

Serum albumin and prevalence of coronary heart disease:

A population-based, cross sectional study

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Det er kjent at lav konsentrasjon av serumalbumin er en risikofaktor for hjerte- og karsykdommer. I Helseundersøkelsen i Oslo inngår blant annet spørsmål om hjerteinfarkt. Konsentrasjonen av serumalbumin ble målt på 5224 individer. Verdiene er nær normalfordelt hos begge kjønn, med en forskyvning mot noe lavere verdier hos kvinner. For menn er det fallende albuminverdier med økende alder. Hos kvinner er det liten forskjell i albuminverdien i de to laveste aldersgruppene (30 og 40+45-åringene), men deretter fallende verdier. Lav albumin (≤ 47 g/l) vs høy (> 47 g/l) er assosiert med økt prevalens av (selvrapportert) hjerteinfarkt (Odds Ratio=1,95; 95% CI=1,30–2,93; $p=0,001$), angina pectoris (OR=1,44; 95% CI=1,04–2,01; $p=0,03$) og brystmerter ved å gå i trapper/bakker (OR=1,36; 95% CI=1,12–1,64; $p=0,002$), etter justering for alder, kjønn og røyking. Albumin er en viktig ekstracellulær antioksidant. Peroksidering av lipider (bl.a. av LDL) kan øke risikoen for koronar hjertesykdom. I materialet ble det funnet positiv sammenheng mellom alder (og LDL) og serum TBARS, som er et mål på lipidperoksidering. Hos menn var det invers sammenheng mellom albumin og TBARS. Lave TBARS-verdier var assosiert med redusert 'risiko' for å rapportere angina pectoris (OR=0,80; 95% CI=0,688–0,933; $p=0,004$), noe som reiser spørsmålet om den antatt beskyttende effekten av serumalbumin mot koronarsykdom delvis kunne bero på beskyttelse mot lipidperoksider. Det forhold at albuminverdien hos kvinner ikke faller før etter menopause, kunne være en medvirkende årsak til at kvinner sjeldnere får koronarsykdom premenopausal. Det er imidlertid ikke mulig å si noe sikkert om kausale sammenhenger på basis av dette tverrsnittsmaterialet.

INTRODUCTION

Phillips et al (1) first reported an inverse relationship between the concentration of serum albumin (within a narrow physiological range) and total mortality, and mortality of cardiovascular diseases, cancer and other diseases (1). Since there was no *a priori* hypothesis, the observation was described as a 'serendipitous' finding. Subsequently, several reports on the apparent protective influence of serum albumin against diseases have appeared (2-7). Indeed, it has been suggested that serum albumin concentration is "the best predictor of mortality in elderly people" (3). The mechanisms behind the apparent cardioprotective effect of albumin remain to be elucidated. One hypothesis could be that albumin protects against harmful lipid peroxidation products, since albumin serves as an important extracellular antioxidant (8).

It seemed, accordingly, of interest to examine whether analysis of data in the Oslo Health Study might be in support of a cardioprotective effect of albumin. In addition, the lipid peroxidation estimate, TBARS, was measured to examine whether variations in lipid peroxidation might fit the hypothesis that albumin works through peroxidation protection.

MATERIAL AND METHODS

In 2000-2001 the Oslo Health Study was conducted under the joint collaboration of the National Health Screening Service of Norway (now the Norwegian Institute of Public Health), the University of Oslo and the Municipality of Oslo. The study population included all individuals in Oslo County born in 1970, 1960, 1955, 1940/41 and 1924/25. A total of 18,770 individuals (45.9% of the invited) participated – in other words 8,404 men (42.4%) and 10,366 women (49.3%) attended the physical examination and/or filled in at least one of the questionnaires. For the five age cohorts the participation rates were 36.1, 43.7, 46.5, 55.4 and 53.2%, respectively. Baseline measurements included height, weight, waist and hip circumference, blood pressure, heart rate, and non-fasting blood tests to analyze serum total cholesterol, HDL-cholesterol, triglycerides, and glucose.

