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# Increased Plasma Markers of Oxidative Stress Are Associated with Coronary Heart Disease in Males with Diabetes Mellitus and with 10-Year Risk in a Prospective Sample of Males

Jeffrey W. Stephens,[1*] David R. Gable,[2] Steven J. Hurel,[3] George J. Miller,[4] Jackie A. Cooper,[2] and Steve E. Humphries[2]

***Background:*** **Increased oxidative stress is associated with coronary heart disease (CHD). We examined the association between plasma markers of oxidative stress and CHD in a cross-sectional sample of patients with diabetes and prospective CHD risk in a sample of men predominantly without diabetes.**

***Methods:*** **Plasma total antioxidant status (TAOS) and the ratio of oxidized LDL (Ox-LDL) to LDL-cholesterol (LDL-C) were determined in a cross-section of 761 Caucasian individuals with diabetes (UDACS study). Plasma TAOS was also determined in 310 baseline samples from a 10-year prospective cohort of 3012 healthy males (NPHSII).**

***Results:*** **Within UDACS, males with CHD had lower mean (SD) plasma TAOS [no CHD, 43.4 (13.2)%; CHD, 40.3 (13.8)%; $P = 0.04$]. The prevalence of CHD was higher in the lowest compared with the upper quartiles (32.7% vs 19.7%; $P = 0.004$). We observed a significant association between plasma Ox-LDL:LDL-C and CHD status [no CHD vs CHD, 16.9 (3.1) vs 19.3 (5.0) units/mmol; $P = 0.04$], with the prevalence of CHD being higher among men in the upper compared with lower quartiles (18.4% vs 35.1%; $P = 0.003$). No association was observed in females. In NPHSII, TAOS was lower in those who developed CHD [35.1 (8.0)% vs 37.1 (7.9)%; $P = 0.04$]. The odds ratio for CHD in the lowest compared with the upper quartile was 1.91 (95% confidence interval, 0.99–3.70; $P = 0.04$). This remained unchanged after adjustment for classic risk factors.**

***Conclusions:*** **A cross-sectional and prospective association exists between baseline plasma measures of oxidative stress and CHD risk. The association with prospective CHD risk remained after adjustment for "traditional" risk factors, implying an independent role for oxidative stress in CHD risk.**



Considerable interest has developed in the role of free radical–mediated damage in many major disorders, in particular coronary heart disease (CHD),[5] diabetes, and cancer *(1)*. Oxidative stress results from an imbalance between oxidant production (or the formation of reactive oxygen species) and antioxidant defenses *(2)*. Several studies have demonstrated that plasma markers of oxidative stress are increased in CHD or in the presence of its classic risk factors *(3–5)*. In vitro, numerous adverse effects on the vascular system are associated with increased oxidative stress. The oxidation of vulnerable cell membrane unsaturated lipids *(6)* may modulate diverse signal transduction pathways *(5, 7)*, leading to numerous

[1] The Medical School, University of Wales Swansea, Swansea, United Kingdom.

[2] Centre for Cardiovascular Genetics, British Heart Foundation Laboratories, Royal Free & University College London Medical School, London, United Kingdom.

[3] Department of Diabetes & Endocrinology, University College London Hospitals, London, United Kingdom.

[4] Medical Research Council Cardiovascular Research Group, Wolfson Institute of Preventive Medicine, London, United Kingdom.

* Address correspondence to this author at: The Medical School, University of Wales Swansea, Swansea, SA2 8PP, United Kingdom. Fax 44-1792-703214; e-mail J.W.Stephens@Swansea.ac.uk.

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[5] Nonstandard abbreviations: CHD, coronary heart disease; UDACS, University College London Diabetes and Cardiovascular Disease Study; TAOS, total antioxidant status; Ox-LDL, oxidized LDL; LDL-C, LDL-cholesterol; NPHSII, The Second Northwick Park Heart Study; CRP, C-reactive protein; OR, odds ratio; CI, confidence interval; ACEI, angiotensin-converting enzyme inhibitor; Hb $A_{1c}$, glycohemoglobin; TG, triglyceride; and BMI, body mass index.

adverse effects implicated in the pathogenesis of atherosclerosis. These include increased expression of cell adhesion molecules, induction of proinflammatory pathways, activation of matrix metalloproteinase, vascular smooth muscle cell proliferation and death, endothelial dysfunction, and lipid peroxidation (LDL oxidation). Apart from the global effects associated with increased oxidative stress described above, more specific effects also occur. LDL is an important target of oxidation, and oxidative modification of LDL is a key step in the pathogenesis of atherosclerosis *(8)*. Therefore, as well as the general harmful effects on the vasculature, more specific effects may be seen at the lipoprotein level, which are dependent on the properties of the lipoprotein molecules.

