6.47 ^a
Marital status (%) [*]		
Currently married	80	79
Not married	20	21
Educational status (%) [*]		
Low education	0	0
Medium education	29	28
High education	71	72
Height (m)	1.73 ±0.11 ^a	1.72±0.10 ^a
Body weight (kg)	87.40±10.00 ^a	86.10±11.5 ^a
Body Mass Index (BMI)	25.26±3.0 ^a	25.02±3.2 ^a
Blood pressure (mmHg)		
Diastolic	88.10±3.24 ^a	80.99±2.98 ^b
Systolic	134.22±3.36 ^a	120.20±3.10 ^b
Smoking period (year)	12.89±1.30	-
No. of cigarettes smoked per day	17.50±0.50	-

†Values are expressed as Mean±SD unless indicate it. ^{*}Values expressed as percent with no significant difference between smokers and non-smokers (Chi-square). ^aValues with different letter(s) in the same row are significantly different at p<0.05 (t-test)

Table 2: Serum concentrations of lipid profiles and oxidative stress marker in male active smokers and non-smokers†

Parameters	Active smokers (n = 100)	Non-smokers (n = 100)
HDL-cholesterol (mmol L ⁻¹)	1.10±0.08 ^a	1.23±0.11 ^b
LDL-cholesterol (mmol L ⁻¹)	3.66±0.79 ^a	3.13±0.61 ^b
Apolipoprotein A1 (mmol L ⁻¹)	43.00±1.92 ^a	47.00±1.09 ^b
Apolipoprotein B (mmol L ⁻¹)	2.43±0.44 ^a	2.07±0.51 ^b
Malondialdehyde (nmol L ⁻¹)	2.31±0.41 ^a	1.97±0.49 ^b

†Values are Mean±SD, ^{ab}Values with different letter(s) in the same row are significant

Table 3: Serum concentrations of α-tocopherol, Lycopene and β-carotene in male active smokers and non-smokers†

Parameters	Active smokers (n = 100)	Non-smokers (n = 100)
α-tocopherol (μmol L ⁻¹)	29.80±4.24 ^a	29.65±5.42 ^a
Lycopene (μmol L ⁻¹)	0.30±0.09 ^a	0.41±0.08 ^b
β-carotene (μmol L ⁻¹)	0.27±0.11 ^a	0.38±0.10 ^b

†Values are Mean±SD, ^{ab}Values with different letter(s) in the same row are significantly different at p<0.05

Table 4: Correlation between oxidative stress marker and serum concentrations of α-tocopherol, Lycopene and β-carotene in male active smokers and non-smokers†

Parameters	Oxidative stress marker	
	Active smokers (n = 100)	Non-smokers (n = 100)
α-tocopherol	-0.14	-0.13
Lycopene	-0.78*	-0.69*
β-carotene	-0.81*	-0.74*

†Values are correlation (r). *Significant correlation at (p<0.05)

slightly lower (not significant) in smokers than in non-smokers (Table 3). The lycopene and β-carotene serum concentrations were inversely (p<0.05) correlated with oxidative stress marker (Malondialdehyde) in both groups, but there was a weak inverse correlation between α-tocopherol and the oxidative marker MAD (Table 4).

DISCUSSION

Cigarette smoking is the most popular form of smoking and is one of the most prevalent social habits worldwide. This makes smoking the leading preventable cause of death and disability in the world. In addition to mortality, smoking was shown to deplete the body of its endogenous antioxidants. Antioxidant depletion was shown to increase individual vulnerability to free radicals and other oxidant species produced by cigarette smoking (Elsayed and Bendich, 2001). Thus, the present study was carried out to determine the effect of cigarette smoking on hypertension serum levels of antioxidants (lycopene, β-carotene and α-tocopherols) and the concentration of the oxidative biomarker (MAD) as well as to investigate the correlation between serum levels of antioxidants and MAD and determine the influence of

cigarette smoking on serum LDL-C, HDL-C, apolipoprotein A1 and apolipoprotein B.

Cigarette smoking and anthropometric measurements and hypertension: The data in this study shows that there were no significant differences in height, weight and Body Mass Index (BMI) between smokers and non-smokers. These results are in conformity with those previously published by Al-Numair (2006), who mentioned that there was no significant effect on weight, height and body mass index of healthy Saudi men from Riyadh city, the capital of Saudi Arabia under cigarette smoking, while Kim *et al.* (2003) found that the slightly lower body weight of smokers was probably secondary to a lower caloric intake in smoking groups.

