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Short communication

Plasma malondialdehyde is induced by smoking: a study with balanced antioxidant profiles

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It has been reported that smokers have higher plasma malondialdehyde concentrations compared with non-smokers. However, smokers have also consistently been shown to have a lower intake of fruits and vegetables as well as lower plasma antioxidant concentrations. Since both the latter issues may well influence the malondialdehyde concentration, we wanted to investigate if the observed difference between smokers and non-smokers was a result of differences in antioxidant status or if a more direct effect of smoking could also be isolated. In the present study, the plasma malondialdehyde and antioxidant profiles of a cohort of smokers (*n* 48) and non-smokers (*n* 32) were compared. While there was no significant difference in the major plasma antioxidants measured, i.e. ascorbic acid, α- and γ-tocopherol and uric acid, we found a significant effect of smoking on plasma malondialdehyde ($P=0{\cdot}0003$). Consequently, the present study suggests that lipid peroxidation as measured by plasma malondialdehyde is induced by smoking *per se*. While poor antioxidant status presumably also affects lipid peroxidation, it is only partly responsible for the increased level found in smokers in general.

Malondialdehyde: Antioxidants: Smoking

Smoking has long been associated with an increased risk of developing several chronic diseases including atherosclerosis (McGill, 1990). The process of atherosclerosis is believed to be initiated by lipid peroxidation (Steinberg, 1997).

There are several reasons why smokers would be expected to have a higher steady-state level of lipid peroxidation compared with non-smokers. First of all, smokers are prone to oxidation from the inhalation of large numbers of gas-phase and other radicals giving rise to increased oxidative damage (Frei *et al.* 1991; Pryor & Stone, 1993). Second, depletion of plasma antioxidants otherwise protecting against oxidative damage such as lipid peroxidation has consistently been observed among smokers (Lykkesfeldt *et al.* 1996, 1997, 2000). Third, smokers have been shown to have a lower intake of fruits and vegetables (Morabia & Wynder, 1990; Preston, 1991; Serdula *et al.* 1996) also known to protect against oxidative damage (Hininger *et al.* 1997; Harats *et al.* 1998; La Vecchia & Tavani, 1998).

In agreement with the above rationale, smokers have indeed been shown to have higher levels of lipid peroxidation compared with non-smokers, as measured by increased plasma and urinary concentrations of F_2-isoprostanes, malondialdehyde (MDA) and conjugated dienes (Duthie *et al.* 1993; Block *et al.* 2002; Dietrich *et al.* 2002). However, because of the concurrently observed depletion of plasma antioxidants resulting both directly from smoking and from dietary differences (Lykkesfeldt *et al.* 2003), it has been difficult to distinguish between the effects of antioxidant status and smoking *per se*.

Using MDA as a marker of lipid peroxidation, we conducted a study to test if smoking results in elevated lipid peroxidation in a cohort of smokers (*n* 48) and non-smokers (*n* 32) in which dietary recommendations 1 month before sampling were included in order to balance their antioxidant profiles.

Methods

Subjects and study design

The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee of Copenhagen. Subjects were recruited from the

Abbreviation: MDA, malondialdehyde.

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greater Copenhagen area using newspaper advertisements. At the pre-screening interview 1 month before the study, the participants were given dietary recommendations regarding the intake of foodstuffs for the period up until the time of blood sampling. The purpose was to diminish the dietary differences between the smokers and non-smokers. The subjects were instructed to avoid a variety of fruits and vegetables particularly rich in antioxidants, i.e. to consume a diet more comparable with that of smokers in general. Inclusion criteria were as follows: >18 years of age, smoking more than five cigarettes per d (smokers only), normal weight ($20\,kg/m^2 \leq BMI \leq 30\,kg/m^2$) and signing the informed-consent form. Exclusion criteria were chronic diseases, regular use of medicine, any use of vitamin or other dietary supplements after pre-screening, and smoking within the last 2 years (non-smokers only). Finally, eighty healthy male volunteers (forty-eight smokers and thirty-two non-smokers) between 21 and 63 years of age were included in the study. Information on any previous use of vitamin supplements was recorded. No subjects withdrew from the study after recruitment.

Sample collection and biochemical analyses

An overnight fasting blood sample was collected from each subject by using EDTA as an anticoagulant. The blood samples were immediately centrifuged at 2000 ***g*** for 5 min (4°C) and plasma was separated and processed for the following analyses.

Plasma samples for ascorbic acid measurement were immediately stabilised with an equal amount of 10 % (w/v) *meta*-phosphoric acid containing 2 mM-disodium–EDTA. The precipitate was removed by centrifugation at 16 000 ***g*** for 1 min (4°C) and the supernatant fractions were stored at −80°C for less than 1 month until analysis. The concentration of ascorbic acid in plasma was measured by using reversed-phase HPLC with coulometric detection as described elsewhere (Lykkesfeldt, 2000, 2001*a*, 2002). The remaining plasma was frozen neat in samples at −80°C for the analysis of α- and γ-tocopherol by HPLC with amperometric detection as described by Sattler *et al.* (1994) or for the analysis of MDA as dithiobarbituric acid adduct by HPLC with fluorescence detection as described previously (Lykkesfeldt, 2001*b*). The detection limit of the MDA assay was 0·1 μmol/l and the within- and between-day CV were 1·2 and 6·2 %, respectively (*n* 12). These values represent the entire assay including derivatisation and are based on authentic standards. Plasma cholesterol was measured by using a commercial kit (Sigma, St Louis, MO, USA).

