Plasma lipid peroxides: relationships to cardiovascular risk factors and prevalent cardiovascular disease

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Received 14 July 2003 and in revised form 26 August 2004

Summary

Background: The epidemiology of plasma lipid peroxides, which may play a role in atherogenesis, has not been well defined.

Aim: To study the relationships of plasma lipid peroxides to cardiovascular risk factors in a random population sample.

Design: Random, age- and sex-stratified population sample.

Methods: We studied 739 men and women aged 25–74 years. Lipid peroxides were assayed by the thiobarbituric acid (TBA) assay for malondialdehyde (MDA) in stored plasma samples.

Results: Lipid peroxide levels increased with age. In men, lipid peroxides were significantly associated with smoking habit. Lipid peroxides correlated with non-fasting serum triglycerides (r = 0.33; p < 0.0001) in both sexes. Weaker associations were observed for cholesterol, high-density lipoprotein cholesterol (inversely), body mass index, fibrinogen and white cell count; as well as an inverse association with serum vitamin C in men.

Discussion: These findings clarify the relationships of plasma lipid peroxides to cardiovascular risk factors; and are consistent with the hypothesis that lipid peroxidation may be one mechanism through which several risk factors may promote cardiovascular disease.

Introduction

There is current interest in lipid peroxidation and its relationships to human atherosclerosis and cardiovascular risk factors, including deficiency of antioxidants.¹⁻⁸ Measures of lipid peroxidation include: the thiobarbituric acid (TBA) assay for aldehydes, including malonaldehyde (MDA), which are degradation products of lipid peroxides; measurement of conjugated dienes in low density lipoprotein (LDL); and 8-isoprostanes, which are oxidation products of arachidonic acid.¹⁻⁸ Using the TBA test, Springer et al. reported increased plasma lipid peroxides in patients with coronary or peripheral arterial disease compared to controls, as well as an association with serum triglycerides, but not other cardiovascular risk factors, in the 75 control patients.² In a previous case-control study of peripheral arterial disease in a random sample of the older population, we observed higher plasma lipid peroxide levels (with the TBA assay, but not by the conjugated dienes assay) in cases, as well as associations with smoking, serum lipids, and several haemostatic variables.⁶ Similarly, a study of 35 healthy non-smokers found a stronger relationship of risk factors to the TBA assay than to conjugated dienes.⁹

There is a lack of large epidemiological studies of the distributions of plasma lipid peroxides in the
general population, and their relationships to cardiovascular risk factors or prevalent cardiovascular disease. Therefore, we studied the distributions of plasma lipid peroxide (TBA-MDA) levels in a large, age- and sex-stratified sample of the north Glasgow population aged 25–74 years: the Third WHO-MONICA Survey. We report their relationships to cardiovascular risk factors (measured by standardized MONICA Study methods), prevalent cardiovascular disease, and a wide range of haemostatic and inflammatory variables previously reported from this survey.10–13

Methods

The Third MONICA Survey in north Glasgow was one of a series of cross-sectional random samples of this community conducted as part of the international MONICA project.14 Research ethical committee approval was obtained, and all participants gave informed consent, according to the Declaration of Helsinki. Altogether, 1958 men and women aged 25–74 years were randomly sampled from general practice registers, this representing a 64% response rate among those originally selected with whom contact was made. Subjects were sent a questionnaire, which included socio-demographic questions, questions on past medical history and current medication, questions on smoking and alcohol consumption, the Rose chest pain questionnaire, and the Edinburgh claudication questionnaire.14 Subjects were asked to bring the questionnaire with them, for checking, to a health screening clinic. At the clinic, height, weight and blood pressure were measured, a 12-lead electrocardiogram (ECG) was recorded, carbon monoxide was measured in the subject’s expired air, and a blood sample (non-fasting) was taken. Whenever the sample was sufficiently large, it was separated into two main parts for distribution to Ninewells Hospital, Dundee, and the Glasgow Royal Infirmary. Serum total cholesterol, HDL cholesterol, triglycerides, thiocyanate, cotinine and vitamin C were measured as previously described.10–14 Haemostatic and inflammatory variables were assayed as previously reported.10–13 Lipid peroxides were measured by the TBA-MDA assay as previously described,6 in samples stored at −70°C for <12 months. For logistical reasons, only subjects who attended in the morning (0900–1300 h) were studied.