In vivo, animal studies have shown increased oxidative stress during experimental hypoxia and during cardiac ischemia *(9)*. In humans, increased oxidative stress has also been demonstrated during coronary bypass grafting *(9)*, post-myocardial infarction, and in congestive cardiac failure *(9)*. Hence, there is considerable evidence to support the role of oxidative stress in the pathogenesis of CHD, although no prospective studies have been reported showing increased CHD risk in relation to basal plasma markers of oxidative stress. Hence, whether increased oxidative stress is a "cause" or "effect" of CHD remains to be established in vivo. The aim of this study was to examine the association between plasma markers of oxidative stress and CHD in a cross-sectional sample of individuals with diabetes and with prospective 10-year CHD risk in a sample of men predominantly without diabetes.

## Materials and Methods

Institutional ethics committee approval was obtained, and all participants gave written informed consent.

### CROSS-SECTIONAL SAMPLE OF PATIENTS WITH DIABETES

The group of patients with diabetes comprised 761 Caucasian patients with diabetes recruited into the University College London Diabetes and Cardiovascular Disease Study (UDACS). This is a cross-sectional sample of individuals designed to study the association between common variants in inflammatory/metabolic genes and biochemical risk factors implicated in CHD in patients with diabetes. The sample has been described elsewhere *(10, 11)*. Analysis focused on the 761 Caucasian individuals of the original 1011 to avoid any confounding effects on oxidative stress that may be observed in persons of different ethnic origins *(12, 13)*. The presence of CHD was recorded if any patient had positive coronary angiography/angioplasty, coronary artery bypass, cardiac thallium scan, exercise tolerance test, myocardial infarction, or symptomatic/treated angina. Any individual who was asymptomatic or had negative investigations was categorized as "no CHD". Persons with type 2 diabetes were defined as those patients not requiring insulin within 12 months of diagnosis. Smoking status was defined as current, never, or ex-smoker. The latter comprised those patients who reported having stopped smoking for >12 months. Current smokers included those who reported having stopped smoking within the last 12-month period. None of the participants was knowingly taking any form of vitamin supplementation. Plasma samples were collected within a 12-month period and stored immediately at −80 °C. Of the 761 participants, 457 were males and 304 were females. Plasma total antioxidant status (TAOS) and oxidized LDL (Ox-LDL) was determined on these samples.

### PROSPECTIVE SAMPLE OF PATIENTS

These were males recruited into the Second Northwick Park Heart Study (NPHSII), as detailed elsewhere *(14)*. In brief, 3012 unrelated healthy Caucasian middle-aged men [mean (SD) age, 56.1 (3.5) years] recruited from 9 general practices in the United Kingdom were prospectively followed for a median of 10.2 years (interquartile range, 8.1–11.4 years). Exclusion criteria at baseline were a history of myocardial infarction, cerebrovascular disease, life-threatening malignancy, or regular medication with aspirin or anticoagulants. At entry, a 5-mL EDTA-blood sample was obtained. Time to first CHD event, defined as symptomatic/silent myocardial infarction (the appearance of a new major Q wave on the follow-up electrocardiogram, using Minnesota codes $1_1$,$1_{2.1}$ to $1_{2.7}$, and $1_{2.8}$ plus $5_1$ or $5_2$) or coronary revascularization, was recorded, yielding only 1 event/individual. Baseline plasma was made available to determine plasma TAOS on 310 samples. All samples had been stored under identical conditions at −80 °C for a period of 10 years. These samples were accessed to determine plasma TAOS in relation to 10-year prospective risk. Of these men, 10 had diabetes (7 with CHD at follow-up). The diabetes status in these men was made from direct questioning of symptoms and a previous diagnosis. A biochemical diagnosis could not be obtained. The use of vitamin supplements was not documented at baseline in these individuals. Current smokers and nonsmokers were documented. The latter included never and ex-smokers, i.e., those reporting having stopped smoking more than 12 months prior to the start of the study.