One self-administered questionnaire was part of the letter of invitation, whereas two supplementary questionnaires were handed out at the survey, and sent back in pre-stamped self-addressed envelope. The questionnaires provided information on health status, symptoms, diseases and various aspects of health behaviour.

Up to two reminders were sent to the non-responders of the invitation to participate in the survey. The second reminder invited those living in the suburban parts of the city to mobile screening units parked in the neighbourhood of the invited. For further details, see: <http://www.fhi.no/tema/helseundersokelse/oslo/index.html>. The study protocol was placed before the Regional Committee for Medical Research Ethics and approved by the Norwegian Data Inspectorate. The study has been conducted in full accordance with the World Medical Association Declaration of Helsinki.

As a supplementary project of the study, the concentration of albumin was measured in sera from 6,333 subjects. Serum albumin was determined using the bromocresol green method (9), with a reference interval of 38-54 g/l. The intra- and inter-assay coefficients of variation (CV) were 2% and 6% respectively. Since albumin acts as an antioxidant (8), TBARS (Thiobarbituric Acid Reactive Substances), which is a measure of lipid peroxidation (10), was determined in 5,224 of the available 'albumin sera'. The present results refer to these sera. The intention was to have about equal representation from each sex and from each of the several age groups. It turned out, however, that there was a greater representation of women than of men, and also differences in the number of subjects in each age group, with the highest number of subjects in age group 40+45 years (Table 1). The sex distribution did not vary much between the various age groups.

We previously observed that freezing and thawing before TBARS determination did not have any major influence on the results. Determination was carried out according to Kosugi et al (11). Intra- and inter-assay CV was 7 and 8%, respectively. The serum concentration of low density lipoprotein cholesterol (LDL) was estimated by the Friedewald formula (12).

Statistical analyses were done using SPSS 11.1. Albumin values were divided into low (≤ 47 g/l) and high (>47 g/l) groups, or into tertiles. Using logistic regression, odds ratio and 95% confidence intervals were computed for the 'risk' of reporting myocardial infarction, angina pectoris, or chest pain when walking up stairs/hills ('yes' or 'no'), for low vs high serum albumin, after adjusting for age, sex and smoking. Linear regression was used to estimate association between albumin, LDL and TBARS. ANOVA and Scheffe test for multiple comparisons were performed when evaluating significance of differences between several mean values. A significance level of 0.05 was used. SPSS 11.0 was used to produce the figures.

Table 1. Number and distribution of subjects.

Age (years)	Men			Women		
	n	Row%	Col%	n	Row%	Col%
30	423	39.4	21.0	651	60.6	20.3
40+45	689	38.5	34.2	1101	61.5	34.3
59-60	534	39.7	26.5	812	60.3	25.3
75-76	368	36.3	18.3	646	63.7	20.1

RESULTS

As shown in Figure 1 the serum albumin concentration was fairly normally distributed in both sexes, but the values were distributed towards slightly higher values in men (47.9 ± 3.0 , mean \pm SD) than in women (46.2 ± 2.8). In men there was an age-related decrease in the serum albumin concentration throughout all age groups (Figure 2).

In contrast, for women the serum albumin concentration remained constant in the two lowest age groups, but decreased above age group 40+45 years. For all age groups women had lower values than men. It seems from the figure that the sex difference in serum albumin concentration is reduced with increasing age. By ANOVA a significant interaction ($p < 0.001$) between sex and age was found.

