Increased methionine sulfoxide content of apoA-I in type 1 diabetes

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Abstract Cardiovascular disease is a major cause of morbidity and premature mortality in diabetes. HDL plays an important role in limiting vascular damage by removing cholesterol and cholesteryl ester hydroperoxides from oxidized low density lipoprotein and foam cells. Methionine (Met) residues in apolipoprotein A-I (apoA-I), the major apolipoprotein of HDL, reduce peroxides in HDL lipids, forming methionine sulfoxide [Met(O)]. We examined the extent and sites of Met(O) formation in apoA-I of HDL isolated from plasma of healthy control and type 1 diabetic subjects to assess apoA-I exposure to lipid peroxides and the status of oxidative stress in the vascular compartment in diabetes. Three tryptic peptides of apoA-I contain Met residues: Q^{84}-M^{86}-K^{88}, W^{108}-M^{112}-R^{116}, and L^{144}-M^{148}-R^{149}. These peptides and their Met(O) analogs were identified and quantified by mass spectrometry. Relative to controls, Met(O) formation was significantly increased at all three locations (Met^{86}, Met^{112}, and Met^{148}) in diabetic patients. The increase in Met(O) in the diabetic group did not correlate with other biomarkers of oxidative stress, such as N^{ϵ}-malondialdehyde-lysine or N^{ϵ}-(carboxymethyl)lysine, in plasma or lipoproteins. The higher Met(O) content in apoA-I from diabetic patients is consistent with increased levels of lipid peroxidation products in plasma in diabetes. Using the methods developed here, future studies can address the relationship between Met(O) in apoA-I and the risk, development, or progression of the vascular complications of diabetes.—Brock, J. W. C., A. J. Jenkins, T. J. Lyons, R. L. Klein, E. Yim, M. Lopes-Virella, R. E. Carter, DCCT/EDIC, S. R. Thorpe, and J. W. Baynes. **Increased methionine sulfoxide content of apoA-I in type 1 diabetes. *J. Lipid Res.* 2008.** 49: **847–855.**

Supplementary key words apolipoprotein A-I • high density lipoprotein • oxidation • oxidative stress

Oxidative stress, induced by hyperglycemia (1, 2), is implicated in the progression of cardiovascular disease (CVD) (3, 4), the leading cause of death in diabetes (5). CVD is particularly accelerated in the presence of diabetic nephropathy (6). Increased oxidation of plasma LDL (7) and accumulation of oxidatively modified LDL in macrophages in the arterial wall (8) are characteristic of the early stage of atherogenesis. HDL has a protective role against atherosclerosis: it removes lipid peroxides (LPOs) and cholesterol from oxidized LDL (9, 10) and from cell membranes through the reverse cholesterol transport pathway (11, 12). Once LPOs are absorbed by HDL, they are either transported to the liver, where they are detoxified and excreted into the bile (13–15), or they are reduced directly by HDL to hydroxylipids (16, 17). At least two HDL-bound proteins are involved with LPO detoxification, paraoxonase (PON-1) (18, 19) and apolipoprotein A-I (apoA-I) (20). PON-1 is a lactonase that protects against LDL and HDL oxidation by hydrolyzing oxidized fatty acids to lactones; plasma or serum PON-1 concentration or activity is inversely correlated with CVD and is commonly decreased in diabetes and in renal disease (21–24). ApoA-I, the major protein of HDL, is involved with the mobilization of cholesterol from oxidized LDL and macrophages of the arterial wall during reverse cholesterol transport (25) and also reduces LPOs, using methionine (Met)

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Abbreviations: ACE, angiotensin-converting enzyme; AER, albumin excretion rate; apoA-I, apolipoprotein A-I; CVD, cardiovascular disease; DCCT/EDIC, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications; HODE, hydroxyoctadecadienoic acid; LPO, lipid peroxide; Met, methionine; Met(O), methionine sulfoxide; PON-1, paraoxonase; Q-TOF, quadrupole time-of-flight; RA, relative area; SLO, soybean lipoxygenase; TIC, total ion chromatogram; T1DM, type 1 diabetes mellitus; XIC, extracted ion chromatogram.

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TABLE 1. Clinical characteristics of control and diabetic subjects

Characteristic	Healthy Control (n = 13)	Type 1 Diabetes (n = 26)	*P*
Demographic profile			
Age (years)	37 ± 8	35 ± 6	0.58
Body mass index (kg/m^2)	25 ± 3	26 ± 3	0.40
Diabetes profile			
Duration (years)	0	21 ± 4	—
Hemoglobin A1c (%)	ND	7.9 ± 1	—
Albumin excretion rate (mg/24 h)	ND	176 ± 433	—
Lipid profile			
Total cholesterol (mg/dl)	176 ± 33	186 ± 25	0.43
Triglycerides (mg/dl)	107 ± 44	88 ± 51	0.16
LDL-cholesterol (mg/dl)	102 ± 28	110 ± 23	0.39
HDL-cholesterol (mg/dl)	52 ± 13	58 ± 13	0.23

Data are means ± SD, based on 10 healthy controls and 24 type 1 diabetes patients because of some unavailable data. *P* values are from the Mann-Whitney *U*-test. ND, not determined.

residues as the reductant and producing methionine sulfoxide [Met(O)] (see below).