Subjects were classified as positive for cardiovascular disease (CVD) if: (i) they stated that they had ever been told by a doctor that they had, or had had, angina, a heart attack (myocardial infarction (MI), coronary thrombosis) or a stroke; (ii) they were classified as having angina or possible MI by the Rose questionnaire; (iii) they were classified as having peripheral vascular disease by the Edinburgh questionnaire; or (iv) they had Q/QS patterns or ST of T wave changes on their ECG.15 Everyone else was classified as negative for CVD, except those who had not been classified as CVD-positive, but had declared that they took medication consistent only with prevalent CVD; they were excluded on the grounds that their CVD status was ambiguous.

Statistical methods

Lipid peroxide levels were first compared with commonly-accepted cardiovascular risk factors and then associated with prevalent CVD. Since age is an important determinant of all these outcomes, all analyses were age-adjusted.

Social class was measured using the occupational definition of the Office of Population Censuses and Surveys,14 with married women coded according to their husband’s occupation. Smoking status (never/ex/current) was derived from self-reported current and previous smoking of cigarettes, pipes and cigars. Alcohol status was classified as ‘never’ if the subject reported that they had never taken alcoholic drinks, ‘zero’ if they reported some lifetime alcohol consumption but no consumption in the past 7 days, and then into three positive consumption groups according to the tertiles (within sex groups) of reported consumption over the last 7 days.

Multiple linear logistic regression was used to calculate age- and multiple-adjusted odds ratios for prevalent CVD by sex-specific quarters of lipid peroxides. Only multiple-adjustments for a single set of most-commonly accepted CVD risk factors, without unnecessary redundancy, are included here.

Results

Lipid peroxide results were obtained in 739 persons (368 men, 371 women). Their distributions in men and women are shown in Figure 1. Within each sex group, the distribution of lipid peroxides was found to be well approximated by a normal probability distribution. Table 1 summarizes the distributions by age and sex: at least 57 results were available in each 10-year age and sex group. Median lipid peroxide levels increased by about 10% between 30 and 70 years of age, both in men ($r=0.11$, $p=0.046$) and in women ($r=0.19$, $p=0.0002$). Overall, there was no significant sex difference in lipid peroxide levels, but men had slightly higher
levels than women in the 25–34 year age group ($p=0.04$).

There were no significant relationships between lipid peroxide levels and use of oral contraceptives in women aged 25–34 years, or menopausal or hormone replacement therapy status in women aged 45–54 years (data not shown).

Lipid peroxide levels were significantly higher in male current smokers (mean $4.96\,\mu\text{mol/l}$; SE 0.09) compared to ex-smokers ($4.55\,\mu\text{mol/l}$; 0.11) or never smokers ($4.72\,\mu\text{mol/l}$; 0.13; $p=0.01$); the relationship to smoking in women was not statistically significant ($p=0.20$). Conversely, lipid peroxide levels were unrelated to alcohol consumption in men, but were lower in current drinkers among women (mean $4.62\,\mu\text{mol/l}$, SE 0.07 vs. $5.06\,\mu\text{mol/l}$, 0.16; $p=0.04$). Lipid peroxide levels were higher in women of manual compared to non-manual social class (mean $4.82\,\mu\text{mol/l}$; SE 0.09).

