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Selenium and vitamin E in relation to risk factors for coronary heart disease

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SUMMARY Fasting blood samples taken from 116 apparently healthy men aged 30–50 years were assayed for selenium, glutathione peroxidase activity, vitamin E, cadmium, lead, glucose, lipids, and albumin. Blood pressure was measured in each subject, and details of height, weight, smoking habits, and alcohol consumption were recorded.

Multivariate analysis of the data showed that the decrease in blood and serum concentrations of selenium and the increase in whole blood cadmium concentrations in the cigarette smokers was independent of alcohol consumption. There was no correlation between blood selenium concentrations or glutathione peroxidase activities and the risk factors for cardiovascular disease. Neither alcohol consumption nor smoking had an effect on the vitamin E concentrations. There was a strong association, however, between vitamin E and serum lipid concentrations, although the increase in triglyceride concentrations in the smokers was not matched by a comparable increase in vitamin E. The possible role of selenium in the aetiology of heart disease remains unresolved.

A number of studies have suggested that hyperlipidaemia, hypertension, obesity, diabetes, and cigarette smoking may be possible risk factors in the aetiology of coronary heart disease.[1–3] Although risk factor intervention trials have not been conclusive,[4] there seems to be general agreement on the relation between cigarette smoking and coronary heart disease, together with the improved prognosis that follows cessation of smoking.[5]

The trace elements lead and cadmium have also been implicated in heart disease.[6,7] It has been suggested that the higher incidence of cardiovascular mortality in soft water areas may be due to the increased lead concentrations found in many soft waters.[8] Shaper *et al*[8] have shown a strong association between blood lead concentrations, alcohol consumption, and cigarette smoking. Blood cadmium concentrations are higher in cigarette smokers than in non-smokers,[9] but the evidence linking blood cadmium to hypertension in man is equivocal.[10,11] A pressor effect of cadmium in drinking water fed to rats has been reported. This effect was reversed by the addition to the drinking water of another trace element, selenium.[12]

Recently there has been growing interest in the role of selenium in relation to coronary heart disease. The effects of selenium deficiency in animals have been well documented. For example, rats and lambs fed selenium deficient diets developed abnormal electrocardiograms accompanied by blood pressure changes,[13,14] and young pigs fed a semi-synthetic diet deficient in selenium and vitamin E developed a cardiomyopathy, which was clearly shown by histological techniques.[15]

Epidemiological studies have shown an inverse relation between selenium and mortality due to heart disease.[16] A report from the People's Republic of China suggests the importance of sodium selenite in the prevention and treatment of Keshan's disease, a fatal cardiomyopathy affecting mainly children and pregnant women.[17] Salonen *et al*[18] showed a significant relation between the concentration of selenium in serum and cardiovascular death and myocardial infarction, and Oster *et al*[19] suggested that a deficiency of selenium may be present in a

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number of patients with cardiomyopathy.

In a recent study we found that the concentration of selenium in whole blood and plasma was significantly lower in male cigarette smokers over 30 years of age compared with non-smoking controls of a similar age.[20] The activity of glutathione peroxidase, a selenoenzyme, was also lower in the cigarette smokers. This enzyme is responsible for the removal of hydrogen peroxide and other organic hydroperoxides formed during cellular oxidative metabolism. Glutathione peroxidase has a close metabolic relation with vitamin E, another important antioxidant. This fat soluble vitamin, present in cellular and subcellular membranes, provides a defence against peroxidation of vital phospholipids. The biochemical actions of vitamin E and glutathione peroxidase are therefore concerned with the prevention of peroxidative damage to cells and subcellular elements.[21]

The main aim of this study was to examine the concentrations of selenium and vitamin E in blood samples from a group of healthy men, between 30 and 50 years of age, in relation to other accepted risk factors for coronary heart disease.

Subjects and methods

SUBJECTS

A total of 123 apparently healthy men between 30 and 50 years of age participated in the study. Of these volunteers, 94 were hospital employees and the remaining 29 were employed by a local firm. The subjects were asked to complete a questionnaire[8] which included details of their occupation, height, and weight, as well as alcohol consumption and smoking habits. In addition, the subjects were asked about recent medication and major illnesses; full details are given in Table 1. Blood samples were obtained by venepuncture between 8.00 am and 10.00 am after each subject had abstained from food and drink, other than water, for at least 12 h. Blood pressure was measured with each subject in the sitting position by the same observer using a Reister sphygmomanometer.