### DETERMINATION OF PLASMA TAOS

Plasma TAOS was determined by a photometric microassay previously described by Sampson et al. *(15)*. The plasma TAOS was determined by its capacity to inhibit the peroxidase-mediated formation of the 2,2-azino-bis-3-ethylbensthiazoline-6-sulfonic acid (ABTS+) radical. In the assay, the relative inhibition of ABTS+ formation in the presence of plasma is proportional to the antioxidant capacity of the sample. There thus are 2 arms to the assay, a control arm and a test arm. In the control arm, phosphate-buffered saline is used instead of plasma. The assay is performed in a 96-well ELISA plate with 2.5 μL of plasma. The difference in absorbance (control absorbance minus test absorbance) divided by the control absorbance (expressed as a percentage) was used to represent the percentage inhibition of the reaction by plasma and thus

the antioxidant status relative to saline. Therefore, in general terms, increased oxidative stress within a sample would be associated with consumption of antioxidants and diminished antioxidant status within the sample. The interassay CV was 14%.

### measurement of Ox-LDL in udacs

Ox-LDL was measure by ELISA (Mercodia AB). In this assay, a monoclonal antibody is directed against antigenic determinants in the Ox-LDL molecule (mAB-4E6). Ox-LDL concentrations are strongly correlated with plasma LDL concentrations, and the latter is thus a key factor in determining absolute plasma Ox-LDL concentration. To overcome this, several researchers in the field have considered the ratio of the Ox-LDL (units/L) concentration to the LDL-cholesterol (LDL-C; mmol/L) concentration *(16)*. This gives an Ox-LDL:LDL-C ratio (units per mmol of LDL-C). We chose this approach to express our results.

### statistical analysis

Analysis in UDACS was performed with SPSS (Ver. 10.1; SPSS Inc.). Results are presented as the mean (SD), and for data that were not gaussian distributed as the geometric mean (approximate SD) or median (interquartile range) as indicated in the *Results*. The geometric mean and approximate SD are shown for plasma C-reactive protein (CRP) and Ox-LDL:LDL. In UDACS, the relationships between baseline values and plasma TAOS and Ox-LDL:LDL-C were tested by Pearson correlation. Analysis of covariance was performed to test the association between CHD status with plasma TAOS and Ox-LDL:LDL-C after adjustment for potential confounders, using multiple regression analysis to obtain a residual. $\chi^2$ tests were used to compare differences in categorical variables. In all cases, a $P$ value $<0.05$ was considered statistically significant. In NPHSII, analysis was performed with the "Intercooled STATA" package (Ver. 8.2; STATA Corporation). Results are presented as odds ratios (ORs) with their corresponding 95% confidence intervals (CIs) obtained from logistic regression models.

## Results

### patients with diabetes (udacs): cross-sectional sample

The baseline characteristics of those individuals recruited from UDACS are shown in Table 1. As described previously for this sample *(17)*, a significantly higher proportion of individuals with CHD were taking angiotensin-converting enzyme inhibitors (ACEIs), statins, insulin, and metformin, which potentially accounted for their lower total cholesterol and blood pressure. We observed no difference in glycohemoglobin (Hb $A_{1c}$) and random plasma glucose between those with and without CHD. Of those with CHD, there were a significantly higher proportion of current smokers and those with type 2 diabetes mellitus.

### plasma taos and chd

With respect to sex, we observed no significant difference in plasma TAOS between males and females [43.1 (13.2)% vs 41.7 (13.1)%; $P = 0.14$]. Plasma TAOS was independent of pharmacotherapy but correlated positively with plasma HDL-cholesterol and negatively with triglyceride (TG), glucose, and Hb $A_{1c}$ concentrations and proteinuria [correlation coefficient ($r$) = 0.12, −0.15, −0.11, −0.10, and −0.07, respectively; all $P < 0.05$]. Stepwise backward re-

