Elevated Levels of Authentic Plasma Hydroperoxides in NIDDM

Jaffar Nourooz-Zadeh, Javad Tajaddini-Sarmadi, Sue McCarthy, D. John Betteridge, and Simon P. Wolff

Using a precise technique for measuring authentic plasma lipid hydroperoxides (ROOHs), we show that individuals with non-insulin-dependent diabetes mellitus (NIDDM) have higher levels of ROOH than do control subjects. ROOHs were measured by the ferrous oxidation with xylenol orange assay coupled with the selective ROOH reductant triphenylphosphine. Formation of the ferric xylenol orange complex was determined at 560 nm and calibrated against H_2O_2. For 22 individuals with NIDDM, a concentration of 9.04 ± 4.3 μmol/l (mean ± SD) ROOH was recorded. This concentration was higher ($P < 0.0005$ by separate-variance t test) than that of plasma ROOHs from control subjects (3.76 ± 2.48 μmol/l). There was no difference between concentrations of plasma malondialdehyde measured as thiobarbituric acid–reactive material (TBARM) in NIDDM or control subjects (1.00 ± 0.70 vs. 1.21 ± 0.62 μmol/l, respectively; $P > 0.1$). A trend to lower vitamin E levels in the NIDDM group (9.03 ± 3.31 vs. 10.31 ± 5.02 μg/ml in control subjects) failed to achieve significance at the 95% confidence level. Plasma ROOHs in the diabetic group did not correlate with total plasma cholesterol, triglyceride, fasting glucose, HbA_1, vitamin E, or TBARM levels. These data indicate that measurement of authentic ROOHs shows NIDDM to be associated with oxidative stress, which may be unrelated to abnormalities in lipid metabolism and glycemic control. *Diabetes* 44:1054–1058, 1995

Non-insulin-dependent diabetes mellitus (NIDDM) is postulated to be associated with increased lipid peroxidation, which may contribute to vascular and other complications of the syndrome (1,2). Such oxidative stress has, however, been difficult to assess because specific and reproducible methods for the measurement of products of lipid peroxidation have been unavailable. We have recently developed an assay for lipid hydroperoxides (ROOHs) based upon the selective oxidation of ferrous to ferric ions by hydroperoxides under acidic conditions. Ferric ions generated by ROOHs in the assay are complexed by the ferric ion indicator, xylenol orange [*o*-cresolsulfonphthalein-3′,3″-bis(methyliminodiacetic acid) sodium salt], generating a blue-purple complex with an absorbance maximum of 550–600 nm. A wide range of synthetic hydroperoxides give an extinction coefficient at 560 nm of 4.3×10^4 $mol^{-1} \cdot l \cdot cm^{-1}$ in the assay (3,4). For plasma samples, signal generated using the ferrous oxidation in xylenol orange (FOX) assay is authenticated by prior reduction of plasma ROOHs with triphenylphosphine (TPP), thereby generating an internal control (5).

The FOX technique for hydroperoxide measurement requires no plasma lipid extraction step, in contrast to iodometric (6) and high-performance liquid chromatography (HPLC)-linked (postcolumn) microperoxidase-driven isoluminol chemiluminescence techniques (7–9), and thus suffers no extraction losses. Further, the FOX assay is capable of measuring all classes of hydroperoxide present in plasma with precision, while the HPLC-chemiluminescence techniques may greatly over- or underestimate cholesteryl ester and phospholipid hydroperoxide concentrations. Thus, with very similar HPLC-chemiluminescence techniques, normal levels of phospholipid hydroperoxide in apparently healthy people have been variously reported be 10–500 nmol/l (8), to be at undetectably low levels (9), or to have an upper limit of 30 nmol/l (10). Cholesteryl ester hydroperoxide levels in normal plasma are reported to be as low an 3 nmol/l (11) or as high as 920 nmol/l (12). There appears to have been no attempt to measure hydroperoxide in the triglyceride fraction, perhaps because this fraction is difficult to resolve by HPLC, although it cannot be inert with respect to peroxidation.