METHODS

Haematological parameters were measured within 4 h of blood sampling using a Coulter counter and Hawksley microhaematocrit centrifuge. Samples for glutathione peroxidase, vitamin E, and plasma glucose measurement were stored at 4°C and assayed within 48 h. Serum and whole blood samples were stored at −20°C before analysis for lipids, albumin, and trace elements. Table 2 gives details of the methods, including instrumentation, for the biochemical analysis of the blood samples together with the between batch precision of analysis. An obesity index[32] for each subject was calculated from the formula:

$$\frac{\text{weight in kilograms}}{(\text{height in metres})^2}$$

STATISTICAL ANALYSIS

Data processing was carried out on an ICL 2970 computer and the appropriate statistics were obtained using the SPSS package.[33]

Results

Seven of the original 123 male volunteers who took part in the study were excluded. Current drug treatment or past medical history accounted for five of the subjects who were excluded. The other two subjects excluded were a man who had a fasting plasma glucose concentration of 8·9 mmol/l and a

Table 1 *Details of subjects*

	Age group 30–40 years		*Age group 41–50 years*		*Total*
	No	*(% of total)*	*No*	*(% of total)*	
All subjects	73	(62·9)	43	(37·1)	116
Occupational groups					
Manual workers	17	(68·0)	8	(32·0)	25
Non-manual workers	56	(61·5)	35	(38·5)	91
Tobacco consumption					
Non-smokers	41	(62·1)	25	(37·9)	66
Cigarette smokers	29	(70·7)	12	(29·3)	41
Pipe and cigar smokers	3	(33·3)	6	(66·7)	9
Alcohol consumption					
Non-drinkers*	15	(65·2)	8	(34·8)	23
Weekend drinkers	28	(68·3)	13	(31·7)	41
Daily drinkers	30	(57·7)	22	(42·3)	52

*This group also includes those subjects who drank alcohol only very occasionally

Table 2 *Details of sample analysis*

Analysis	*Methodology*	*Between batch precision (%)*	*Instrumentation*
Albumin	Immunoturbidimetry[22]	3·1	LKB 8600 reaction rate analyser
Glucose	Glucose oxidase[23]	1·1	Technicon autoanalyser
Total cholesterol	Enzymatic assay using BCL kit no: 290319[24]	3·9	Instrumentation laboratory micro-centrifugal analyser Multistat III
HDL cholesterol	Precipitation of LDL and very LDL followed by cholesterol assay of the supernatant[25]	5·2	Instrumentation laboratory microcentrifugal analyser Multistat III
Triglycerides	Enzymatic assay using BCL kit no: 126012[26]	3·4	LKB 8600 reaction rate analyser
Glutathione peroxidase	Coupled reaction system using t-butyl hydroperoxide[27]	7·6	LKB 8600 reaction rate analyser
Selenium	Hydride generation and atomic absorption spectrophotometry[28]	4·9	Perkin Elmer Model 360 atomic absorption spectrophotometer
Vitamin E	High pressure liquid chromatography[29]	3·4	Laboratory Data Control pump and UV detector
Cadmium	Electrothermal atomisation and atomic absorption spectrophotometry[30]	5·8	Perkin Elmer Model 2380 atomic absorption spectrophotometer
Lead	Atomic absorption spectrophotometry[31]	3·2	Perkin Elmer Model 360 atomic absorption spectrophotometer

HDL = high density lipoprotein; LDL = low density lipoprotein.

family history of diabetes and another man whose plasma sample was grossly lipaemic, with a triglyceride value of 8·8 mmol/l and a raised plasma glucose concentration. Some of the remaining 116 subjects had blood lipid concentrations outside the accepted reference ranges: 18 men had fasting triglyceride concentrations greater than 2·2 mmol/l, and five had triglyceride concentrations greater than 3·0 mmol/l. In this group three men had total cholesterol concentrations greater than 8·0 mmol/l. A further group of five men also had total cholesterol concentrations greater than 8·0 mmol/l. As this proportion of abnormal lipid results was similar to the findings of other workers also studying an apparently healthy population,[34 35] all the subjects were included in the study. None of the subjects was hypertensive.