**Table 1. Baseline differences in patients by CHD status in UDACS.**

| Trait | No CHD (n = 607) | CHD (n = 154) | *P* |
|---|---|---|---|
| Age,[a] years | 61.0 (14.0) | 67.8 (10.3) | <0.001 |
| Systolic blood pressure,[b] mmHg | 139 (20) | 139 (22) | 0.99 |
| Diastolic blood pressure,[b] mmHg | 80 (11) | 77 (12) | 0.003 |
| BMI,[b] kg/m$^2$ | 28.3 (5.6) | 29.5 (5.4) | 0.02 |
| Hb $A_{1c}$,[b] % | 7.8 (1.6) | 7.6 (1.5) | 0.14 |
| Glucose,[b] mmol/L | 9.7 (4.8) | 9.6 (4.3) | 0.75 |
| LDL-cholesterol,[a] mmol/L | 2.9 (0.9) | 2.5 (0.90) | <0.001 |
| HDL-cholesterol,[b] mmol/L | 1.4 (0.5) | 1.2 (0.4) | <0.001 |
| TGs,[b] mmol/L | 1.6 (0.9) | 1.9 (1.0) | 0.01 |
| CRP,[b] mg/L | 1.53 (1.33) | 1.82 (1.58) | 0.03 |
| TAOS,[a] % | 42.7 (13.2) | 41.5 (13.1) | 0.32 |
| Sex (F/M), % | 41.2/58.8 | 32.0/68.0 | 0.04 |
| Type of diabetes (I/II), % | 27.0/73.0 | 5.1/94.9 | <0.001 |
| Never/Ex/Current smokers, % | 51.9/30.8/17.3 | 43.7/44.4/11.9 | 0.006 |
| Medications (no/yes), % | | | |
| ACEI | 58.0/42.0 | 44.2/55.8 | 0.002 |
| Aspirin | 62.8/37.2 | 25.7/74.3 | <0.001 |
| Insulin | 54.3/45.7 | 63.6/36.4 | 0.04 |
| Statin | 81.7/18.3 | 40.5/659.5 | <0.001 |

[a] Mean (SD).
[b] Log-transformed data. Geometric mean (approximate SD) shown.

gression showed that glucose and TG concentrations were the strongest independent predictors of plasma TAOS (glucose, $P = 0.004$; TGs, $P = 0.005$). No other significant correlations were observed with blood pressure, type of diabetes, smoking status, other lipid measures, CRP, or body mass index (BMI).

In males, there was a significant association between plasma TAOS and CHD status [no CHD vs CHD, 43.4 (13.2)% vs 40.3 (13.8)%; $P = 0.04$]. After adjustment of plasma TAOS for plasma glucose and TG concentrations, this association was no longer statistically significant ($P = 0.06$). Shown in Fig. 1A is the proportion of men with CHD grouped by quartiles of TAOS. The percentage of men with CHD in the lowest quartile was significantly higher than in the other quartiles, suggesting that there was a threshold at which lower plasma TAOS was associated with CHD. Furthermore, there was a highly significant difference when men in the lower quartile were compared with the other quartiles combined (percentage of men with CHD in the lower quartile vs the other quartiles 32.7% vs 19.7%; $P = 0.004$). In the women, we found no association between plasma TAOS and CHD status [no CHD vs CHD, 41.7 (13.1)% vs 43.9 (11.4)%; $P = 0.26$]. We observed no difference after adjustment and no effect after we divided the group by quartiles of plasma TAOS (Fig. 1B).