The present study shows that systolic and diastolic blood pressure were significantly (p<0.05) higher in cigarette smokers than in non-smokers. The systolic and diastolic values of smokers were arranged to the upper limit of the normal range. These results are confirmed by Al-Numair (2006), who found that the diastolic and systolic blood pressure were significantly (p<0.05) higher in smokers than in non-smokers. In addition, this observation suggests that hypertension, typically reported in smokers, reflects the effect of chronic and long-term vascular damage as mentioned by Kim *et al.* (2003).

Another explanation of smokers' induced hypertension might be attributed to altered trace element metabolism in chronic smokers as mentioned by Al-Numair (2006), who found that cigarette smoking decreased serum zinc and selenium, but increased copper concentration. However, it is not known whether these changes in Zn, Se and Cu are acute effects that occur shortly after the initiation of smoking or whether they are secondary to the development of smoking related to chronic diseases such as hypertension, hypozincemia and hypercopperemia, which can be characteristic of hypertension, independent of smoking.

Cigarette smoking and carotenoids: Carotenoids, one of the most widespread groups of naturally occurring pigments, are found in the red, yellow, orange and green parts of plants (Olson, 1994). The most well known are lycopene and β-carotene. In addition to being found in serum, carotenoids are also found in cell membrane (Chaudiere and Ferrari-Lliou, 1999). Carotenoids are efficient quenchers of singlet oxygen and they can directly scavenge free radicals and inhibit lipid peroxidations, specially lycopene, which has the strongest ability (DiMascio *et al.*, 1989; Liu *et al.*, 2008).

In the present study, cigarette smoking significantly ($p < 0.05$) decreased serum β -carotene. The results of this study confirm those of previous studies (Walmesley *et al.*, 1999; Chopra *et al.*, 2000; Marangon *et al.*, 1998), which reported lower plasma β -carotene concentrations in smokers (ranged from -17.0 to 50.7%) than in non-smokers. Moreover, Fukao *et al.* (1996) showed a dose-dependent decline in the geometric mean of serum β -carotene concentrations with greater smoking intensity. The cause of decreasing plasma β -carotene by smoking attributed to β -carotene was being removed from the circulation as a consequence of its interaction with the free radical load imposed by cigarette smoke and the normal turnover of β -carotene in the body was found to be accelerated by the elevated inflammatory response in smokers (Margetts and Jackson, 1996; Thurnha *et al.*, 1990). The level of lycopene in the current smoking group was significantly lower than in non-smoking group ($p < 0.05$). This was supported by many studies that smokers have lower plasma concentration of most carotenoid than non-smokers (Garg *et al.*, 2006; Van Antwerpen *et al.*, 1995). Pamuk *et al.* (1994) reported that smokers had lower geometric mean serum concentration of β -carotene, α -carotene, lycopene and β -cryptoxanthin -78 to 79% of those for non-smokers. Furthermore, women who smoked more than 10 cigarettes per day were estimated to have a geometric mean serum concentration of α -carotene, β -carotene and lycopene of only 69, 63 and 67%, respectively, of those in women who never smoked ($p < 0.05$). There is a report that lycopene is the most potential antioxidant activity among carotenoids (Weisburger, 2002). It has 2 and 10 times more singlet oxygen quenching activity than β -carotene and α -tocopherol, respectively (Heber and Lu, 2002). Metabolite of lycopene may be bioactive and responsible for the beneficial effects because isomers of lycopene may have different biological activities than α -tocopherol (Frohlich *et al.*, 2006).

Cigarette smoking and α -tocopherols: The current smoking group had lower serum α -tocopherol level than the non-smoking one, but not statistically significant. Evidence of an association between cigarette smoking and α -tocopherol status is controversial. Some studies found that smokers had significantly lower serum levels of α -tocopherol (Mezzetti *et al.*, 1995; Bolton-Smith *et al.*, 1991; Bashar and Mitra, 2004; Paula *et al.*, 2004; Helmersson *et al.*, 2005), whereas others reported no differences between smokers and non-smokers (Stryker *et al.*, 1988; Ascherio *et al.*, 1992; Sobczak *et al.*, 2004; Suwannalert *et al.*, 2007). Lycopene and α -tocopherol

may be used to neutralize the free radicals generated from smoking. In conclusion, lycopene may be more effective to scavenge free radical in these subjects who had risk of oxidative stress from smoking, than α -tocopherol and β -carotene.

Cigarette smoking and oxidative stress marker (MAD):

The current smokers had higher MAD levels than non-smokers. These results are consistent with previous studies (Suwannalert *et al.*, 2007; Dautzenberg, 2004; Del Rio *et al.*, 2005), which reported that the oxidative stress biomarker (MAD) was significantly higher in smokers than in non-smokers. It appears that smoking plays a major role in lowering plasma antioxidants and exhibit a greater degree of lipid peroxidation in smokers.