Levine and colleagues (26) have proposed that Met residues in protein serve as endogenous antioxidants, protecting functionally important amino acids from oxidation. In apoA-I, Met appears to have the additional function of reducing LPOs. There are three Met residues on apoA-I, two of which are reported to be susceptible to oxidation by LPOs (27). These residues are oxidized at a rate parallel to the rate of reduction of cholesteryl ester hydroperoxides to cholesteryl ester hydroxide (28, 29). In apoA-I, Met^{86} and Met^{112} are thought to be important for cholesterol efflux and Met^{148} is believed to be involved in LCAT activation (30); oxidation of apoA-I Met residues has no effect on the affinity of HDL for cholesterol (31). Although Met(O) reductase activity is found in all cells and reverses the oxidation of Met(O) in intracellular compartments (30), this enzyme is absent from the plasma compartment (32). Thus, we hypothesized that Met(O) formation in apoA-I might serve as a biomarker for exposure of HDL to LPOs in plasma and as an integrator of oxidative stress in the vascular compartment. We describe here the quantification of oxidation of specific Met residues in apoA-I isolated from patients with type 1 diabetes mellitus (T1DM), including patients with and without renal disease, and healthy nondiabetic controls, using liquid chromatography-mass spectrometry analysis of tryptic peptides.

EXPERIMENTAL PROCEDURES

Materials

Unless indicated otherwise, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Organic solvents were of the highest purity available from Acros Chemicals (Atlanta, GA).

Study subjects

Twenty-six T1DM patients, including 13 with and 13 without nephropathy [defined as urinary albumin excretion rate (AER) > 40 mg/24 h], were selected from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications (DCCT/EDIC) cohort (33), and 13 healthy nondiabetic controls were recruited as part of a Program Project Grant, as described previously (34). All hemoglobin A1c, conventional lipid profiles, and renal function measures were performed by the DCCT/EDIC central laboratory, as described previously (33). Urine specimens used to calculate AER were obtained in the EDIC year that preceded the collection of the serum used to measure Met(O) oxidation levels (**Table 1**). Other measures of apolipoproteins, inflammation, oxidative stress, and advanced glycation end products were performed in the authors' laboratories as described previously (34–39). Lipoprotein subclasses were measured by NMR (LipoScience, Raleigh, NC) (33).

HDL isolation

HDL (d = 1.063–1.21 g/ml) was isolated from EDTA-anticoagulated plasma from fasting subjects by sequential ultracentrifugation, after removal of VLDL/intermediate density lipoprotein and LDL, as described previously (40). The HDL supernatant layer was removed, washed and concentrated, dialyzed, sterile-filtered (0.22 μm), and stored under nitrogen at −70°C in a lipoprotein preservative solution containing 2.8 mM EDTA (40). Plasma from both control and diabetic patients was collected and HDL was isolated during 1997–1999, and Met(O) was quantified in 2005. A long-term control pool, prepared by sequential ultracentrifugation in 1998, also had levels of Met(O) similar to those in freshly prepared samples of HDL from a separate group of control subjects: pool Met(O) 86:112:148 (triplicate analyses): 0.104 ± 0.037, 0.038 ± 0.004, 0.023 ± 0.002; freshly prepared controls (n = 3): 0.082 ± 0.009, 0.054 ± 0.032, 0.034 ± 0.016. The low levels of Met(O) in both

TABLE 2. Mass-charge ratios of the charged forms of unmodified and oxidized apolipoprotein A-I tryptic peptides

Peptide	Charged Forms +1	+2	+3
Unmodified peptides			
Q^{84}-M^{86}-K^{88}	622	312	ND
W^{108}-M^{112}-R^{116}	1,284	642	429
L^{144}-M^{148}-R^{149}	1,032	516	ND
Q^{216}-K^{226} (reference peptide)	1,231	616	411
Oxidized peptides			
Q^{84}-Met(O)^{86}-K^{88}	638	320	ND
W^{108}-Met(O)^{112}-R^{116}	1,300	650	434
L^{144}-Met(O)^{148}-R^{149}	1,048	524	ND

Met(O), methionine sulfoxide; ND, not determined.

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controls and pools, compared with fresh samples, support the stability of these samples during storage. HDL from the same group of subjects was also prepared by the (same day) procedures of vertical spin ultracentrifugation and size exclusion chromatography [as described previously (17)], and Met(O) levels did not differ significantly from those in stored frozen samples, supporting a lack of in vitro oxidation during the more prolonged sequential ultracentrifugation procedures.

Oxidation of HDL

Oxidized HDL was prepared using the method of Garner et al. (28). Briefly, HDL prepared from pooled plasma from a separate group of five healthy subjects (2 mg/ml, 0.3 mM Met) was incubated with soybean lipoxygenase (SLO; 4,000 U/ ml) for 12 h at 37°C in a reciprocating water bath at 30 rpm. Aliquots were taken at 0, 1, 2, 6, and 12 h. Butylated hydroxytoluene (1 mM) was added immediately, and the HDL was then delipidated as described below.

Trypsin digestion of HDL

HDL (500 μg of protein, ~2 mg/ml) was delipidated by the addition of 5 volumes of ice-cold methanol-ether (3:1), followed by centrifugation (5,000 *g*, 5 min); the supernatant was discarded and the protein pellet was washed with 500 μl of ice-cold ether, followed by recentrifugation. The supernatant was discarded, and the pellet was dried gently under a stream of N_2, then dissolved in 300 μl of 0.6 M urea/50 mM ammonium bicarbonate, pH 7.2. Trypsin (Sigma Sequencing Grade; T8658), 4.5:100 (w/w), was added followed by incubation overnight at 37°C. Digestion was terminated by freezing at −20°C.