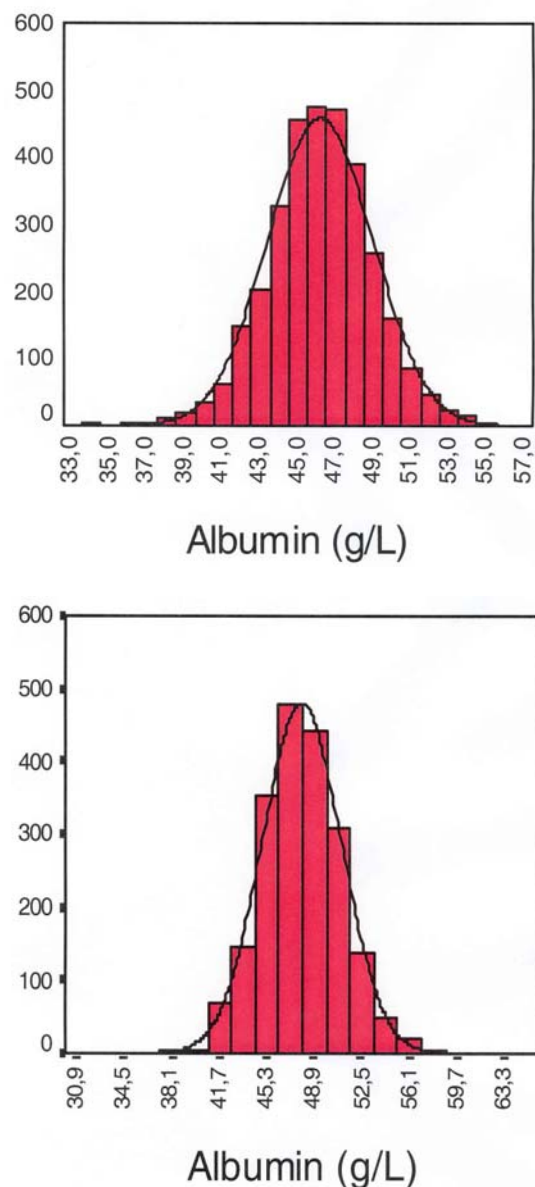


Figure 1. Distribution of the serum albumin concentration in women (top; mean \pm SD: 46.2 ± 2.8 ; $n=3210$), and men (bottom; 47.9 ± 3.0 , $n=1014$).

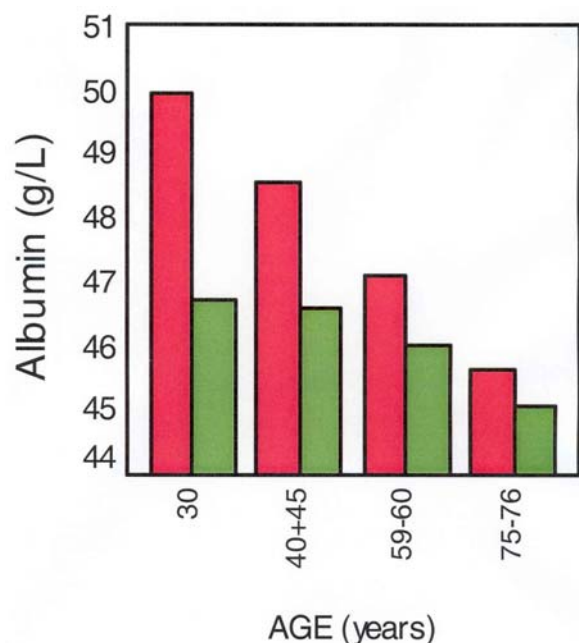


Figure 2. Mean serum albumin concentration in men (dark grey bars, left) and women (lighter grey bars, right), by age.

For each sex and age group the mean albumin concentration in the self reported cases and 'controls' was calculated (Figure 3 and 4). For both women and men the mean albumin concentration was lower in cases than in those without myocardial infarction. The OR for reporting myocardial infarction was increased in the low (≤ 47 g/l) vs high (> 47 g/l) albumin group (OR=1.95; 95% CI=1.30–2.93; $p=0.001$), when adjusting for age (≥ 59 vs ≤ 45 years), sex and smoking. Age was dichotomized due to an apparent non-linear relationship between age and serum albumin concentration. Also when considering subjects aged 59-60 years separately, low albumin increased the OR for reporting MI (OR=2.26; 95%CI=1.06–4.79; $p=0.034$). A similar finding was done for subjects aged 75-76 (OR=2.10; 95%CI=1.18–3.75; $p=0.01$).

To further examine the association between low albumin and (self-reported) OR for coronary heart diseases we studied albumin and odds ratio in subjects reporting angina pectoris. Also for reporting this clinical condition low albumin was a risk factor (OR=1.44; 95%CI=1.04–2.01; $p=0.03$), when adjusting for age, sex and smoking.