Correlation between serum concentrations of oxidative stress marker (MAD) and antioxidant concentrations (lycopene, β -carotene and α -tocopherol): In the present study, we found a significant ($p < 0.05$) inverse correlation between the concentration of serum MAD and β -carotene or lycopene in both smokers ($r = -0.78$ and -0.81) and non-smokers ($r = -0.69$ and -0.74). Moreover, these relations were strong in smokers than in non-smokers and also stronger between MAD and lycopene than between MAD and β -carotene while α -tocopherol shows a weak inverse correlation with MAD levels in both smokers and non-smokers ($r = -0.14$ and -0.13 , respectively). These results coincide with the result of Suwannalert *et al.* (2007), who found that serum lycopene was inversely correlated ($r = -0.38$) with MAD levels in healthy Thai elderly and also α -tocopherol showed no correlation with MAD levels. In the same respect, Sobczak *et al.* (2004) and Stryker *et al.* (1988) found a weak inverse ($r = -0.11$) relation only in women and concluded that cigarette smoking had no effect on plasma α -tocopherol concentration while Farchi *et al.* (2001) found a significant inverse relation between exposure to smoke and plasma β -carotene. Thus, lycopene may be more effective to scavenge free radical and prevent peroxidation in smokers than β -carotene and α -tocopherol.

Cigarette smoking and lipoproteins and apolipoproteins

A1 and B: Cigarette smoking significantly ($p < 0.05$) increases serum LDL-C and its specific apolipoprotein B, but significantly ($p < 0.05$) decreases HDL-C and its specific apolipoprotein A1 concentration. These results are consistent with our previous results on water-pipe smoking (Al-Numair *et al.*, 2007). We mentioned that smoking decreased vitamin C concentration, which led to a significant increase in LDL-C and its apo B concentrations and a decrease in HDL-cholesterol and its

specific apo A1 concentrations. These results are in agreement with the results of other investigators, who mentioned that vitamin C status has a significant positive association with HDL-cholesterol and apo A1 levels and an inverse association with LDL-C and apo B levels (Knishkowsky and Amitai, 2005; Vincent and Avedis, 1993). Sorei-Thomas *et al.* (1988) mentioned that vitamin C deficiency in Guinea pigs lowered serum apo A1 concentration by lowering its mRNA level and suppression of its synthesis in the liver. Ginter (1989) proposed that vitamin C influences HDL-cholesterol concentration through the regulation of lipoprotein lipase activity. Apolipoproteins were suggested as better indicators of cholesterol metabolism than lipoprotein. The apolipoproteins are more stable than their respective lipoproteins during acute changes and play an active role in lipoprotein metabolism (Avogaro *et al.*, 1979; Lahoz *et al.*, 2003). Apo A1 is an important structural protein of HDL-C (Avogaro *et al.*, 1979; Srinivasan and Berenson, 1995; Lahoz *et al.*, 2003).

Apo A1 functions as an activator of lecithin-cholesterol acetyl transferase, a key enzyme in reverse transport of cholesterol from peripheral tissues to the liver (Srinivasan and Berenson, 1995).

Apolipoprotein B facilitates cholesterol delivery to tissues and is an essential structural component of LDL-C. The hepatic LDL-apolipoprotein B activity and its receptors were a major determinant for hepatic cholesterol uptake and plasma cholesterol levels. The risk of cigarette smoking increased due to greater synthesis of LDL apolipoprotein B. Consequently, the result significantly increases of LDL-C. Al-Numair (2006) and Hughes *et al.* (1997) found that cigarette smoking is related to higher LDL-C and lower HDL-C, though there is a close response relationship. Hughes *et al.* (1997) and Fievet and Fruchart (1991) mentioned that the lowering effect of cigarette smoking on HDL-C related to increased lipase activity, lipoprotein lipase and hepatic lipase, which is associated with HDL-C metabolism. A unifying mechanism of smoking-induced higher serum LDL-C concentrations might be due to the effect of smoking on copper metabolism, which increases LDL-C peroxidation (oxLDL-C) (Esterbauer *et al.*, 1992). The peroxidation of LDL-C prevents its uptake by the body cells, causing an elevated concentration in serum (Al-Numair, 2006).

CONCLUSION

Smokers had significant lower plasma lycopene and β -carotene levels than non-smokers while plasma α -tocopherol level showed no significant difference

between smokers and non-smokers. MAD level was significantly higher in smokers than in non-smokers. Plasma lycopene and β -carotene were inversely related with the MAD level, whereas α -tocopherol showed weak inverse correlation with MAD level. Cigarette smoking increased atherogenic risk factors, which included elevation of hypertension, LDL-C and apo B and lower HDL-C and apo A1 levels in smokers compared with non-smokers.

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