ESI-LC-MS

Experimental conditions for LC-MS analysis of tryptic peptides are described in detail elsewhere (41). Briefly, peptides were analyzed using an Agilent (Palo Alto, CA) Series 1100 liquid chromatograph interfaced to a Micromass (Manchester, UK) triple quadrupole (Quattro) or quadrupole time-of-flight (Q-TOF) mass spectrometer. Peptides were fractionated on an ES Industries (West Berlin, NJ) AquaSep C_{18} column (250 × 2 mm) using a gradient from 0.1% aqueous trifluoroacetic acid to 50% acetonitrile in water for 50 min at a flow rate of 0.2 ml/min. The mass spectrometer was set in full scan mode (200–1,800 *m/z*), and masses of interest were extracted using MassLynx (Micromass) software. All assays were carried out in positive ion mode. The extent of modification of Met-containing peptides, expressed as relative area (RA), was calculated by dividing the sum of the peak areas of the different charged forms of the Met(O) peptide of interest by the sum of the peak areas of the charged forms of an internal reference peptide, Q^{216}-K^{226}, chosen because of its strong signal and resolution from other peptides.

Statistical analyses

Data are summarized throughout as means ± SD and are plotted using SigmaPlot software (Systat Software, Inc., Point

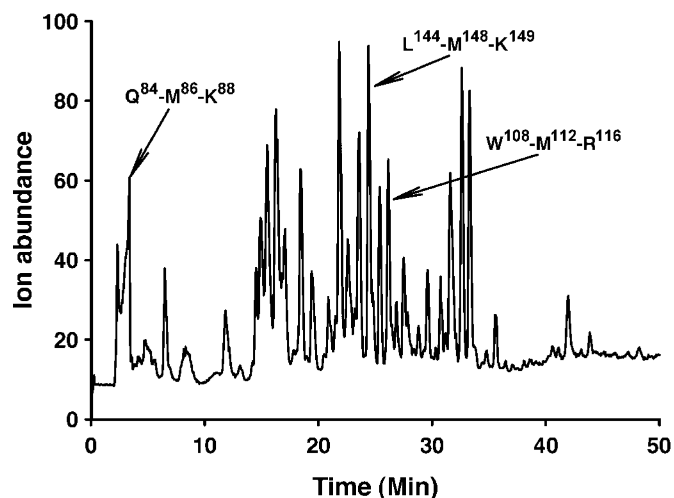

Fig. 1. Typical ESI-LC-MS chromatogram of HDL tryptic peptides. HDL isolated from a type 1 diabetes mellitus (T1DM) subject was delipidated, digested with trypsin, fractionated by reverse-phase LC, and analyzed on a Quattro mass spectrometer set in survey mode (200–1,800 *m/z*). The methionine (Met)-containing peptides Q^{84}-M^{86}-K^{88}, L^{144}-M^{148}-K^{149}, and W^{108}-M^{112}-R^{116} elute at 3.3, 24.4, and 27.5 min, respectively.

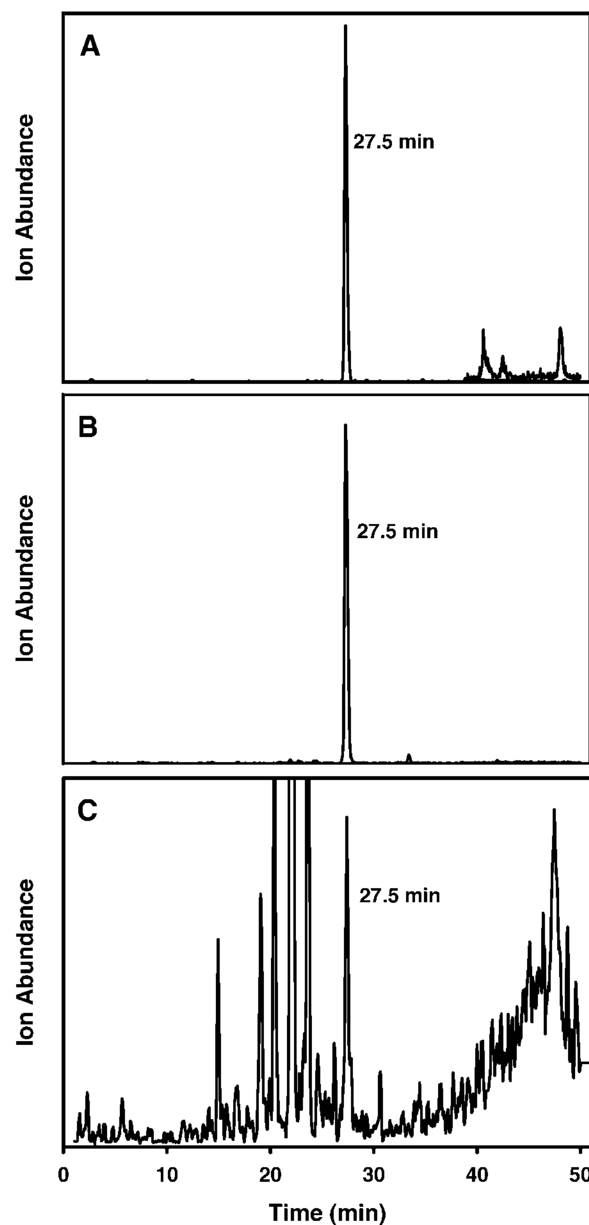

Fig. 2. Typical extracted ion chromatograms (XICs) of peptide W^{108}-M^{112}-R^{116}. XICs are shown for the +1 (1,284 *m/z*) (A), +2 (642 *m/z*) (B), and +3 (429 *m/z*) (C) charge states of peptide W^{108}-M^{112}-R^{116}, for the sample shown in Fig. 1, with an elution time of 27.5 min.

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Richmond, CA). Statistical analyses were performed using the SAS System (Cary, NC). Differences between groups were analyzed using the nonparametric Mann-Whitney *U*-test. Correlations were analyzed by the Spearman nonparametric procedure.