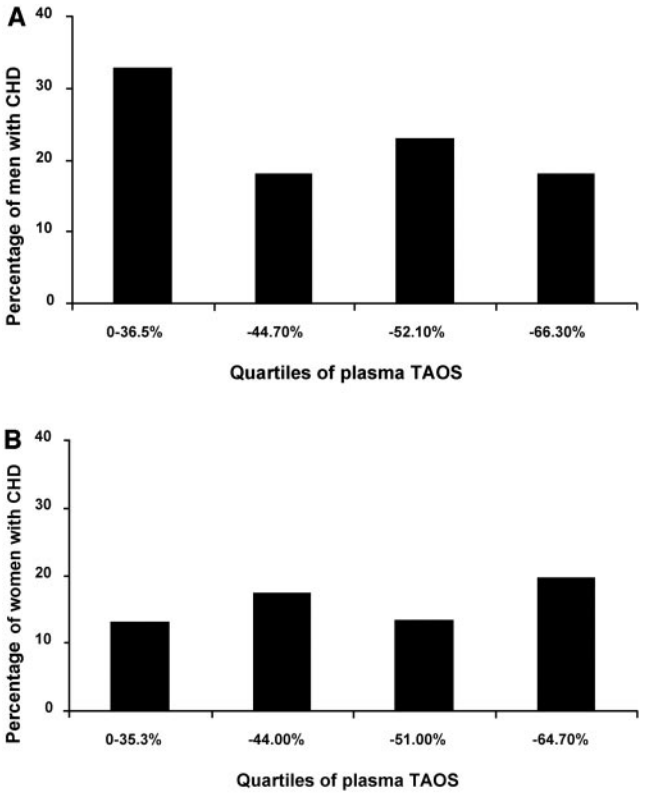


Fig. 1. Association of plasma TAOS with CHD (UDACS).

(*A*), males. $\chi^2$ test for difference between groups, $\chi^2 = 9.93$ ($P = 0.03$). Linear trend, $P = 0.03$. (*B*), females. $\chi^2$ test for difference between groups, $\chi^2 = 1.75$ ($P = 0.63$).

### PLASMA Ox-LDL AND CHD

In UDACS, no correlations between plasma TAOS and Ox-LDL:LDL-C ($r = 0.04$; $P = 0.36$) were observed. In both sexes, Ox-LDL:LDL-C showed a strong negative correlation with LDL ($r = -0.49$; $P < 0.001$). This is expected because LDL is the denominator for the calculation of Ox-LDL:LDL-C. We observed no other significant correlations with blood pressure, type of diabetes, CRP, or BMI. In males, Ox-LDL:LDL-C was negatively correlated with HDL ($r = -0.2$; $P < 0.001$) and positively correlated with plasma TGs ($r = 0.13$; $P = 0.02$). These associations were not significant in females (HDL, $r = 0.08$; $P = 0.24$; TGs, $r = 0.11$; $P = 0.13$). We observed no significant differences in plasma Ox-LDL:LDL-C in those taking or not taking ACEIs, statins, or aspirin (data not shown). In all participants, plasma Ox-LDL:LDL-C was significantly higher in those with CHD [no CHD vs CHD, 16.8 (7.4) vs 19.0 (10.0) units/mmol; $P = 0.02$]. In males, there was a significant association between plasma Ox-LDL:LDL-C and CHD status [no CHD vs CHD, 16.9 (3.1) vs 19.3 (5.0) units/mmol; $P = 0.04$]. After adjustment for HDL and TG concentrations, this association was no longer significant ($P = 0.10$). The proportion of males with CHD grouped by quartiles of Ox-LDL:LDL-C is

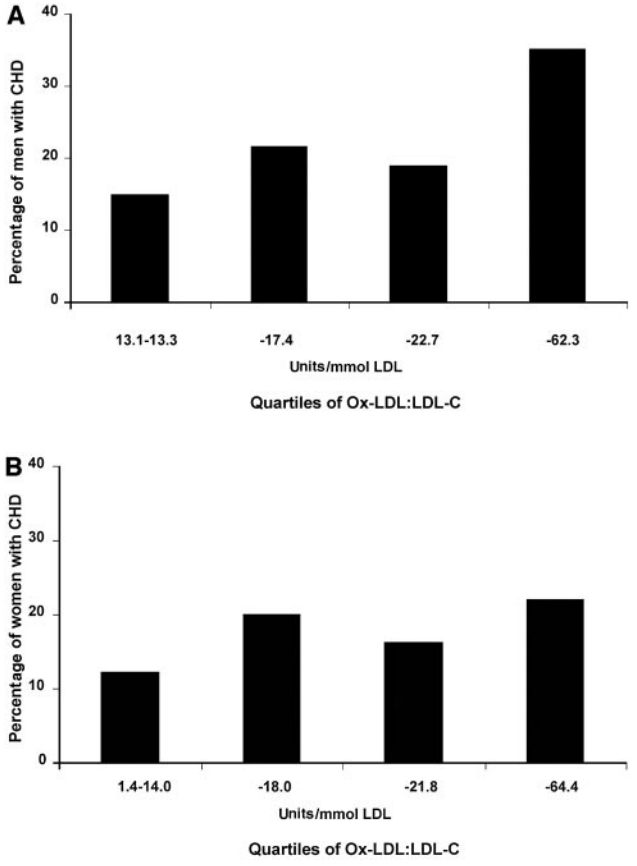


Fig. 2. Association of Ox-LDL:LDL-C with CHD (UDACS).