To test the homogeneity of the sample population the subjects were divided into groups according to age, then occupation, and finally place of employment. The results for the various groups were compared by parametric (t test) and non-parametric (Mann-Whitney) analysis. Table 1 shows that there was a higher proportion of subjects in the 30–40 age group (63%) compared with the 41–50 age group (37%). No significant differences were found for any of the parameters except for plasma glucose, which was higher in the older age group (mean 5·03 mmol/l, SD 0·494) compared with the younger group (mean 4·86 mmol/l, SD 0·423), $p < 0·05$, and serum albumin, which was lower in the older age group (mean 43·8 g/l, SD 3·92) compared with the younger group (mean 45·5 g/l, SD 3·14), $p < 0·02$. There were fewer manual workers (21·5%) than non-manual workers (78·5%), and a higher proportion of the manual workers admitted to smoking than the non-manual workers. No significant differences were found in the parameters for the two groups. Similarly, significant differences were not found between the parameters for the subjects from

Table 3 *Comparison of results according to smoking groups*

Analysis	*Non-smokers* (n = 66)		*Cigarette smokers* (n = 41)	
	Mean	(SD)	Mean	(SD)
Haemoglobin (g/dl)	15·3	(0·91)	15·9*	(1·03)
Plasma glucose (mmol/l)	4·9	(0·45)	4·9	(0·44)
Serum albumin (g/l)	44·7	(3·33)	45·0	(3·40)
Total cholesterol (mmol/l)	5·7	(1·17)	5·8	(1·12)
HDL cholesterol (mmol/l)	1·25	(0·273)	1·20	(0·318)
Triglycerides (mmol/l)	1·32	(0·659)	1·84†	(0·795)
Obesity index	23·5	(2·20)	24·5	(2·96)
Diastolic blood pressure (mmHg)	76·2	(10·8)	77·1	(9·2)
Systolic blood pressure (mmHg)	119·7	(14·3)	120·9	(13·6)

HDL = high density lipoprotein. *$p < 0·005$, †$p < 0·001$.

Table 4 *Vitamin E and trace elements according to smoking groups*

Analysis	*Non-smokers* (n = 66)		*Cigarette smokers* (n = 41)		*Pipe and cigar smokers* (n = 9)	
	Mean	*(SD)*	*Mean*	*(SD)*	*Mean*	*(SD)*
Vitamin E (μmol/l)	25·4	(6·77)	24·4	(6·04)	23·7	(4·60)
Glutathione peroxidase (U/g Hb)	17·9	(3·45)	16·5*	(3·61)	18·4	(4·36)
Whole blood selenium (μg/l)	134·3	(20·43)	115·1†	(10·84)	121·9	(21·29)
Serum selenium (μg/l)	114·5	(15·71)	98·4†	(11·01)	105·9	(13·30)
Whole blood cadmium (μg/l)	0·95	(0·422)	2·11†	(1·287)	0·44	(0·442)
Selenium:cadmium (μmol/l)	249	(159)	111†	(83)	265	(252)
Selenium:lead (μmol/l)	3·3	(1·00)	2·6*	(0·98)	2·9	(0·99)

*Cigarette smokers *v* non-smokers, $p < 0.05$.
†Cigarette smokers *v* non-smokers, $p < 0.001$.

either place of employment. Consequently all 116 subjects were treated as one group, regardless of age or occupation.

The smoking habits and alcohol consumption for each subject had been recorded in detail, but as only nine men admitted to smoking more than 20 cigarettes a day all the cigarette smokers were combined into one group. Alcohol consumption was divided into three categories; occasional and non-drinkers, weekend drinkers, and daily drinkers. Only four men who drank alcohol daily admitted to consuming three or more pints of beer, or its equivalent, each day.