RESULTS

Clinical characteristics

Characteristics of the control and T1DM subjects are shown in Table 1. The healthy controls were comparable to the 26 diabetic patients with respect to age, body mass index, and lipid profile; however, there was significant variation in renal function within the diabetic patients by design. Compared with diabetic patients with normal renal function, the 13 diabetic patients with nephropathy (AER > 40 mg/24 h) exhibited urinary AER (mean ± SD) of 343 ± 576 versus 10 ± 6 mg/day ($P < 0.01$) but similar serum creatinine (1.1 ± 0.5 vs. 0.9 ± 0.1 mg/dl; $P = 0.19$) and glomerular filtration rate (84 ± 24 vs. 99 ± 20 ml/min/1.73 m^2; $P = 0.26$). Of the 13 nephropathic diabetic patients, only 4 were taking angiotensin-converting enzyme (ACE) inhibitors at the time of blood collection. None of the 13 T1DM patients with an AER < 40 mg/24 h were taking ACE inhibitors. Only one diabetic subject was taking lipid-lowering drugs, an HMG-CoA reductase inhibitor. No subjects were taking antioxidant vitamin supplements.

Characterization of Met- and Met(O)-containing peptides of apoA-I

ApoA-I contains three Met residues, Met^{86}, Met^{112}, and Met^{148}, which are located on three different tryptic peptides (**Table 2**). To determine the site specificity of Met oxidation, apoA-I isolated from a healthy subject was digested with trypsin and the peptides were analyzed by reverse-phase HPLC in-line with an ESI tandem mass spectrometer (Quattro or Q-TOF). **Figure 1** shows a typical total ion chromatogram

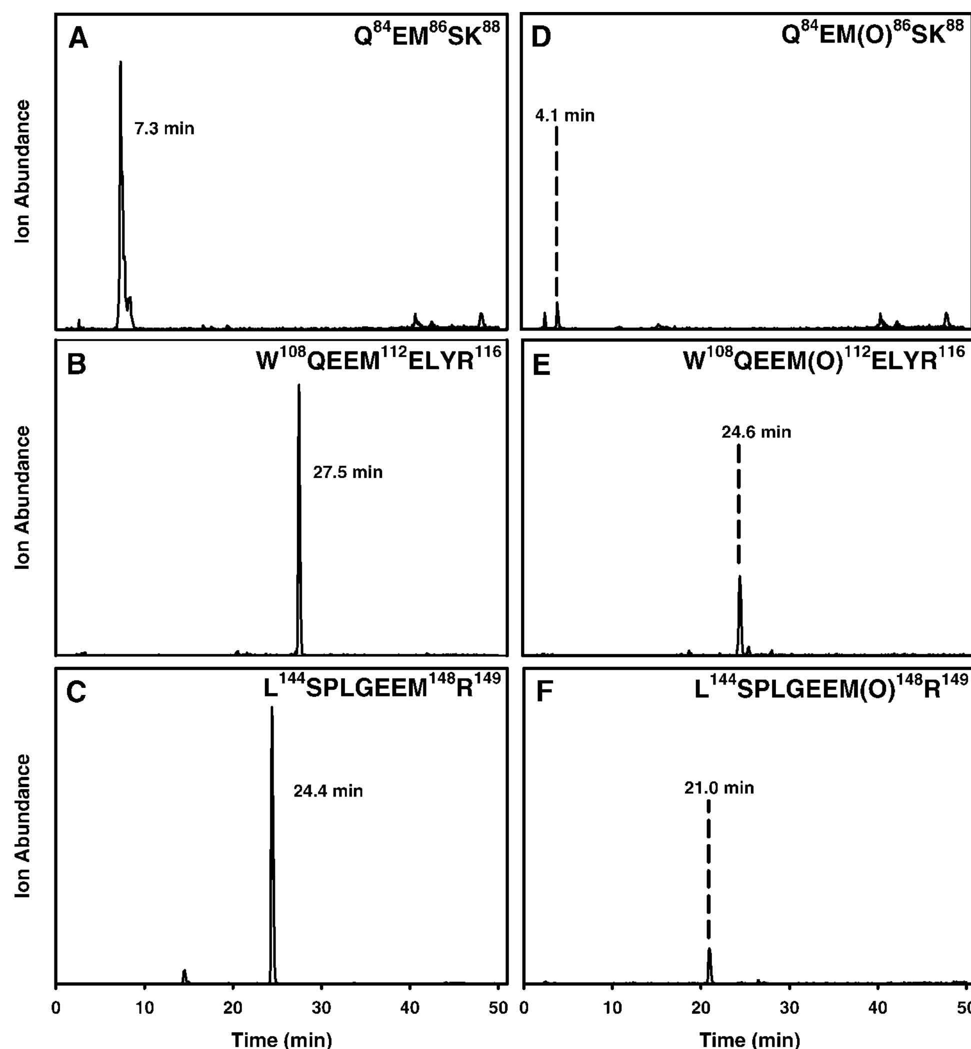

Fig. 3. Typical XICs of the +2 charge state of the nonoxidized and oxidized peptides Q^{84}-M^{86}-K^{88}, W^{108}-M^{112}-R^{116}, and L^{144}-M^{148}-R^{149}. XICs are shown for the +2 charge state of the peptides Q^{84}-M^{86}-K^{88}, W^{108}-M^{112}-R^{116}, and L^{144}-M^{148}-R^{149} (A–C) and the +2 charge state of the peptides Q^{84}-Met(O)^{86}-K^{88}, W^{108}-Met(O)^{112}-R^{116}, and L^{144}-Met(O)^{148}-R^{149} (D–F) from the sample shown in Fig. 1. The methionine sulfoxide [Met(O)] peptides eluted on average 3 min earlier than the nonoxidized (Met) peptides.