**Table 1** Lipid peroxide levels ($\mu\text{mol/l}$) by age and sex, and correlation of lipid peroxides with age

<table>
<thead>
<tr>
<th>Age band</th>
<th>Men</th>
<th>Women</th>
<th>$\rho$</th>
</tr>
</thead>
<tbody>
<tr>
<td>25–34 years</td>
<td>Median (1st, 3rd quartiles)</td>
<td>4.56 (4.00, 5.18)</td>
<td>4.38 (3.70, 4.85)</td>
</tr>
<tr>
<td></td>
<td>5th–95th percentiles</td>
<td>3.05–6.53</td>
<td>3.01–5.79</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td>35–44 years</td>
<td>Median (1st, 3rd quartiles)</td>
<td>4.49 (3.86, 5.25)</td>
<td>4.43 (3.78, 5.23)</td>
</tr>
<tr>
<td></td>
<td>5th–95th percentiles</td>
<td>2.85–6.16</td>
<td>2.80–5.70</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>60</td>
<td>86</td>
</tr>
<tr>
<td>45–54 years</td>
<td>Median (1st, 3rd quartiles)</td>
<td>4.75 (4.15, 5.35)</td>
<td>4.75 (4.05, 5.26)</td>
</tr>
<tr>
<td></td>
<td>5th–95th percentiles</td>
<td>3.55–6.53</td>
<td>3.45–7.20</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>67</td>
<td>86</td>
</tr>
<tr>
<td>55–64 years</td>
<td>Median (1st, 3rd quartiles)</td>
<td>4.75 (4.00, 5.65)</td>
<td>4.88 (4.31, 5.77)</td>
</tr>
<tr>
<td></td>
<td>5th–95th percentiles</td>
<td>3.23–6.85</td>
<td>3.00–7.40</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>78</td>
<td>68</td>
</tr>
<tr>
<td>65–74 years</td>
<td>Median (1st, 3rd quartiles)</td>
<td>4.90 (4.05, 5.62)</td>
<td>4.72 (4.00, 5.78)</td>
</tr>
<tr>
<td></td>
<td>5th–95th percentiles</td>
<td>3.33–7.30</td>
<td>3.15–7.65</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>86</td>
<td>74</td>
</tr>
<tr>
<td>Total</td>
<td>Median (1st, 3rd quartiles)</td>
<td>4.71 (4.02, 5.39)</td>
<td>4.65 (3.90, 5.34)</td>
</tr>
<tr>
<td></td>
<td>5th–95th percentiles</td>
<td>3.27–6.75</td>
<td>3.05–6.97</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>348</td>
<td>371</td>
</tr>
<tr>
<td>Spearman $r$</td>
<td>0.11</td>
<td>0.19</td>
<td>($n=368$, $p=0.046$)</td>
</tr>
</tbody>
</table>
Table 2  Age-adjusted Spearman rank correlations ($n, p$) of lipid peroxides with conventional cardiovascular risk factors, which are continuous variables

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>0.33 (344, 0.0001)</td>
<td>0.33 (369, 0.0001)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.12 (344, 0.02)</td>
<td>0.19 (369, 0.0003)</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-0.13 (331, 0.01)</td>
<td>-0.12 (357, 0.02)</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.02 (344, 0.68)</td>
<td>0.15 (369, 0.003)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.11 (346, 0.05)</td>
<td>0.12 (369, 0.02)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.04 (348, 0.45)</td>
<td>0.08 (371, 0.10)</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.06 (348, 0.28)</td>
<td>0.07 (371, 0.20)</td>
</tr>
<tr>
<td>Plasma vitamin C</td>
<td>-0.15 (341, 0.007)</td>
<td>-0.06 (362, 0.30)</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>0.18 (341, 0.0009)</td>
<td>0.09 (366, 0.09)</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>0.16 (344, 0.003)</td>
<td>0.11 (368, 0.04)</td>
</tr>
<tr>
<td>Cotinine</td>
<td>0.21 (342, 0.0002)</td>
<td>0.08 (365, 0.14)</td>
</tr>
</tbody>
</table>

Table 2 shows that lipid peroxides were significantly associated with whole-blood viscosity and its two major determinants, haematocrit and plasma viscosity. The association with blood viscosity remained significant after correction of viscosity to a standard haematocrit of 45%. There was no significant association with relative viscosity (corrected blood viscosity/plasma viscosity). Lipid peroxides were also significantly associated with several other variables that are positive acute-phase reactants: red cell aggregation (in men only), white blood cell count, fibrinogen and C-reactive protein. However, lipid peroxide levels were not associated with levels of interleukin-6. Because lipid peroxides, as well as many haemostatic or inflammatory variables, are related to age and serum triglycerides,6,9–13 Table 3 also shows their age- and triglyceride-adjusted associations with lipid peroxides. Blood and plasma viscosity and haematocrit remained significantly associated with lipid peroxides following such adjustment. The associations with C-reactive protein, corrected viscosity (in women), and red cell aggregation, white cell count and fibrinogen (in men), became non-significant after such adjustment.

For haemostatic variables, significant associations were observed with coagulation factor IX and antithrombin in men and women; and also with protein C, protein S and activated partial thromboplastin time (APTT) in men. However, all these associations became non-significant after adjustment for age and triglycerides; with the exception of factor IX in women ($r = 0.16, n = 349; p = 0.02$) and APTT in men ($r = 0.16, n = 214, p = 0.02$).

Table 4 shows that plasma lipid peroxides were significantly associated with prevalent cardiovascular disease (CVD) in men: the age-adjusted odds ratio for CVD in the highest quarter compared to the lowest quarter was $1.97$ (95% CI 1.00–3.80). On multiple adjustment for major risk factors, this odds ratio fell to $1.36$ (0.61–3.02) ($p = 0.27$). The age-adjusted association of lipid peroxides was weaker and not statistically significant in women, and was totally absent after multiple risk factor adjustment.