Techniques that measure total ROOHs suggest higher values for lipid peroxidation than those obtained by assessment of phospholipid or cholesteryl ester hydroperoxides. Thus, total ROOHs in pooled plasma samples have been estimated to be 4.0 ± 1.7 μmol/l by a complex but exact iodometric technique (13) and 2.1–4.6 μmol/l ($n = 5$; mean = 3.1 μmol/l) by an automated iodometric technique (14). These values are in reasonable agreement with our own estimate for total plasma ROOHs using the FOX assay with TPP authentication of signal. We have previously reported a range of 0.22–7.8 μmol/l (3.02 ± 1.85 μmol/l) for 23 apparently healthy individuals (5). This value for total plasma hydroperoxides was only slightly lower (range, 0.22–6.22 μmol/l; 2.52 ± 1.65 μmol/l) when the plasma was first subjected to total lipid extraction with ethyl acetate before analysis of hydroperoxides in the lipid fraction. There was a strong correlation (Pearson's $r = 0.78$; $P < 0.005$) between the hydroperoxide values determined in individual plasma

From the Division of Clinical Pharmacology and Toxicology, Department of Medicine, University College London Medical School, London, U.K.

Address correspondence and reprint requests to Dr. Simon P. Wolff, Division of Clinical Pharmacology and Toxicology, Department of Medicine, UCL Medical School, 5 University Street, London WC1E 6JJ, U.K.

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BHT, butylated hydroxytoluene; FOX, ferrous oxidation in xylenol orange; 5-HPETE, [5(*S*),6*E*,8*Z*,11*Z*,14*Z*]-5-hydroperoxyeicosatetraenoic acid; HPLC, high-performance liquid chromatography; 9-HPODE, [9(*S*),10*E*,12*Z*]-9-hydroperoxyoctadecadien-1-oic acid; 13-HPODE, [9*Z*,11*E*,13(*S*)]-13-hydroperoxyoctadecadien-1-oic acid; LDL, low-density lipoprotein; NIDDM, non-insulin-dependent diabetes mellitus; PAP, peroxidase-antiperoxidase; ROOH, lipid hydroperoxide; TBA, thiobarbituric acid; TBARM, thiobarbituric acid–reactive material; TPP, triphenylphosphine.

samples by both methods. Having established the feasibility of measuring ROOH in plasma using the FOX assay, we now propose to determine applicability of the method to the study of lipid peroxidation in disease processes, including NIDDM, in which oxidative stress is postulated.

RESEARCH DESIGN AND METHODS

Reagents. Ammonium ferrous sulfate, H_2O_2, butylated hydroxytoluene (BHT), *tert*-butylhydroperoxide, and thiobarbituric acid (TBA) were obtained from Sigma (Poole, Dorset, U.K). Xylenol orange [*o*-cresolsulfonphthalein-3′,3″-bis(methyliminodiacetic acid sodium salt)] and TPP were purchased from Aldrich (Gillingham, Dorset, U.K.). The commercially available hydroperoxides [5(*S*),6*E*,8*Z*,11*Z*,14*Z*]-5-hydroperoxyeicosatetraenoic acid (5-HPETE), [9(*S*),10*E*,12*Z*]-9-hydroperoxyoctadecadien-1-oic acid (9-HPODE), and [9*Z*,11*E*,13(*S*)]-13-hydroperoxyoctadecadien-1-oic acid (13-HPODE) were purchased from Cascade (Reading, Berkshire, U.K.). Cholesterol color reagent (CHOD-iodide method) was obtained from Merck (Darmstadt, Germany). All general chemicals and reagents were of the highest purity available.

Clinical laboratory measurements. Glycemic control was measured according to fasting plasma glucose levels by the glucose oxidase method, and HbA_1 was measured by agar gel electrophoresis (15). Total plasma cholesterol was measured by the cholesterol-C high-performance CHOD–peroxidaze-antiperoxidase (PAP) method (Boehringer Mannheim, Mannheim, Germany). Total triglyceride was measured using the GPO-PAP high-performance enzymatic colorimetric test (Boehringer Mannheim).