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(TIC) of peptides from apoA-I, on the Quattro, using a full-scan analysis between 200 and 1,800 Da.

ESI-LC-MS produces multiply charged species (i.e., +1, +2, and +3), with each species contributing to the TIC. To quantify the peptides of interest, each multiply charged species was extracted from the TIC using MassLynx software, giving an extracted ion chromatogram (XIC). **Figure 2** shows a typical XIC for the three charged species of the nonoxidized peptide W^{108}-M^{112}-R^{116}. All peptides of interest had multiple charged forms that were extracted. **Figure 3** shows a series of typical XICs for the +2 charged species of the nonoxidized and oxidized peptides Q^{84}-M^{86}-K^{88}, L^{144}-M^{148}-K^{149}, and W^{108}-M^{112}-R^{116}. The Met(O) peptides eluted ~3 min earlier than the nonoxidized (native) form, which is attributable to increased hydrophilicity of Met(O), compared with Met. All of the Met(O) peptides were detectable in this and other samples of HDL from control and diabetic subjects.

To confirm the assignment of the charged species, the Met and Met(O) peptides were sequenced using the Q-TOF mass spectrometer. **Figure 4** shows representative sequencing spectra of the nonoxidized and oxidized W^{108}-M^{112}-R^{116} peptide. The insets represent sequencing bars showing the masses for each y and b ion. A complete series of y ions and immonium ions was obtained for both peptides. There was a neutral loss of 64 amu for all y ions containing Met(O), indicative of a loss of methyl sulfoxide from Met(O) (Fig. 4B).

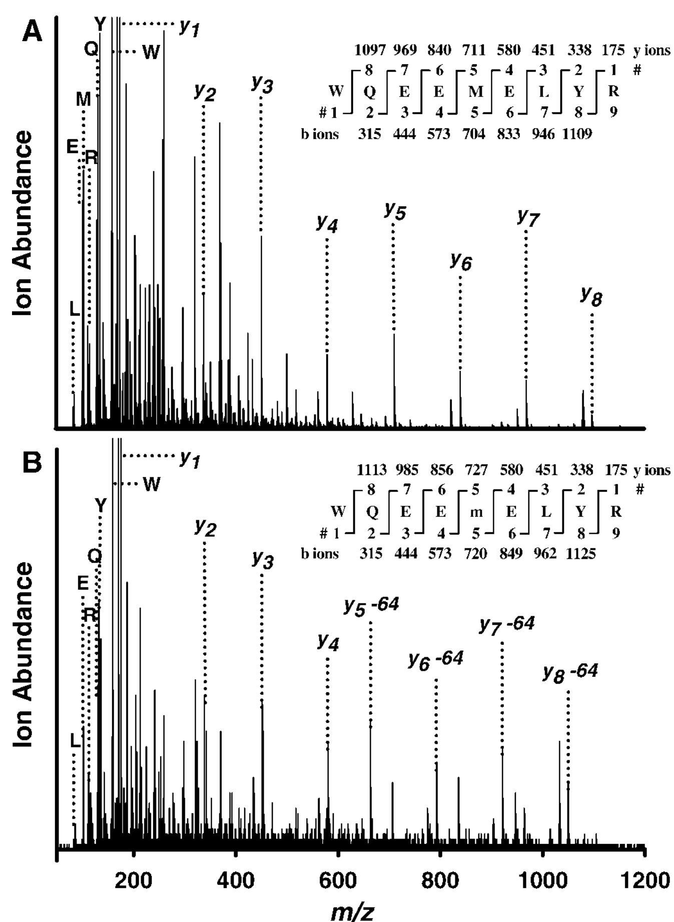

Fig. 4. Sequencing spectra of the nonoxidized and oxidized peptide W^{108}-M^{112}-R^{116}. Sequencing spectra of the +2 ion of peptides W^{108}-M^{112}-R^{116} (A) and W^{108}-Met(O)^{112}-R^{116} (B). All y ions and immonium ions are represented. All y ions containing Met(O) (y5–y8) have a neutral loss of methyl sulfoxide (−64 *m/z*), indicative of Met(O). Insets show amino acid sequences of peptides W^{108}-M^{112}-R^{116} and W^{108}-Met(O)^{112}-R^{116}, showing the theoretical masses of the b and y ions.

Site specificity of Met oxidation on apoA-I during in vitro oxidation of HDL

To determine the site specificity of Met(O) formation on apoA-I, we treated isolated HDL with SLO to generate LPOs with concomitant oxidation of Met, followed by analysis of tryptic peptides on the Quattro mass spectrometer. As indicated by the increase in RA values, all three of the Met residues on apoA-I were susceptible to oxidation by LPOs (**Fig. 5**), with the order of increase in RA for $Met^{112} > Met^{148} > Met^{86}$. There was a slight lag phase in Met oxidation, most apparent for the oxidation of Met^{112} and Met^{148}, probably because HDL contains α-tocopherol, which would temporarily protect against Met oxidation.