**Discussion**

While the thiobarbituric acid (TBA) assay of malondialdehyde (MDA) and other aldehydes has its...
Limitations as a measure of lipid peroxidation in vivo, as with other assays, significant associations with coronary or peripheral arterial disease in case-control studies have been reported, as have associations with some cardiovascular risk factors in smaller studies. These previous reports suggest that this assay is one measure of lipid peroxidation that can be studied in larger epidemiological surveys, to examine the hypothesis that lipid peroxidation may be a mechanism through which known risk factors may promote atherothrombotic cardiovascular disease (i.e. coronary heart disease (CHD), peripheral arterial disease, and stroke). The present study appears to be the largest reported epidemiological survey of this assay of lipid peroxidation in a random sample of the general population (n = 739). We report the associations of lipid peroxides with most major cardiovascular risk factors in men and women, using the internationally-standardized methods of the WHO-MONICA Study, with a wide range of haemostatic and inflammatory variables, assayed using international or national standards where applicable, and with prevalent cardiovascular disease.

The distribution of plasma lipid peroxides in men and women was found to be approximately normal. The median level increased by about 10% between 30 and 70 years of age in both men and women, which may be relevant to the increasing prevalence of atherosclerosis with age. While overall there was no significant sex difference in lipid peroxides over the whole age range studied, the higher level in men compared to women in the youngest 10-year

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted*</td>
</tr>
<tr>
<td>Blood viscosity</td>
<td>0.28 (278, 0.0001)</td>
<td>0.23 (275, 0.0002)</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>0.26 (289, 0.0001)</td>
<td>0.22 (286, 0.0001)</td>
</tr>
<tr>
<td>Corrected viscosity</td>
<td>0.22 (255, 0.0005)</td>
<td>0.18 (252, 0.004)</td>
</tr>
<tr>
<td>Relative viscosity</td>
<td>0.12 (255, 0.06)</td>
<td>0.12 (252, 0.06)</td>
</tr>
<tr>
<td>Plasma viscosity</td>
<td>0.24 (314, 0.0001)</td>
<td>0.16 (310, 0.006)</td>
</tr>
<tr>
<td>Red cell aggregation</td>
<td>0.18 (287, 0.002)</td>
<td>0.05 (284, 0.42)</td>
</tr>
<tr>
<td>White cell count</td>
<td>0.14 (260, 0.02)</td>
<td>0.10 (257, 0.10)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.12 (305, 0.04)</td>
<td>0.09 (302, 0.11)</td>
</tr>
<tr>
<td>CRP</td>
<td>0.16 (184, 0.03)</td>
<td>0.09 (181, 0.21)</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>0.00 (138, 0.99)</td>
<td>—0.04 (136, 0.66)</td>
</tr>
</tbody>
</table>

*For age and triglycerides.

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Adjusted for age OR (95%CI)</td>
</tr>
<tr>
<td></td>
<td>CVD Overall</td>
<td>p</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>24 87</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>23 86</td>
<td>1.04 (0.51–2.13)</td>
</tr>
<tr>
<td>3</td>
<td>31 86</td>
<td>1.53 (0.76–3.06)</td>
</tr>
<tr>
<td>4</td>
<td>41 89</td>
<td>1.97 (1.00–3.80)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>29 91</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>35 92</td>
<td>1.28 (0.69–2.38)</td>
</tr>
<tr>
<td>3</td>
<td>27 94</td>
<td>0.82 (0.43–1.56)</td>
</tr>
<tr>
<td>4</td>
<td>42 94</td>
<td>1.47 (0.79–2.72)</td>
</tr>
</tbody>
</table>

*Adjusted for age, total cholesterol, diastolic blood pressure, smoking status, cotinine, body mass index, alcohol status, triglyceride, menopause status, contraceptive pill status and HRT status.
age group (25–34 years) is of interest, because of the higher prevalence of early atherosclerotic lesions at necropsy in men in this age group.

The present study confirms significant associations between current cigarette-smoking and plasma lipid peroxides suggested by previous, smaller studies of the TBA assay or of 8-isoprostanes. These associations were observed among men both for self-reported smoking habit, and for all three objective biochemical measures of smoking exposure. They were weaker in women. Vitamin C may be an important anti-oxidant defence mechanism against atherosclerosis, and serum levels are lower for any given dietary vitamin C level in smokers. It is therefore of interest that lipid peroxide levels in the present study also showed a significant inverse correlation with plasma vitamin C in men. Studies of the effect of vitamin C supplementation on lipid peroxides are therefore indicated.