Preparation of plasma. Twenty-two patients with NIDDM (9 with no evidence of micro- or macroangiopathy, 10 with microangiopathy, and 3 with macroangiopathy) were recruited from well-characterized patients attending the University College London Hospitals Diabetic Service. Control subjects were recruited from staff at the Department of Medicine, University College London Medical School. Blood was collected by venipuncture into sampling vials containing heparin. Platelet-depleted plasma was prepared by centrifugation at 2,000*g* for 10 min at room temperature.

Measurement of lipid peroxidation products

Hydroperoxides. The hydroperoxide content of whole plasma was determined with the FOX Version II assay for lipid ROOHs (FOX2) (8). The FOX2 reagent was prepared by dissolving ammonium ferrous sulfate (9.8 mg) in 250 mmol/l H_2SO_4 (10 ml) to give a final concentration of 250 μmol/l ferrous ion in acid. This solution was then added to 90 ml of HPLC-grade methanol containing 79.2 mg BHT. Finally, 7.6 mg xylenol orange were added with stirring to make the final working reagent (250 μmol/l ammonium ferrous sulfate, 100 μmol/l xylenol orange, 25 mmol/l H_2SO_4, and 4 mmol/l BHT in 90% vol/vol methanol in a final volume of 100 ml). The working reagent was routinely calibrated against solutions of H_2O_2 of known concentration. Reagent was also obtained as the commercial preparation (PeroXOquant quantitative peroxide assay: lipid compatible formulation) from Pierce (Rockford, IL).

Aliquots (90 μl) of plasma were transferred into six 1.5-ml microcentrifuge vials. TPP in methanol (10 μl of 10 mmol/l) was added to three vials to remove hydroperoxides. Methanol (10 μl) was added to the remaining three vials. This generated blank and test samples, respectively. All vials were then vortex-mixed and incubated at room temperature for 30 min before the addition of 900 μl FOX2 reagent with mixing. After incubation at room temperature for a further 30 min, the vials were centrifuged at 12,000*g* for 10 min. The absorbance of the supernatant was then determined at 560 nm. Hydroperoxide content in the plasma samples was determined as a function of the mean absorbance difference of samples with and without elimination of ROOH by TPP. The SD was taken as the larger of the SDs of the measurements obtained with or without TPP treatment. The coefficient of variation for individual plasma samples with this method is typically <5%.

For the iodometric measurement of ROOHs, synthetic hydroperoxides (20 μl) were mixed with 2 ml of CHOD-iodide reagent in a 3-ml cuvette at room temperature. The absorbance at 290 nm was continuously monitored for 15 min and calibrated against standardized hydrogen peroxide solutions (16).

Thiobarbituric acid-reactive material (TBARM). Malondialdehyde and related aldehydes (TBARM) in whole samples were determined by mixing of plasma samples (100 μl) with 0.67% TBA (1,000 μl) and 20% trichloroacetic acid (500 μl) followed by incubation at 100°C for 20 min. The absorbance of the supernatant was monitored at 532 nm after centrifugation at 12,000*g* for 5 min. The concentration of lipid peroxidation products was calculated as malondialdehyde equivalents using the extinction coefficient for the malondialdehyde-TBA complex of $1.56 \times 10^5\ mol^{-1} \cdot l \cdot cm^{-1}$ (17). Inclusion of butylated hydroxytoluene in plasma sample/reagent, as recommended by some authors, did not affect the data obtained under our experimental conditions (18).

Vitamin E (α-tocopherol) analysis. For analysis of vitamin E, plasma samples (200 μl) were first mixed with 500 μl ethanol (containing 50 ng/ml of γ-tocopherol as internal standard), 1,000 μl hexane, and 300 μl water. After centrifugation at 2,500*g* for 5 min, the hexane (upper layer) was transferred to a glass vial and dried under a stream of nitrogen. The residue was then redissolved in 200 μl acetonitrile. HPLC separation was performed on a Hypersil-ODS column (10 cm × 5 mm, particle size 5 μmol/l; Chrompack, the Netherlands) using acetonitrile:tetrahydrofuran: water (80:14:6 by volume) at a flow rate of 0.8 ml/min. Tocopherols were monitored fluorometrically (emission, 295 nm; excitation, 340 nm) (19).