Extent of Met(O) formation on apoA-I in HDL isolated from control and diabetic subjects

HDL isolated from T1DM patients and nondiabetic controls (Table 1) was analyzed to determine whether Met(O) was increased in T1DM versus control subjects. Met(O) was detectable in apoA-I from healthy nondiabetic individuals, with the RA for W^{108}-Met(O)^{112}-R^{116} ≈ L^{144}-Met(O)^{148}-K^{149} >> Q^{84}-Met(O)^{86}-K^{88} (**Fig. 6**), in reasonable agreement with the in vitro data for the zero-time sample (Fig. 5). The RAs for all three of the Met(O) peptides were increased significantly in diabetic subjects compared with nondiabetic controls (Fig. 6); there was also a strong correlation between the RA values for

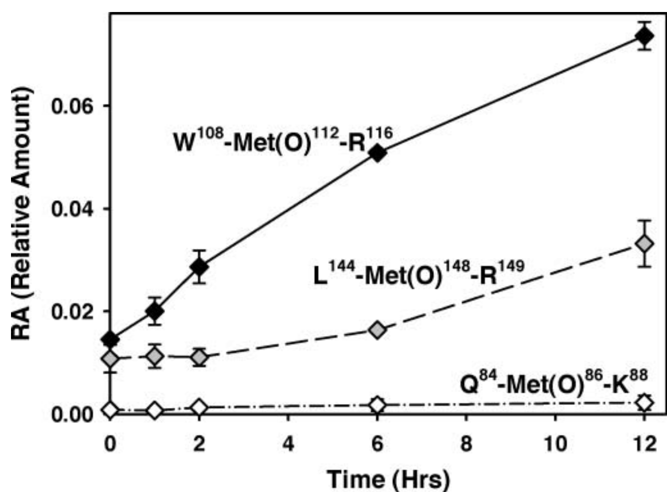

Fig. 5. Analysis of the site specificity of Met oxidation on apolipoprotein A-I (apoA-I) in lipoxygenase-treated HDL. Met(O)^{112} is the predominant site of oxidation during lipoxygenase treatment of HDL, followed by Met^{148} and Met^{86}. Relative area (RA) values (means ± SD; n = 3) are calculated relative to the reference peptide Q^{216}-K^{226}, as described in Experimental Procedures.

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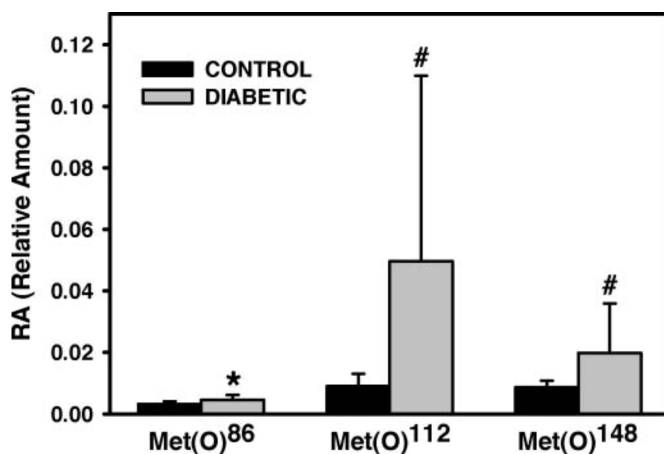

Fig. 6. Met(O) concentration is significantly increased in apoA-I peptides from T1DM patients. HDL isolated from healthy subjects (n = 13) and T1DM subjects (n = 26) was delipidated and digested with trypsin for ESI-LC-MS analysis. Data are expressed relative to the peptide Q^{216}-K^{226} (see Experimental Procedures). Data are means ± SD; statistical analysis was performed by Dunn's (nonparametric) test (* $P = 0.01$ vs. healthy control; $^{\#}$ $P < 0.01$ vs. healthy control).

$Met(O)^{112}$ and $Met(O)^{148}$ (**Fig. 7**), comparable to that observed in LPO-treated HDL (Fig. 5; $r^2 = 0.84$).

Correlation between Met(O) on apoA-I and risk markers for CVD

There were no statistically significant correlations between the RAs for Met(O) peptides and urinary AER, serum creatinine, or glomerular filtration rate. Met(O) levels also did not differ significantly between diabetic subjects with or without nephropathy or between subjects taking or not taking ACE inhibitors or HMG-CoA reductase inhibitors (data not shown). This does not exclude Met(O) as an independent risk factor for CVD, so a cross-sectional analysis was also performed between Met(O) peptide RAs and potential mediators of CVD, including other markers of oxidative stress and inflammation [C-reactive protein, soluble vascular cell adhesion molecule-1, soluble intracellular adhesion molecule, soluble E-selectin, smoking, LDL susceptibility to in vitro oxidation (oxidizibility), and serum PON-1 activity]. No statistically significant correlations were found between these measures and RAs for Met(O) peptides (data not shown). As there are several size-based HDL subclasses, the relationship between Met(O) RAs and NMR-determined HDL profile (including HDL particle number, mean HDL size, concentration of five HDL subclasses, and ratio of large to small HDL) was also assessed. Again, there was no correlation found between Met(O) formation on apoA-I and these measurements. Nor was there any correlation with measures of plasma antioxidant status (albumin, bilirubin, urate, PON activity, or iron concentration) or levels of advanced glycation and lipoxidation end products [N^{ϵ}-(carboxymethyl)lysine, N^{ϵ}-(carboxyethyl)lysine, hydroxynonenal-lysine, malondialdehyde-lysine, or pentosidine] in plasma, LDL, or HDL. The clinical CVD status of the patients is not yet available, so the predictive value of Met(O) in apoA-I or of other biomarkers for CVD cannot be assessed at this time.