Moreover, low albumin was associated with increased OR for reporting chest pain when walking up stairs or hills (OR=1.36; 95% CI=1.12–1.64; $p=0.002$).

Results with TBARS

Some analyses were made to examine whether variations in lipid peroxidation might fit the hypothesis that albumin exerts some of its effect via protection against lipid peroxidation, as estimated by TBARS. There was a positive correlation between age and TBARS ($r=$

0.101; $p<0.001$). Mean TBARS concentration in men and women in the age groups is shown in Figure 5.

In age group 75-76 years the TBARS concentration was significantly higher in men than in women ($p=0.004$).

The observation that serum TBARS levels rose with increasing age, as the albumin concentration fell, would suggest that the two variables might be inversely related. In men, but not in women, there was a weak, but significant inverse relationship between individual values of the two variables ($r=-0.052$; $p=0.01$; $n=2,014$). Mean values of TBARS in tertiles of albumin for men are shown in Figure 6. For each tertile the mean TBARS concentration was significantly ($p<0.001$) different from mean values in the two other tertiles (oneway ANOVA, Scheffe multiple comparison test). No association was found between TBARS level and OR for reporting myocardial infarction. There was, however, an association between low TBARS concentration and reduced OR for reporting angina pectoris (OR=0.80; CI=0.692–0.934; $p=0.004$). Additionally, for subjects above age 45 years, low TBARS was significantly ($p=0.02$) associated with reduced OR for reporting chest pain when walking up stairs/hills, but the association was weak (OR=0.86; 95% CI=0.74–0.99).

When examining separately the 134 subjects reporting myocardial infarction there was a significant ($r=0.182$; $p=0.035$) inverse relationship between serum albumin and TBARS (Figure 7).

In contrast to this, in the 4,951 subjects answering no to the question about myocardial infarction there was no association between the two variables ($r=0.005$; $p=0.721$).

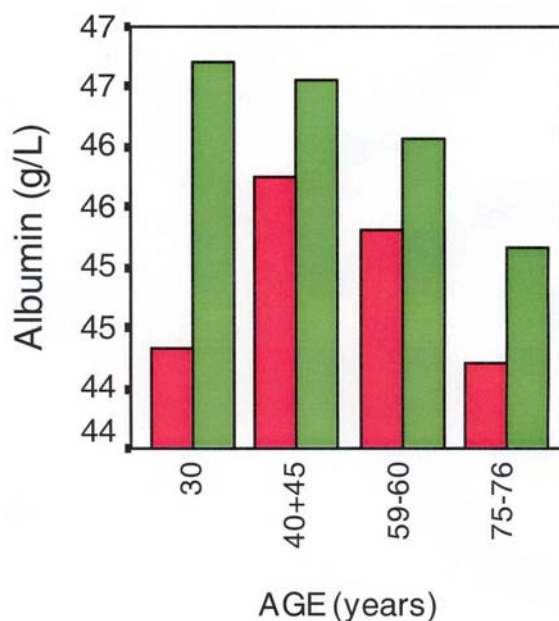


Figure 3. Mean serum albumin concentration in (self-reported) MI cases (dark grey bars, left) and 'controls' (lighter grey bars, right) by age, women. Number of cases/controls in the 4 age groups: 2/632, 1/1073, 9/765, 33/595.

LDL and TBARS

Lipid peroxidation, as estimated by TBARS, could be related to the serum level of low density lipoproteins (LDL). Including subjects (n=4,453) answering yes or no to the question about myocardial infarction, with positive LDL values within the physiological range, i.e. ≤ 6 mmol/L (as estimated by the Friedewald formula, see Methods), and excluding subjects reporting use of cholesterol lowering drugs, there was a weak but

highly significant ($r=0.086$; $p<0.001$) positive linear association between LDL and TBARS. This relationship might, however, be spurious since both variables increased with increasing age. Significance levels for the linear relationship in each of the 4 age groups were: $p=0.41$ (n=1,013), $p=0.064$ (n=1,624), $p=0.005$ (n=1,085), and $p=0.192$ (n=731). Thus, LDL was significantly associated with TBARS only in age group 59-60 years. There was no significant association between serum triglycerides (and cholesterol) and TBARS.