(*A*), males. $\chi^2$ test for difference between groups, $\chi^2 = 9.90$ ($P = 0.02$). Linear trend, $P = 0.008$. (*B*), females. $\chi^2$ test for difference between groups, $\chi^2 = 1.83$ ($P = 0.60$).

**Table 2. Baseline differences in patients by CHD status in NPHSII.**

| Trait | No CHD (n = 211) | CHD (n = 99) | *P* |
|---|---|---|---|
| Age,[a] years | 56.3 (3.6) | 56.4 (3.58) | 0.83 |
| Systolic blood pressure,[b] mmHg | 138 (19) | 143 (21) | 0.03 |
| Diastolic blood pressure,[a] mmHg | 85 (12) | 86 (11) | 0.37 |
| Body mass index,[b] kg/m$^2$ | 26.8 (3.4) | 26.8 (3.4) | 0.82 |
| Current smoking, % (n) | 25.6 (54) | 46.5 (46) | <0.0001 |
| Cholesterol,[a] mmol/L | 5.8 (1.00) | 6.1 (1.00) | 0.02 |
| TGs,[b] mmol/L | 1.9 (1.0) | 2.1 (1.1) | 0.17 |
| CRP,[b] mg/L | 1.15 (1.28) | 2.05 (2.26) | <0.0001 |
| Fibrinogen,[b] g/L | 2.73 (0.55) | 2.93 (0.56) | 0.003 |
| Plasma TAOS,[a] % | 37.1 (7.9) | 35.1 (8.0) | 0.04 |

[a] Mean (SD).
[b] Log-transformed data. Geometric mean (approximate SD) shown.

shown in Fig. 2A. The percentage of men with CHD was significantly higher in the highest quartile compared with the lower 3 quartiles, suggesting a threshold at which Ox-LDL:LDL-C was associated with CHD. Consistent with this, there was a highly significant difference when men in the upper quartile was compared with the other quartiles combined (percentage of men with CHD in the lower quartiles vs the upper quartiles, 18.4% vs 35.1%; $P = 0.003$). In the females, we found no significant association between plasma Ox-LDL:LDL-C and CHD [no CHD vs CHD, 16.7 (3.4) vs 18.4 (2.8) units/mmol; $P = 0.25$]. We also observed no effect when we divided the group by quartiles of plasma Ox-LDL:LDL-C (Fig. 2B).

### prospective sample (nphsii)

The baseline characteristics of the NPHSII individuals, grouped by CHD status, are shown in Table 2. Of note, there were 10 patients with diabetes in this sample. Of these, 7 went on to develop CHD and 3 did not. We observed no difference in plasma TAOS between these patients [nondiabetes vs diabetes, 36.4 (7.9) vs 39.9 (8.1); $P = 0.08$]. We have included these in the overall analysis because the numbers are small. No further subgroup analysis has been performed in relation to diabetes status because of these small numbers. As shown, the expected baseline measures were associated with prospective CHD risk. In NPHSII, plasma TAOS was negatively correlated with TG concentration ($r = -0.20$; $P = 0.06$). No glucose values were available on this group of individuals. There was a nonsignificant borderline association with fibrinogen concentration ($r = 0.18$; $P = 0.07$). No other significant associations were observed with blood pressure, other lipid measures, or smoking status. Of the 310 samples analyzed, 99 patients went onto develop a CHD event over the following 10 years, and mean plasma TAOS was significantly lower in this group (Table 2). A 1 SD decrease in TAOS of 7.9% was associated with a modest increase in CHD risk (OR = 1.30; 95% CI, 1.02–1.64; $P = 0.04$). This association remained significant after adjustment for classic risk factors associated with increased risk in the NPHSII (age, systolic blood pressure, BMI, TG and fibrinogen concentrations, diabetes, and smoking). After adjustment for these risk factors, the OR for CHD associated with a 1 SD decrease in plasma TAOS was 1.30 (95% CI, 1.08–1.45; $P = 0.01$), suggesting that a lower plasma TAOS had an independent effect on 10-year CHD risk. The ORs for CHD by quartiles of plasma TAOS [the upper quartile (quartile 4) being the reference point; OR = 1.00] are shown in Table 3. The pattern is similar to that observed for the prevalence of CHD in males from UDACS (described above). Again a threshold effect was observed, with the OR for CHD being significantly higher in the lowest quartile of plasma TAOS.