Recent epidemiological studies in industrialized countries have suggested that manual social class is associated with increased risk of CVD, while alcohol consumption is associated with decreased risk. We observed that plasma lipid peroxide levels were associated with manual social class, and with lower alcohol consumption, in women. These associations were not observed in men, possibly because of the confounding effects of smoking in men, due to their higher levels of smoke inhalation.

As in previous studies, the strongest association among cardiovascular risk factors with plasma lipid peroxide levels was serum triglyceride levels. Smaller, denser low-density lipoprotein (LDL) particles, which are more susceptibility to peroxidation, are associated with triglyceride levels and with coronary heart disease, as are oxidized LDL levels. Hence it is possible that hypertriglyceridaemia promotes oxidation of lipids (including LDL), and that this may be one mechanism through which increased serum triglycerides increase the risk of coronary heart disease. As in previous studies, weaker correlations of plasma lipid peroxides were observed with total or HDL cholesterol. The inverse correlation with HDL cholesterol has been observed previously and may reflect a role for HDL in reducing oxidation of LDL.

No correlations of lipid peroxide levels were observed with blood pressure. Lipid peroxides correlated with body mass index, and with blood glucose in women: these correlations are consistent with a previous report, with reports that glycaemia may promote lipid peroxidation through several mechanisms, and with a report of increased MDA levels in non-insulin-dependent diabetes patients, especially those with microalbuminuria.

Plasma lipid peroxides were significantly associated with whole-blood viscosity and its two major determinants: haematocrit and plasma viscosity, which are risk predictors for CHD and stroke. These associations were independent of the potential confounders, age and serum triglyceride (Table 3). Lipid peroxidation may increase endothelial permeability, resulting in haemoconcentration and elevated haematocrit; triglycerides may also increase blood viscosity, possibly through oxidant damage to erythrocytes. Lipid peroxidation may promote acute-phase reactions by increasing endothelial/monocyte interactions and release of cytokines such as interleukin-6, which are key mediators of inflammatory reactions including elevations of white cell count, fibrinogen and C-reactive protein. However, after adjustment for age and triglyceride levels, associations of lipid peroxides with C-reactive protein were not significant; and for white cell count and fibrinogen were significant only in women. There was no significant association of lipid peroxide levels with interleukin-6.

Overall, there were few associations between lipid peroxide levels and plasma levels of haemostatic variables, indicating no major effect of lipid peroxidation upon activation of blood coagulation.

On univariate analyses, trends were seen for age-adjusted associations between lipid peroxide levels and prevalent cardiovascular disease, especially in men (Table 4). These findings are consistent with those of two previous case-control studies. However, these associations became non-significant after adjustment for a set of conventional cardiovascular risk factors (Table 4).

Our study has several potential limitations. First, the TBA assay is recognized to have limitations of sensitivity and specificity. TBA reacts not only with MDA, but also with a number of molecules not produced by lipid peroxidation. However, the assay used in the present study used an HPLC separation of the products of the TBA reaction, so that only the TBA-MDA adduct was measured, thereby increasing the specificity for lipid peroxide breakdown to MDA during the TBA reaction. While there is a large within-subject day-to-day variation in plasma MDA, on a group basis it is a potential biomarker of oxidative stress, hence it should give valid information in a study of this size (n = 739). Second, MDA levels increase during storage of frozen plasma samples; however, our samples were stored at −70°C for <12 months, hence any increase during storage would be both negligible and consistent. Third, non-fasting samples were collected (as part of the standard International MONICA study protocol), hence it is possible that plasma lipid peroxides might in part be derived from ingested food.
but we have not found references to support this hypothesis.

In conclusion, the findings of the present study are broadly consistent with the hypothesis that lipid peroxidation may be one mechanism through which several cardiovascular risk factors (e.g. smoking, serum triglycerides, low HDL, low vitamin C) may promote cardiovascular disease. Further, larger studies; as well as large randomized trials of antioxidants; are required to test this hypothesis.

Acknowledgements

This study was partly funded by the Chief Scientist Office, Department of Health, Scottish Office. The views expressed here are those of the authors and not necessarily of this office. The work was also supported by a grant from the British Heart Foundation.

We thank Hugh Tunstall-Pedoe and Caroline Morrison of the Scottish MONICA Project; Roger Tavendale, who ran the Dundee laboratory; Pamela Griffiths and Catherine Stewart for technical assistance; and Ruth Simpson and Ann Harold, who typed the manuscript.

References


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