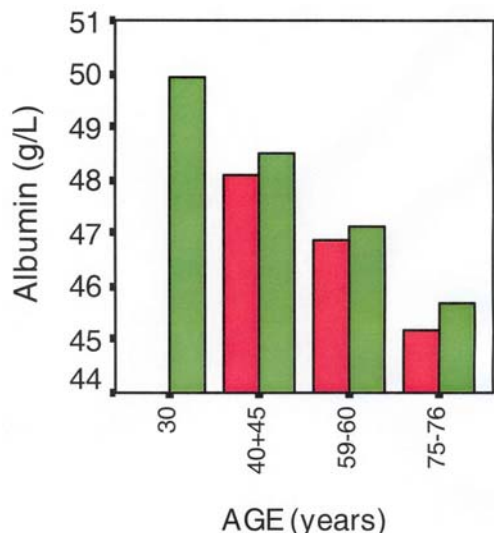


Figure 4. Mean serum albumin concentration in (self-reported) MI cases (dark grey bars, left) and 'controls' (lighter grey bars, right) by age, men. Number of cases/controls in the 4 age groups: 0/417, 6/668, 33/488, 51/315.

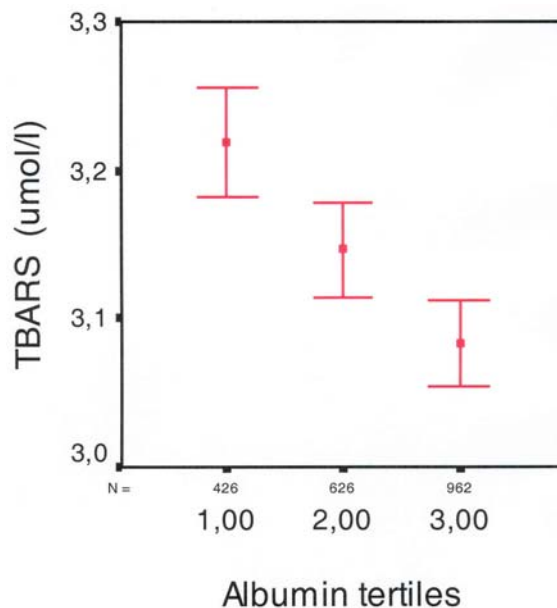


Figure 6. Serum TBARS in albumin tertiles, men. Mean \pm SEM.

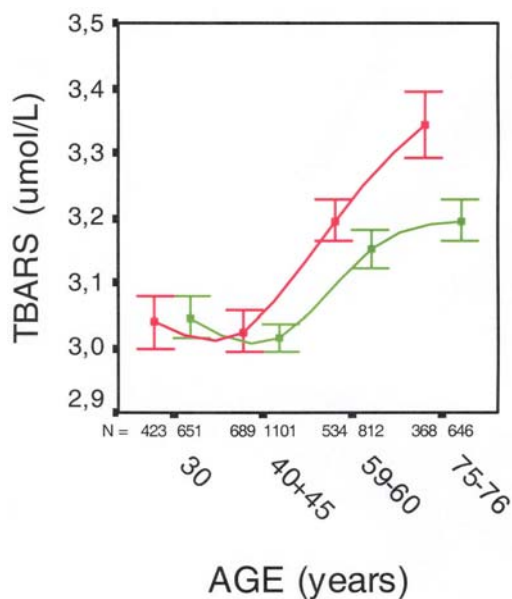


Figure 5. Age-related changes in TBARS (mean values \pm SEM for men (upper curve) and women (lower curve). Note that SPSS does not allow corresponding mean values to be shown at the expected points on the abscissa; curves are added to clarify groups.