Plasma fatty acid composition. The fatty acid profile of plasma samples was determined by the analysis of total fatty acids as their methyl esters. Plasma samples (200 μl) were mixed with 100 μg heptadecanoic acid in ethanol (250 μl) as an internal standard. Ethyl acetate (500 μl) and water (300 μl) were then added with vigorous mixing, and samples were centrifuged at 2,500*g* for 5 min. The organic (upper) layer was then transferred to a glass vial. Ethyl acetate (500 μl) was added to the remaining aqueous layer with vortex mixing followed by centrifugation at 2,500*g*. The organic layers from the first and second extractions were pooled and dried under a nitrogen stream. Boron trifluoride–methanol (14%) solution (500 μl) was then added, and the vials were heated at 60°C for 30 min. Water (500 μl) and hexane (1 ml) were then added. The hexane was evaporated to dryness under a stream of nitrogen. The residue was redissolved in 100 μl hexane, of which 1 μl was injected onto a Stabilwax column (30 m × 0.53 mm, film thickness 1.0 μm) using a temperature gradient of 140–210°C at 2°C/min. The signal was detected by a flame ionization detector.

Lipoprotein purification. Lipoproteins were purified sequentially from platelet-depleted plasma as described previously (20). In brief, platelet-depleted plasma was prepared by centrifugation at 2,000*g* for 10 min at room temperature in the presence of 1 mg/ml EDTA to prevent oxidation. After centrifugation for 18 h at 100,000*g*, the supernatant (density <1.006), containing very-low-density lipoprotein and chylomicrons, was removed. The density of infranatant was adjusted to 1.063 g/ml by addition of solid potassium bromide and recentrifuged as before. The low-density lipoprotein (LDL), which floats at a relative density of 1.006–1.063, was collected and exhaustively dialyzed against phosphate-buffered saline (pH 7.4). The remaining infranatant (1.063–1.210), containing high-density lipoprotein, was also exhaustively dialyzed. Hydroperoxide content in the various lipoprotein fractions was determined as a function of protein content determined by the Lowry assay.

Statistical analysis. Statistical analyses (correlations, multivariate analyses) were performed using the Unistat Desktop Statistical Analysis package (Unistat, London, U.K.).

RESULTS

Diurnal variation in plasma content. We were concerned that plasma lipid ROOHs might demonstrate substantial diurnal variation. Possible diurnal variation was thus examined in two individuals (one man, one woman, ages 36 and 30 years, respectively) under fasting and nonfasting conditions (Fig. 1). In both subjects, there was some fluctuation around a mean value but little gross variation associated with diurnal variation or with a fasted versus fed state. In addition, one of the subjects had been tested 4 months previously as part of another study. This previous point lay within 1 SD of the mean value obtained for the individual (subject 1) shown in Fig. 1. It cannot be excluded that dietary input of hydroperoxides contributes to plasma hydroperoxide status, but this contribution appears to be small by comparison with metabolic hydroperoxide production.

Recovery of synthetic hydroperoxides from plasma. The FOX method as adapted for plasma requires measurement of the absorbance differences generated from plasma aliquots incubated with the assay reagent with and without pretreatment with TPP (5). TPP selectively reduces ROOHs and thus

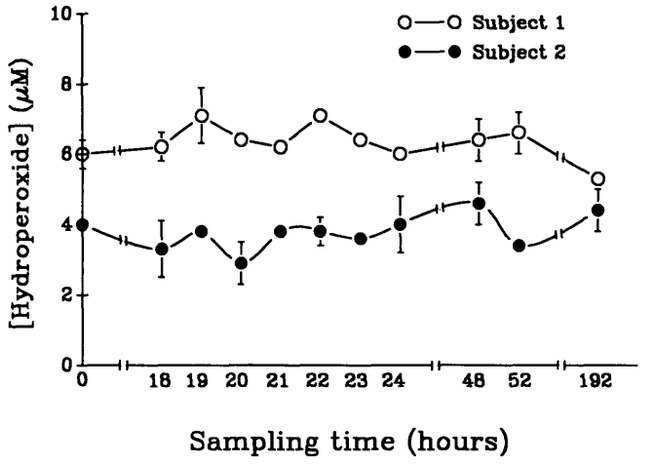

FIG. 1. Plasma hydroperoxide levels do not show substantial diurnal variation. Plasma was analyzed for hydroperoxide in two individuals (subject 1, 30-year-old woman; subject 2, 36-year-old man) as described in METHODS.