The haemoglobin, albumin, glucose, and lipid concentrations for the cigarette smokers and non-smokers are shown in Table 3, together with the results for the obesity index and the blood pressure measurements for each group. Haemoglobin and triglyceride concentrations were significantly higher in the cigarette smokers compared with the non-smokers but were not significantly different for the pipe and cigar smokers when compared with either

Table 5 *Effects of cigarette smoking without alcohol consumption*

Analysis	*Non-drinkers*			
	Non-smokers (n = 15)		*Cigarette smokers* (n = 7)	
	Mean	*(SD)*	*Mean*	*(SD)*
Serum triglyceride (mmol/l)	1·18	(0·568)	1·63	(0·401)
Glutathione peroxidase (U/g Hb)	19·1	(3·85)	15·7*	(2·22)
Whole blood selenium (μg/l)	132·8	(17·30)	114·7†	(8·60)
Serum selenium (μg/l)	112·4	(11·64)	102·0	(10·41)
Whole blood cadmium (μg/l)	1·06	(0·643)	1·84	(0·643)
Whole blood lead (μg/dl)	11·7	(3·64)	10·2	(3·08)

*$p < 0.05$, †$p < 0.02$.

Table 6 *Effects of cigarette smoking with daily alcohol consumption*

Analysis	*Daily drinkers*			
	Non-smokers (n = 29)		*Cigarette smokers* (n = 18)	
	Mean	*(SD)*	*Mean*	*(SD)*
Serum triglyceride (mmol/l)	1·24	(0·473)	1·81*	(1·011)
Glutathione peroxidase (U/g Hb)	17·9	(3·65)	17·3	(3·88)
Whole blood selenium (μg/l)	137·7	(23·30)	113·5†	(12·49)
Serum selenium (μg/l)	114·2	(18·58)	95·1†	(10·02)
Whole blood cadmium (μg/l)	0·89	(0·341)	2·16†	(1·120)
Whole blood lead (μg/dl)	12·1	(2·72)	14·5	(4·56)

*$p < 0.05$, †$p < 0.001$.

the non-smokers or the cigarette smokers.

The effect of cigarette smoking on glutathione peroxidase activity and concentrations of selenium, cadmium and lead is shown in Table 4. The plasma and whole blood selenium concentrations were significantly lower in the cigarette smokers compared with the non-smokers, whereas the concentrations of lead and cadmium were significantly higher in the cigarette smokers. These differences had an appreciable effect on the selenium:cadmium and selenium:lead ratios. The concentrations of each element in the cigar and pipe smoking group fell between those of the non-smokers and cigarette smokers, but these differences were not statistically significant. There appeared to be no difference in the vitamin E concentrations for any of the smoking groups.

Tables 5 and 6 show the effect of classifying the non-smokers and cigarette smokers according to their alcohol consumption. Glutathione peroxidase activity and serum and blood selenium concentrations were lower in the cigarette smokers compared with the non-smokers, but no significant differences were found between the non-drinking cigarette smokers and the smokers who drank alcohol daily. Similarly, no significant differences were found between the non-drinkers and the daily drinkers in the non-smoking group. The concentrations of cadmium and serum triglyceride were higher in the cigarette smoking groups, whereas daily alcohol consumption seemed to have a greater effect on the concentration of lead in blood. The independent effects of cigarette smoking and alcohol consumption on the results were confirmed by analysis of variance. No significant differences were found for blood pressure, obesity index, total cholesterol, albumin, glucose, or vitamin E.

The possibility existed that cadmium and lead might have had an effect on blood selenium concentration. Analysis of covariance, however, showed that neither cadmium nor lead had a significant effect on blood selenium concentrations.

The data were examined for possible associations between selenium concentration, vitamin E, and the cardiovascular risk factors. Glutathione peroxidase was not associated with any of the risk factors (apart from smoking); however, both whole blood and serum selenium concentrations showed a weak negative association with blood cadmium concentrations ($r = -0{\cdot}24$, $p = 0{\cdot}01$). Vitamin E showed a strong association with total cholesterol ($r = 0{\cdot}67$, $p < 0{\cdot}001$) and to a lesser extent with serum triglyceride ($r = 0{\cdot}44$, $p < 0{\cdot}001$). There was also a weak correlation between vitamin E and plasma selenium concentration ($r = 0{\cdot}32$, $p < 0{\cdot}001$).

Discussion

All the men included in the study presented as apparently healthy subjects without a history of diabetes, hypertension, or heart disease. Blood lipid concentrations above the accepted reference ranges were found in 23 subjects. Several other workers have reported similar abnormal lipids in an apparently healthy population,[34,35] and we therefore included all 116 subjects as they seemed to be representative of a "normal" population.