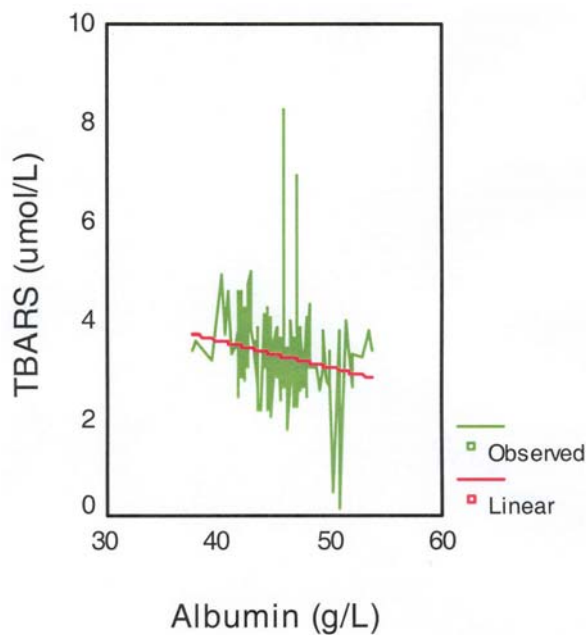


Figure 7. Regression plot for serum albumin vs TBARS in subjects reporting MI (n=134; $r=0.182$; $p=0.035$).

DISCUSSION

The present results would seem to be in support of a cardioprotective role for serum albumin, thus apparently supporting the original work by Phillips et al (1). It is emphasized, however, that the results are obtained in a cross sectional study, implying that conclusions about causal relationships are not permitted. In particular, it should be kept in mind that low serum albumin values is a well known clinical feature accompanying various diseases. This raises the question whether the lower albumin levels in MI cases than in 'controls' could have been caused by their disease. Working against this suggestion is the observation that the variation in serum albumin concentration was within a narrow physiological range. In other words, by considering only the albumin values of the MI patients they would not be classified as patients. More importantly, prospective epidemiological studies (and experimental studies to explore mechanisms, *vide infra*) provide supportive evidence in favour of a cause-effect relationship between low levels of serum albumin and cardiovascular diseases.

Questionnaire information about occurrence of diseases could lead to erroneous conclusions. Among the questions considered in the present work the question about myocardial infarction probably is more reliable than those about angina pectoris, and pain when walking up stairs/hills. Noticeably, low serum albumin concentration increased the 'risk' of reporting all three atherosclerosis-related conditions.

In the population sample studied there was a higher percentage of women than of men, but with a fairly similar sex distribution in all age groups. Percentage of subjects was highest in age group 40+45 years. This distribution difference could make it difficult to generalize the observed quantitative relationship between serum albumin concentration and self reported risk of coronary heart disease. The purpose of the work was, however, only to study associations.

The method for determination of serum albumin has a high accuracy, as indicated by the low coefficients of variation. A high precision was ensured using standard reference sera. Furthermore, we previously observed no alteration in the albumin concentration upon storage even for 3-6 years at -25°C in the dark (13).

The statistical analyses to evaluate associations between serum albumin (and TBARS) and self reported coronary heart disease were robust since, in general, similar observations were done in the whole material, and in each sex separately, and also in more than one age group.

In the study of Phillips et al (1) there was no a priori albumin hypothesis. In the present work we have considered whether the results would fit the hypothesis that albumin might work through protection against lipid peroxidation.

In general, albumin has an important role in regu-

lating the colloid osmotic pressure in blood, and serves as a vehicle for the transport of many substances in blood, such as hormones, drugs, amino acids and free fatty acids (FFA). In addition, albumin acts also as an important extracellular antioxidant (8). It would seem reasonable to attribute the apparent protective role of albumin to one or more of its several physiological functions (8,14-16).

The role of albumin to bind and transport FFA, and to protect against lipid peroxidation, seem of particular interest. The various types of fatty acids serve as an energy source for muscle contraction, and are also important building material for cell membranes. Additionally, some fatty acids may influence several cell functions, such as gene expression and intracellular signal transduction. Some fatty acids are precursors of eicosanoids (16).

The release of FFA from adipose tissue is enhanced by physical activity due to increased adrenergic stimulation, in order to ensure energy substrate for the working muscle (14). The increased extraction of FFA during muscle activity prevents high and possibly toxic levels of plasma FFA concentrations. However, immediately post exercise there is a rapid increase in the FFA concentration (18,19).