## Discussion

Plasma TAOS and Ox-LDL:LDL-C are measures of plasma oxidation and specific LDL oxidation, respectively. Although plasma TAOS is not a highly specific measure of plasma oxidative stress, for larger studies such as UDACS, it is a practical and inexpensive assay. By comparison, more specific assays of plasma oxidation (e.g., plasma $F_2$-isoprostanes) are time-consuming and

**Table 3. CHD risk by baseline plasma TAOS in NPHSII.**

| Quartile (plasma TAOS) | No CHD, n (%) | CHD, n (%) | OR (95% CI) | Adjusted OR[a] (95% CI) |
|---|---|---|---|---|
| 1 (<31%) | 43 (20.4) | 35 (35.4) | 1.91 (0.99–3.70) | 2.22 (1.08–4.56) |
| 2 (31%–37.3%) | 57 (27.0) | 20 (20.2) | 0.82 (0.41–1.67) | 0.89 (0.41–1.91) |
| 3 (37.4%–42.1%) | 57 (27.0) | 21 (21.2) | 0.86 (0.43–1.74) | 0.88 (0.41–1.89) |
| 4 (>42.1%) | 54 (25.6) | 23 (23.2) | 1.00 | 1.00 |
| *P* | | | 0.04 | 0.03 |

[a] Adjusted for age; systolic blood pressure; BMI; cholesterol, Tg, and fibrinogen concentrations; diabetes; and smoking.

expensive. Of interest, we have previously observed a strong correlation ($r = -0.65$; $P = 0.003$) *(18)* between plasma TAOS and esterified $F_2$-isoprostane in a subsample from UDACS *(17)*. This observation requires replication in a larger study.

The strongest correlates with plasma TAOS were random glucose and TG concentrations, 2 of the common abnormalities associated with type 2 diabetes mellitus. Plasma TG concentrations are negatively associated with HDL (in UDACS, $r = -0.52$), which may account for the association between TAOS and HDL. This is in line with previous observations suggesting that HDL exhibits antioxidant properties *(19)*, and increasing plasma glucose is associated with increased oxidative stress *(20)*. Interestingly, increased TG concentrations are also associated with the increased preponderance of small, dense LDL, which is more labile to oxidation *(21)*. We observed no significant effects with respect to treatment with ACEIs, statins, or aspirin. CHD was associated with lower plasma TAOS in males. With respect to CHD, we observed a "threshold"; ~13% more men in the lower quartile of plasma TAOS had CHD compared with the combined upper quartiles. In the females, we observed no such association with plasma TAOS or Ox-LDL:LDL-C. The association between plasma markers of oxidative stress and CHD in men has previously been described in cross-sectional studies *(21)*, but no studies have yet been published looking at this association in females. This is an interesting observation because it is well established that women have relative protection from CHD compared with men. However, in women with diabetes, the risk is similar to that of males. This observation requires replication in a future study before any generalized comments can be made.

We also explored the association with CHD in the prospective NPHSII sample of men. Patients in the lowest quartile of plasma TAOS had an approximate doubling in prospective CHD risk, even after adjustment for well-established risk factors associated with CHD. Again, in this sample of patients, there was a threshold at which risk was increased. This risk is substantial and equates to that of cigarette smokers or a family history of CHD *(22)*. Our results indicate that oxidative stress is associated with CHD risk in both groups of patients. Because UDACS is a cross-sectional study, we are unable to ascertain the contribution to prospective risk in this study. One might anticipate that the risk associated with a 1 SD decrease in plasma TAOS would be greater in a diabetic sample. Since recruitment of UDACS completed in December 2002, 38 participants have died. To date we do not have prospective data on CHD outcome; however, of interest, when death was considered as the outcome, for the whole sample the OR for death was 3.26 (95% CI, 1.05–10.15) for persons in the lowest quartile of TAOS compared with the upper quartile ($P = 0.03$). This is a preliminary observation only without any adjustment for confounders, and no data are available on the cause of death at present. This will be followed up in due course. Determining plasma TAOS in another prospective sample is required to confirm this observation. No previous reports have been published reporting the association between baseline measures of plasma oxidative stress and prospective risk.