eliminates the ROOH-derived absorbance increase in the FOX assay, thereby generating a blank sample (5). This maneuver is necessary because plasma samples can contain variable amounts of ferric ion detectable by xylenol orange, thus contributing to high background absorbances, particularly if there is hemolysis. We have shown previously, however, that the amount of hydroperoxide detected with TPP in whole plasma corresponds very closely with the amounts of hydroperoxide measurable if total lipid in plasma is first separated from water-soluble material, including ferric ion, by extraction of plasma with ethyl acetate (5).

Figure 2 shows typical data for a plasma sample before and after TPP treatment and also after prior introduction to the plasma of defined amounts of synthetic free fatty acid

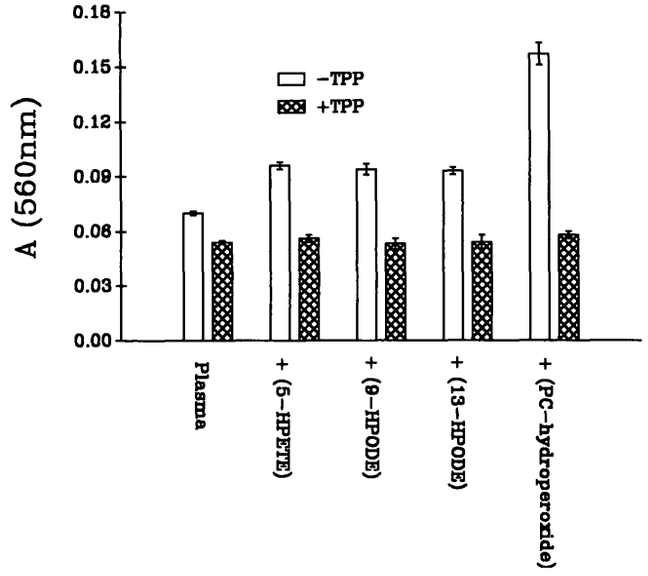

FIG. 2. Authentic synthetic ROOHs can be detected in plasma. Plasma samples (80 μl) were transferred to 1.5-ml microcentrifuge vials and spiked with 10 μl 5-HPETE, 9-HPODE, 13-HPODE, peroxidized phosphatidylcholine (PC-hydroperoxide), or methanol. The samples were then incubated with 10 μl TPP (10 mmol/l in methanol) or methanol alone for 30 min at room temperature. FOX2 reagent (900 μl) was added, and the samples were incubated for 30 min at room temperature. Data represent the mean of quadruplicate analysis ± SD. PC-hydroperoxide, hydroperoxide synthesized by exposure of phosphatidylcholine liposomes to 10 μmol/l copper sulfate for 3 h at 37°C in phosphate-buffered saline, pH 7.4.

TABLE 1
Comparison of the extinction coefficient for synthetic hydroperoxides using the FOX2 assay and the iodometric technique

Compound	Extinction coefficient ($\times 10^4$ $mol^{-1} \cdot l \cdot cm^{-1}$) FOX2 (560 nm)	Iodometric (290 nm)
H_2O_2	4.4	3.9
5 (*S*)-HPETE	4.6	3.8
9 (*S*)-HPODE	4.6	3.8
13 (*S*)-HPODE	4.6	3.7

hydroperoxides and phosphatidylcholine hydroperoxides. The assay generates approximately equivalent signals with equal concentrations of exogenous ROOHs. The signals can be uniformly reduced to a background level by treatment with TPP before addition of the reagent. These data reconfirm that physiological ROOHs of varying structure generate the same signal in the FOX assay. This is reassuring, since it is feasible that different individuals may have different classes of ROOHs present in their plasma. Table 1 compares the extinction coefficients generated for these ROOHs in the FOX assay according to the values obtained using the iodometric assay for ROOH described in METHODS.