Fatty acids are released in high amounts during various stress conditions without an accompanying increased utilization, such as myocardial infarction, serious burns, and preeclampsia (14,20-22). Increased mortality is observed at serum FFA concentrations above 1.2 mmol/l in myocardial infarction patients, probably due to ventricular fibrillation caused by the high FFA concentrations (20,21).

In geriatric patients we observed a positive correlation between the serum FFA/albumin ratio and some indicators of cell damage (23). A high FFA/albumin ratio has toxic effects on red blood cells in vitro, and can strongly inhibit the growth of liver, kidney and endothelial cells in culture (24-28).

In vitro studies suggest that it is the easily peroxidized, polyunsaturated fatty acids which have the strongest toxic potential. In this context it would seem of interest that albumin is an important extracellular antioxidant (8). One hypothesis to explain the observed apparent protective function of high levels of serum albumin against coronary heart diseases could accordingly be that albumin binds free fatty acids so as to prevent toxic FFA effects, and also formation of oxidized low density lipoproteins (LDL).

Unfortunately, there are no data on serum fatty acids and oxidized LDL in the present study. However, it would appear that the present TBARS results are relevant in this context, since this variable is a crude measure of lipid peroxidation (10). The level of TBARS should reflect overall FFA levels (free and esterified, for example in LDL), provided similar conditions otherwise. To study relationships with LDL it seems appropriate to exclude subjects reporting cholesterol lowering drugs. Including all age groups

there was a positive linear relationship between LDL and TBARS. However, when studying each age group separately, this relationship was significant only in subjects aged 59-60 years, presumably indicating a 'loose coupling' between the variables.

The TBARS data could be interpreted in support of the idea that lipid peroxidation is associated with coronary risk. Also the results with self reported angina pectoris, and chest pain during physical activity, would be in line with an apparent cardioprotective role for low serum concentration of TBARS, presumably due to albumin protection. Again, conclusions about causal relationships are not appropriate in a cross-sectional questionnaire study.

Serum TBARS levels increased with increasing age, as does the incidence of coronary heart diseases. However, the variation in serum TBARS concentration was large, probably reflecting considerable biological variation and the unspecific nature of TBARS as a measure of lipid peroxidation (10). It seems reasonable to suppose considerable variation in the antioxidant state of the subjects. Nevertheless, the finding that TBARS increased with age could possibly be attributed to a reduced albumin protection of lipid peroxidation. In men there was a significant inverse correlation between individual TBARS and albumin values. We previously observed a strong protective effect of albumin against appearance of TBARS both in cell cultures, and in a cell free system with free fatty acids (25,28). Hypothetically, increased oxidative stress due to lower albumin protection in higher age groups could be a causative mechanism behind the age-related increased occurrence of coronary heart disease. We have no explanation for the lack of an inverse relationship between albumin and TBARS in women.

Thus, it would appear that the well known age-related increase in occurrence of myocardial infarction is accompanied by a decrease in serum albumin levels.

It is therefore tempting to speculate that the age-related increase in coronary heart disease could partly be attributed to reduced albumin levels. In this regard it would also seem of interest that an age-related reduction in serum albumin was not observed in women when the age increased from the lowest age group (30 years) to the next (40+45 years). This raises the question whether the apparent cardioprotective premenopausal age period in women is related to their constant albumin (and TBARS) level. After this age the serum albumin concentration fell in both sexes.

Oxidation of LDL seems to be involved in the development of atherosclerosis. Also a toxic effect of a high FFA/albumin ratio on endothelial cells could promote atherosclerosis and thrombosis. As observed in vitro, albumin can strongly counteract the inhibitory effect of fatty acids on growth of endothelial cells (28).

As pointed out above, conclusions about cause-and-effect associations are not appropriate based upon results of a cross sectional study. Self reported outcome variables are unreliable. Also, a cross sectional study implies differences other than age *per se* in the various age groups. In spite of this, and with reference to prospective epidemiological studies as well as many experimental studies, it is tempting to suggest that the present results of the Oslo Health Study would be in support of a cardioprotective role of serum albumin. Among several mechanisms of action it is hypothesized that albumin may exert part of its effect through protection against lipid peroxidation, but further evidence is needed to substantiate this hypothesis.

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