Statistical analysis

Data were analysed by using Statistica 6 (StatSoft, Tulsa, OK, USA). Homogeneity of variances was verified by Levene's test. Differences between smokers and non-smokers were analysed by one-way ANOVA. A two-tailed *P* value of <0·05 was considered statistically significant. Values are reported as means and standard deviations.

Results and discussion

In a large recent survey, it was demonstrated that tobacco smoking is a major predictor of plasma MDA and F_2-isoprostanes, two commonly used biomarkers of lipid peroxidation (Block *et al.* 2002). However, higher levels of lipid peroxidation among smokers in Block's and several previous studies could in fact primarily be a secondary effect of the poorer antioxidant status also found in smokers. That antioxidant status, in particular that of vitamin C, is a major factor in lipid peroxidation is supported by several intervention studies. These studies link antioxidant supplementation to lower lipid peroxidation among smokers (Duthie *et al.* 1993; Rifici & Khachadurian, 1993; Nyyssonen *et al.* 1994; Mezzetti *et al.* 1995; Howard *et al.* 1998; Kaikkonen *et al.* 2001; Kim & Lee, 2001; Block *et al.* 2002; Dietrich *et al.* 2002).

In the present study, we wanted to investigate the effect of smoking in a cohort of smokers and non-smokers with similar antioxidant profiles. The smokers did not differ from the non-smokers in age or BMI (Table 1). At the pre-screening interview 1 month before the blood sampling, the subjects had been instructed to avoid fruits and vegetables particularly rich in antioxidants. At 1 month later, there was no significant difference in antioxidant status between the smokers and non-smokers as measured by plasma ascorbic acid, α- and γ-tocopherol and uric acid (Table 2). However, in contrast, the smokers had significantly higher plasma levels of MDA ($P=0{\cdot}0003$; Table 2). Cholesterol has been shown to correlate with plasma MDA (Block *et al.* 2002). However, the

Table 1. Characteristics of the study population
(Mean values and standard deviations)

	Non-smokers (*n* 32)		Smokers (*n* 48)	
	Mean	SD	Mean	SD
Age (years)	36·1	12·4	38·6	12·1
BMI (kg/m^2)	24·3	2·6	23·7	2·8
Smoking habits (no. cigarettes/d)	–		17·5	8·1
Pack years (years)*	–		16·5	14·1

*Pack years is the number of packs of cigarettes smoked per day multiplied by the number of years the person has smoked.

Table 2. Plasma concentrations of ascorbic acid, tocopherols, uric acid and malondialdehyde (MDA) in overnight fasting samples†
(Mean values and standard deviations)

	Non-smokers (*n* 32)		Smokers (*n* 48)	
	Mean	SD	Mean	SD
Ascorbic acid (μmol/l)	45·3	16·2	44·9	17·3
α-Tocopherol (μmol/l)	31·4	8·6	30·8	9·6
γ-Tocopherol (μmol/l)	1·82	0·86	1·73	0·78
Uric acid (μmol/l)	386	87	352	76
MDA (μmol/l)	0·82	0·18	1·06**	0·34
MDA corrected for total cholesterol	0·17	0·03	0·22*	0·09

Mean value was significantly different to that for non-smokers by one-way ANOVA: *$P=0{\cdot}0022$, **$P=0{\cdot}0003$.
† For details of subjects and procedures, see Table 1 and p. 204.

difference in plasma MDA observed in the present study persisted when using cholesterol-corrected values ($P=0{\cdot}0022$; Table 2). Thus, the present data suggest that smoking itself induces lipid peroxidation. Block's group also found an inverse correlation between plasma ascorbic acid and MDA (Block *et al.* 2002), further supporting the link between antioxidant status and MDA, but such a correlation was not found in the present cohort. However, we did observe a positive correlation between plasma MDA and age (r 0·24; $P=0{\cdot}034$). Such a correlation has recently been reported in a study in which antioxidant intakes were matched between smokers and non-smokers (Jacob *et al.* 2003).

Information on previous use of vitamin supplements was recorded for the smokers, while the non-smokers (controls) had no history of supplement use. Twenty of the forty-eight smokers had taken various forms of vitamin supplements up to the time of pre-screening. However, at the time of the blood sampling 4 weeks later, there was no difference in plasma antioxidant concentrations between the smokers who had taken supplements and those who had not (data not shown). Consistent with this, there was no effect of previous supplementation history on plasma MDA (data not shown).

In conclusion, we show in the present study that smokers have higher plasma concentrations of MDA compared with non-smokers in spite of balanced antioxidant profiles. This suggests that the observed increase in plasma MDA was induced by smoking *per se*. While poor antioxidant status presumably also affects lipid peroxidation as shown in several previous studies, the present study shows that it is only partly responsible for the increased level found in smokers in general.

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