Characteristics of individuals with NIDDM and their comparison with normal individuals. Table 2 shows the clinical characteristics for patients and control subjects as well as the differences in levels of plasma linolenic acid, arachidonic acid, vitamin E, TBARM, and ROOHs. The expected differences in fasting glucose, triglyceride, and cholesterol levels agreed with those of other studies. There was a small difference in the proportion of linolenic acid in the patient and control groups and a much greater difference in the level of ROOH, but no difference in the levels of vitamin E or TBARM. We had initially been concerned that the age-matching between patients and control subjects was imprecise. However, we found that there were no strong relationships between age and hydroperoxide levels in either the patient or control group (Fig. 3). In fact, there was a slight negative correlation, with a very shallow slope, between age and hydroperoxide levels in the control group (Fig. 3).

TABLE 2
Clinical characteristics of control and NIDDM subjects

	Control	NIDDM	*P*
Age (years)	43.7 ± 12	57.5 ± 9.3	<0.0005
Sex (M/F)	12, 11	13, 9	—
Fasting glucose (mmol/l)	4.89 ± 0.36	8.68 ± 3.2	<0.0005
HbA_1 (%)	(5–8)	10 ± 1.76	—
Diabetes duration (years)	—	11.2 ± 8.8	—
Triglycerides (mmol/l)	0.92 ± 0.37	2.59 ± 1.45	<0.0005
Cholesterol (mmol/l)	4.97 ± 0.83	6.36 ± 1.52	<0.002
Linoleic acid (%)	28.9 ± 3.2	25.2 ± 4.9	<0.01
Arachidonic acid (%)	8.2 ± 1.9	8.7 ± 2.0	NS
Vitamin E (μg/ml)	10.31 ± 5.02	9.03 ± 3.31	NS
TBARM (μmol/l)	1.21 ± 0.61	1.00 ± 0.70	NS
Hydroperoxides (μmol/l)	3.76 ± 2.48	9.04 ± 4.31	<0.0005

Data for linoleic and arachidonic acids are the percentage contribution to total plasma fatty acids (16:0, 18:0, 18:1, 18:2, 18:3, 20:4, 20:5, and 22:6). Other peroxidizable unsaturated fatty-acid components analyzed accounted for no more than 2% of the total. Numbers in parentheses are normal range.

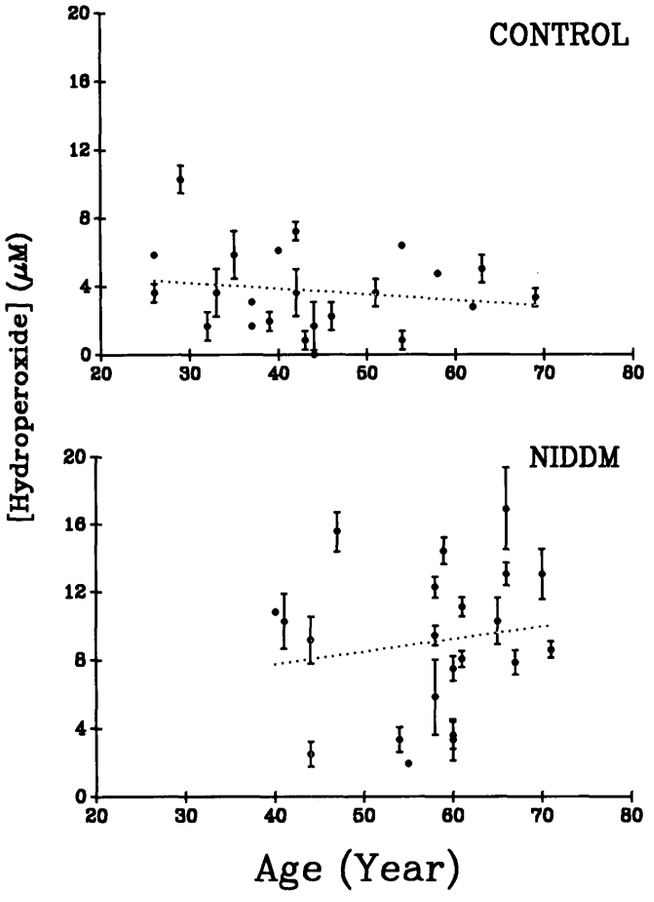

FIG. 3. Lack of a positive association between age and hydroperoxide levels in patients and control subjects. Control group, $r = -0.36$, $P < 0.05$; NIDDM group, $r = 0.17$, NS.