The age group for the study was limited to 30–50 years to minimise changes in the parameters which might be due to age alone. Only plasma glucose, which was higher in the older age group, and serum albumin, which was lower, showed significant differences between the two groups. The mean concentrations of both serum triglyceride and total cholesterol were higher in the older age group, but the differences were not significant.

The metabolic changes due to smoking and alcohol consumption have been reviewed elsewhere.[36] Previous workers have reported an association between alcohol consumption and high density lipoprotein cholesterol,[37] a finding confirmed in this study. The mean concentration of high density lipoprotein cholesterol was 1·12 mmol/l for the non-drinkers (SD 0·276, n = 23) compared with 1·33 mmol/l for the daily drinkers (SD 0·326, n = 52), $p < 0{\cdot}01$.

Although only nine men admitted to smoking more than 20 cigarettes a day, we found the expected increase in haemoglobin and triglyceride concentrations in the smoking group.[37] Significant increases in the concentrations of cadmium and lead were also found in the smokers, as well as a decrease in selenium concentration. Multivariate analysis of the data showed that the decrease in selenium and the increase in cadmium in the cigarette smokers was independent of alcohol consumption. The results for the pipe and cigar smoking group were intermediate between those of the cigarette smokers and the non-smokers. These results may lack statistical significance because only nine men smoked pipes or cigars. Alternatively, the fact that cigar and pipe smokers do not usually inhale smoke may have a considerable influence on the results.

The reason for the lower concentrations of selenium in the cigarette smokers is not yet understood. Selenium undergoes a complex chemical transformation in the red cells which is dependent on an adequate supply of glutathione. Competition of cadmium with selenium for the SH sites or the formation of a cadmium/selenium complex may interfere with the metabolism of selenium. Early studies showed that selenium is initially transported

by serum albumin and then bound to α_1- and α_2-globulins, and studies have also shown an association of selenium with plasma lipoproteins.[38] We did not find reduced serum albumin concentrations in the smoking group, but confirmed an increase in triglyceride concentrations. The lower concentrations of selenium found in the cigarette smokers did not therefore relate to lower albumin or lipid concentrations. Motsenbocker and Tappel[39] have identified a selenium binding protein in rat plasma and plasma from a Rhesus monkey, which has a molecular weight of 80 000 with a selenopolypeptide size of 45 000. Experimental evidence showed that the selenium was present in the form of selenocysteine. They suggest that this selenium binding plasma protein is engaged in selenium transport and that it delivers selenium in the form of selenocysteine to various body tissues. If this protein also exists in human blood then increased concentrations of cadmium in blood may affect the binding of selenium to this protein and hence the transport of selenium to the tissues. York *et al*[40] described the effect of in vivo cigarette smoke exposure on glutathione peroxidase related enzyme systems in rat lung. After 21 days' exposure the activity of glutathione peroxidase was increased by 34%, although detectable histological lesions were not found. They suggested that short term, low level exposure to cigarette smoke is capable of initiating metabolic changes in lung cells.[40] Such changes in alveolar cells may lead to an increased excretion of selenium via the breath, particularly after long term chronic inhalation.

Neither alcohol consumption nor smoking had any effect on the vitamin E concentrations in this study. Because vitamin E is not synthesised in the body the concentration found in plasma is partly dependent on the amount of biologically active vitamin E being consumed. Behrens *et al*[41] showed that low density and high density lipoproteins were the main carriers of vitamin E in plasma. It is therefore not surprising that we found a strong association between serum lipid and vitamin E concentrations. The significant increase in the triglyceride concentrations of the smokers, however, was not matched by a comparable increase in vitamin E. The weak association of vitamin E with plasma selenium may also be a reflection of dietary intake.

The results for all the factors measured in this study were consistent with the expected results from an apparently healthy group of men aged 30–50 years. We were unable to show an association between selenium concentration and the risk factors for cardiovascular disease with the exception of cigarette smoking, but a similar association was not seen with vitamin E. None of the subjects studied were selenium deficient; nevertheless, the blood selenium concentrations in the cigarette smokers were significantly lower than in the non-smokers. If, as many reports now suggest, selenium and vitamin E are protective elements against coronary heart disease, then an explanation of the effect of smoking on selenium concentration would be useful in understanding the aetiology of coronary heart disease.

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