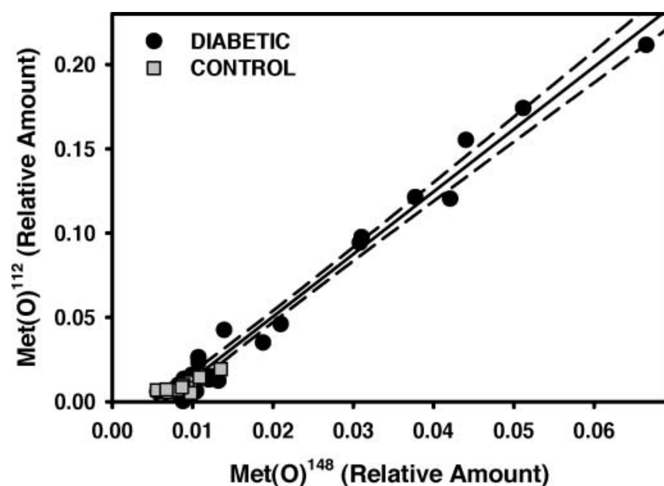

Fig. 7. Correlation between $Met(O)^{112}$ and $Met(O)^{148}$ formation on apoA-I. $Met(O)^{112}$ and $Met(O)^{148}$ oxidation are strongly correlated in HDL from T1DM patients, indicating an overall increase in oxidative damage to the HDL particle. $Met(O)^{112}$ is the predominant site of oxidation in T1DM. Two-tailed $P < 0.0001$ with a 99% confidence interval is shown (n = 39).

DISCUSSION

In this study, we used LC-MS/MS to measure the oxidation of specific Met residues in apoA-I. This protein has three Met residues, Met^{86}, Met^{112}, and Met^{148}. The relative rates of oxidation of the three peptides containing these Met residues are in agreement with earlier work based on chromatographic separation of intact apoA-I molecules containing $Met(O)^{112}$ or $Met(O)^{148}$ (42), which also identified Met^{112} as the primary site of oxidation of HDL that had been chemically oxidized with chloramine-T. In other studies, Met^{86} was identified as the major site of oxidation of apoA-I initiated by the aqueous peroxyl radical generator 2,2′-azo-bis-(2-amidinopropane)dihydrochloride. Thus, although the differences in relative rates of oxidation of these Met residues are attributable to differences in oxidant and methodology, in the present study the oxidation of Met residues in apoA-I by SLO-generated LPOs is consistent with the relative extents of oxidation of Met residues in apoA-I isolated from human plasma (Fig. 5 vs. Fig. 6).

In in vitro studies with SLO-generated LPOs (Fig. 5) and among the diabetic subjects (Fig. 7), there was a strong correlation between the extent of oxidation of Met^{112} and Met^{148}, suggesting oxidation by LPOs, both in vitro and in vivo. The greater oxidation of Met^{112} compared with Met^{148} may be attributed to structural features of the protein, such as exposure of the Met residues to bound lipids or sites of docking with cellular or protein donors of LPOs. Recent studies by Shao et al. (43) indicate that Met^{112} oxidation in HDL may also be enhanced by the MxxY motif in the protein. In this case, Met may protect tyrosine from chlorination by serving as a sacrificial anti-

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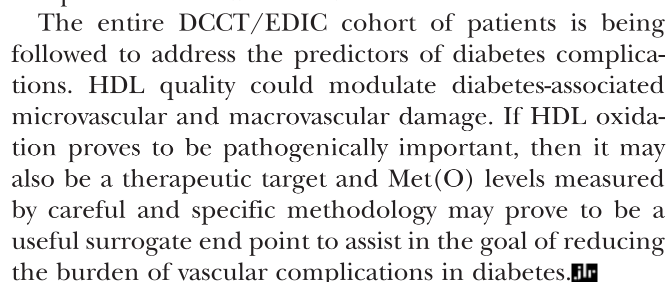
oxidant, reducing HOCl, a product of myeloperoxidase in macrophages. During reverse cholesterol transport, HDL interacts with foam cells, which are laden with oxidized LDL, and this interaction could also expose apoA-I to HOCl as well as to LPOs, both of which may drive the oxidation of Met^{112}.

If LPOs were the primary source of apoA-I oxidation, hydroxylipids, such as 9- or 13-hydroxyoctadecadienoic acids (HODEs) or cholesterol oxides, might increase in HDL in diabetes. Jira et al. (44, 45), in fact, have reported increases in HODEs in LDL in diseases associated with increased oxidative stress, such as rheumatoid arthritis (44) and atherosclerosis (45). In obese type 2 diabetic patients, plasma 9- and 13-HODE levels are decreased by troglitazone therapy (46), but no information is available regarding plasma or lipoprotein-related HODE concentrations in T1DM. However, Ferderbar et al. (47) recently reported significant increases in total cholesterol oxides and 7-hydroxycholesterol in plasma of children and young adults with T1DM, even in the absence of hypercholesterolemia. Martin-Gallan et al. (48) have also reported increased levels of LPOs and the lipid peroxidation product, malondialdehyde, in plasma of T1DM patients, independent of complication status. Thus, although little information is available regarding levels of HODEs in patients with diabetes, there is some evidence for increased cholesterol oxidation and oxidative stress in T1DM, even in the absence of dyslipidemia or complications. These results are consistent with increased peroxidation of lipids in diabetes and suggest that measurement of Met(O) in apoA-I in HDL, which has an average half-life of ~5 days in plasma (49), may provide a useful intermediate-term index of exposure to oxidative stress in diabetes.

Despite the evidence for increased oxidative stress in diabetes (2–4, 50), in the present study diabetic patients with nephropathy, which is thought to increase oxidative stress and CVD risk (50–52), did not show a statistically significant increase in Met oxidation. Relative to control subjects, Met(O) was increased in the HDL of all diabetic subjects (irrespective of nephropathy status). In contrast, in a recent study of skin collagen, Met(O) levels were increased in diabetic patients with complications but did not differ significantly between complication-free diabetic patients and controls (39). The studies of Met(O) in apoA-I and collagen are not readily compared because of differences in patient populations and the complications evaluated: there were fewer patients with renal complications and more with retinal complications in the collagen study. Nonetheless, both of these studies indicate that extracellular proteins are exposed to increased oxidative stress in T1DM.