Within NPHSII, plasma for the measurement of TAOS was obtained from samples selected as a nested case–control study. Therefore, by design there was a greater proportion of cases included in this analysis than in the overall cohort. Compared with the unmeasured individuals, the measured controls had a marginally higher mean (SD) BMI [26.1 (3.4) vs 26.7 (3.4) $kg/m^2$; $P = 0.03$], whereas the cases had a higher proportion of smokers (32.5% vs 45.3%; $P = 0.04$). Because the TAOS difference between cases and controls remained statistically significant after adjustment for BMI, smoking is not a major confounder. The use of vitamin supplements was not documented in the NPHSII study; hence, this could not be considered in the analysis. Therefore, any possible antioxidant effects by such supplements have not been considered, and the data should be interpreted with this in mind. One potential concern with the NPHSII data was that mean plasma TAOS was lower in the NPHSII sample compared with UDACS. It is likely that this is a result of the fact that the samples from NPHSII had been stored at −80 °C for a period of 10 years, compared with ~12 months for the samples from UDACS. Thus, the TAOS measures cannot be compared directly between the 2 cohorts, but within each, the results are interpretable. It is not possible to predict the degree of autooxidation that might have occurred in the NPHSII samples over the storage period, and it is possible that total antioxidant status may decay at a variable rate, depending on the initial antioxidant content and availability of prooxidant factors. Although we are unable to provide direct evidence to support the suggestion that autooxidation will proceed at the same rate in all stored samples, we believe that this is unlikely to be a major confounder of the case–control difference seen because all samples within each study were stored under identical conditions and the analysis was performed blindly and yielded robust results. However, the samples thus cannot be compared between the 2 cohorts, but within each, the results are interpretable.

Within UDACS, Ox-LDL:LDL-C was also studied in relation to CHD status. The latter provides an estimate of the proportion of oxidatively modified LDL per LDL-C. Ox-LDL:LDL-C was significantly higher in males with CHD, although no significant difference was observed in females. In males, there appeared to be a threshold at which men in the upper quartile had a significantly increased prevalence of CHD (~17% higher in the upper quartile compared with the lower quartiles). No such samples were available from NPHSII to allow prospective analysis, and further work is required in this area. Depending on these results, the measurement of Ox-LDL:LDL-C might be considered as an aid in assessing CHD risk.

In line with previous studies *(21, 23)*, LDL oxidation was not correlated with plasma TAOS, suggesting that they are independently associated with risk and other biochemical risk factors. This may be accounted for by the fact that Ox-LDL does not provide a large contribution to overall plasma oxidation but is a specific measure of LDL oxidative modification. Both measures may, however, be clinically useful in identifying at-risk individuals. Ox-LDL is associated with processes involved in plaque formation (e.g., increased foam cell, LDL aggregation, and enhanced monocyte chemotaxis), whereas the TAOS of plasma may influence or regulate other processes involved in the pathogenesis of atherosclerosis (e.g., matrix metalloproteinase activation, apoptosis, expression of cell adhesion molecules, and altered vasomotor activity).

In summary, this study shows a cross-sectional and prospective association between baseline plasma measures of oxidative stress and CHD risk. The association with prospective CHD risk remained after adjustment for "traditional" risk factors, suggesting an independent role for oxidative stress in CHD risk. The recruitment of UDACS completed in December 2002; since then, 38 participants have died. Of interest, when death was considered as the outcome, for the whole sample the OR for death was 3.26 (1.05–10.15) for persons in the lowest quartile of TAOS compared with the upper quartile ($P = 0.03$). This is a preliminary observation only without any adjustment for confounders, and no data are available of the cause of death at present. This will be followed up in due course.

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