Hydroperoxide, TBARM, and vitamin E. Figure 4 shows the data spread for ROOH, TBARM, and vitamin E levels in the two groups. Hydroperoxide levels varied from 0 to 10.27 μmol/l (3.76 ± 2.48 μmol/l; $n = 23$) in the control group and from 1.94 to 16.94 μmol/l (9.04 ± 4.31 μmol/l; $n = 22$) in the diabetic group. The mean level of plasma lipid hydroperoxide in the control group in this study was not different (separate-variance t test, $P > 0.05$) from the level (3.02 ± 1.85 μmol/l; $n = 23$) determined in our earlier study with a different set of control subjects (5).

The greater level of ROOH in plasma from NIDDM patients did not reflect a greater plasma content of peroxidizable unsaturated fatty acids, since the fatty acid composition of the NIDDM patients and control subjects varied only slightly with respect to linoleic (18:2) and arachidonic (20:4) acids, the major plasma fatty acid fractions associated with peroxidation (Table 2). Furthermore, there was no significant correlation between triglycerides and hydroperoxides in normal (Pearson's $r = -0.09$; $P = 0.35$) or patient groups ($r = 0.29$; $P = 0.11$). Exclusion of individuals from the higher range of triglyceride levels in the patient group and from the lower range in the normal group generated a subgroup ($n = 8$) of patients with a plasma triglyceride level of 1.47 ± 0.19 mmol/l and a subgroup of control subjects ($n = 9$) with a plasma triglyceride level of 1.10 ± 0.47 mmol/l, which was thus crudely matched at the 95% confidence level. The level of hydroperoxide in this edited patient subgroup remained significantly higher than in the edited control subgroup

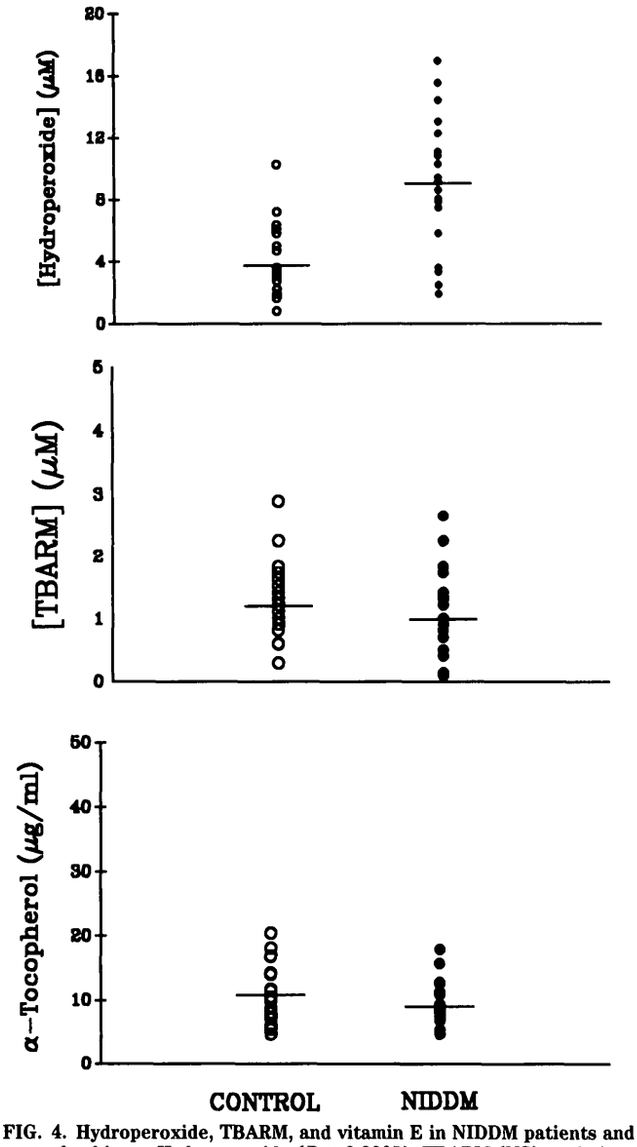

FIG. 4. Hydroperoxide, TBARM, and vitamin E in NIDDM patients and control subjects. Hydroperoxide ($P < 0.0005$), TBARM (NS), and vitamin E (NS) were determined as described in METHODS. Hydroperoxide and TBARM data points represent the means of quadruplicate experiments. The data points given for vitamin E are based on single measurements.

(7.52 ± 3.51 vs. 3.96 ± 2.14 μmol/l; $P = 0.03$) suggesting that elevated ROOHs in NIDDM occur independently of higher triglycerides. This crude analysis cannot, of course, exclude the possibility that a higher mean triglyceride level in patients may contribute to their higher level of ROOHs through a greater availability of peroxidizable substrate. However, it is also reasonable to suggest that the elevated level of hydroperoxides occurs independently of elevated triglyceride status.

Hydroperoxide levels did not correlate with vitamin E levels in patients ($r = -0.08$; $P = 0.37$) or control subjects ($r = -0.18$; $P = 0.24$), suggesting that it might be simplistic to consider that hydroperoxide levels are controlled by vitamin E availability. There was also no correlation between hydroperoxide levels and TBARM in the patient ($r = 0.09$; $P = 0.35$) and control groups ($r = -0.12$; $P = 0.29$), which is in agreement with earlier work (5) and consistent with the conclusion that plasma TBARM is a poor measure of lipid peroxidation in plasma. There was also no association between total cholesterol and hydroperoxide levels in the

patient ($r = 0.07$; $P = 0.39$) and control groups ($r = -0.30$; $P = 0.098$).

These data taken together suggest that hydroperoxide levels are not a simple function of total lipids despite the fact that the majority (>80%) of ROOHs in pooled plasma were found to reside in the LDL fraction, which is elevated in NIDDM (data not shown). There was, however, a significant negative correlation of vitamin E with total cholesterol in both patient ($r = -0.63$; $P < 0.02$) and control groups ($r = -0.44$; $P < 0.05$), which might explain the nonsignificant trend to lower vitamin E levels in the patient group as a result of their higher cholesterol levels (Table 2). There was no significant correlation of fasting glucose and HbA_{1c} with ROOHs ($r = 0.10$; $P = 0.32$), suggesting that hyperglycemia per se is not the cause of the increased hydroperoxide levels reported here. The possibility that hyperglycemia does not contribute to oxidative stress in NIDDM, despite the availability of hypothetical mechanisms, has been raised previously (21,22).

Our patient population was too small in this preliminary study to address the question of any relationship between plasma hydroperoxides and later complications of the disease. However, a crude separation of patients into subgroups with and without macro- or microangiopathy suggested a trend toward higher levels of hydroperoxides in the complications group. Those individuals with macro- or microangiopathy had total plasma lipid hydroperoxide levels of 10.00 ± 4.22 μmol/l ($n = 13$), while those without complications had hydroperoxide levels of 7.65 ± 4.28 μmol/l ($n = 9$). The difference, however, was not significant ($P < 0.25$). Indeed, a crude power calculation suggests that a significant difference between patients with and without complications may be achieved only with a patient base exceeding 100 in this type of cross-sectional survey.

DISCUSSION

A number of studies have compared plasma lipid peroxidation in control subjects and those with NIDDM using the nonspecific TBA assay and have reported higher values of TBARM in NIDDM patients (23–25). In our hands, however, a group of 22 NIDDM patients had levels of TBARM that were not significantly different from TBARM levels in control subjects. We examined the correlation between TBARM and hydroperoxide levels within both groups and found no correlation between these measures of lipid peroxidation. We observed no significant difference between plasma vitamin E levels in NIDDM and control subjects and observed no correlation between hydroperoxide levels and vitamin E levels within the two groups. However, we have observed that NIDDM patients have a higher level of plasma hydroperoxide than do control subjects and that patients with complications may have higher levels of hydroperoxides than those free of secondary disease. The finding of increased levels of plasma ROOH in NIDDM provides direct evidence that NIDDM is associated with oxidative stress. The data also support the view that hydroperoxide measurement is more useful for assessing increased oxidative stress in disease processes where its presence is suspected, such as diabetes, than indirect measures such as antioxidant status or TBARM.

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