Differences in the oxidation of Met in apoA-I and collagen may result from differences in location (plasma vs. extracellular matrix), the different half-lives of the lipoproteins and collagen (days for apoA-I, years for collagen), or the nature of the oxidant (LPOs in apoA-I vs. water-soluble or cell-derived oxidants in the extracellular matrix). It is also possible that alterations in lipoprotein metabolism in diabetes may obscure differences in oxidative stress in diabetic patients with or without complications. In the present, small cross-sectional study, we found no evidence of differences in lipemia, apoprotein composition, or lipoprotein size distribution between the nephropathic and nonnephropathic groups of patients, nor any statistically significant correlations with Met(O), but in a larger cross-sectional study of DCCT/EDIC subjects, in which Met(O) was not quantified, we found multiple differences in lipoproteins between such groups (34). Coronary artery disease is the major cause of morbidity and mortality in T1DM patients, and HDL oxidation, as indicated by the increased Met (O) in diabetic compared with healthy subjects, may contribute to accelerated atherosclerosis. Even young people with T1DM without clinically evident complications have increased carotid intima medial thickness and coronary artery atheroma, as detected by intravascular ultrasound (53–55). Atherosclerosis may also contribute to the increased levels of circulating HDL oxidation products by providing a source of pro-oxidants; thus, future research will examine the association of Met(O) in apoA-I and measures of CVD. Potential modulators that might also be evaluated in future clinical studies, in addition to antioxidants, include ACE inhibitors, angiotensin receptor blockers (56–58), and HMG-CoA reductase inhibitors (59), which are more likely to be used by patients with complications and which have antioxidant effects.

The entire DCCT/EDIC cohort of patients is being followed to address the predictors of diabetes complications. HDL quality could modulate diabetes-associated microvascular and macrovascular damage. If HDL oxidation proves to be pathogenically important, then it may also be a therapeutic target and Met(O) levels measured by careful and specific methodology may prove to be a useful surrogate end point to assist in the goal of reducing the burden of vascular complications in diabetes.



APPENDIX

Participants of the DCCT/EDIC Research Group

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REFERENCES

1. Giardino, I., D. Edelstein, and M. Brownlee. 1996. BCL-2 expression or antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation endproducts in bovine endothelial cells. *J. Clin. Invest.* **97:** 1422–1428.
2. Nishikawa, T., D. Edelstein, X. L. Du, S. Yamagishi, T. Matsumura, Y. Kaneda, M. A. Yorek, D. Beebe, P. J. Oates, H. Hammes, et al. 2000. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature.* **404:** 787–790.
3. Jay, D., H. Hitomi, and K. K. Griendling. 2006. Oxidative stress and diabetic cardiovascular disease. *Free Radic. Biol. Med.* **40:** 183–192.
4. Yorek, M. 2003. The role of oxidative stress in diabetic vascular and neural disease. *Free Radic. Res.* **37:** 471–480.
5. Toto, R. D. 2005. Heart disease in diabetic patients. *Semin. Nephrol.* **25:** 372–378.
6. McCullough, P. J., G. L. Bakris, W. F. Owen, Jr., P. S. Klassen, and R. M. Califf. 2004. Slowing the progression of diabetic nephropathy and its cardiovascular consequences. *Am. Heart J.* **148:** 243–251.
7. Holvoet, P. 2004. Oxidized LDL and coronary heart disease. *Acta Cardiol.* **59:** 479–484.
8. Witztum, J. L., and D. Steinberg. 2001. The oxidative modification hypothesis of atherosclerosis: does it hold for humans? *Trends Cardiovasc. Med.* **11:** 93–102.
9. Sangvanich, P., B. Mackness, S. J. Gaskell, P. Durrington, and M. Mackness. 2003. The effect of high-density lipoproteins on the formation of lipid/protein conjugates during in vitro oxidation of low-density lipoprotein. *Biochem. Biophys. Res. Commun.* **300:** 501–506.
10. Rousselot, D. B., P. Therond, J. L. Beaudeux, J. Peynet, A. LeGrand, and J. Delattre. 1999. High density lipoproteins (HDL) and the oxidative hypothesis of atherosclerosis. *Clin. Chem. Lab. Med.* **37:** 939–948.
11. Christison, J. K., K. Rye, and R. Stocker. 1995. Exchange of oxidized cholesteryl linoleate between LDL and HDL mediated by cholesteryl ester transfer protein. *J. Lipid Res.* **36:** 2017–2026.
12. De Vries, R., M. N. Kerstens, W. J. Sluiter, A. K. Groen, A. van Tol, and R. P. F. Dullaart. 2005. Cellular cholesterol efflux to plasma from moderately hypercholesterolaemic type 1 diabetic patients is enhanced, and is unaffected by simvastatin treatment. *Diabetologia.* **48:** 1105–1113.
13. Bowry, V. W., K. K. Stanley, and R. Stocker. 1992. High density lipoprotein is the major carrier of lipid hydroperoxides in human blood plasma from fasting donors. *Proc. Natl. Acad. Sci. USA.* **89:** 10316–10320.
14. Christison, J., A. Karjalainen, J. Brauman, F. Bygrave, and R. Stocker. 1996. Rapid reduction and removal of HDL- but not LDL-associated cholesteryl ester hydroperoxides by rat liver perfused in situ. *Biochem. J.* **314:** 739–742.
15. Flutier, K., H. Vietsch, E. A. L. Biessen, G. M. Kostner, T. J. C. van Berkel, and W. Sattler. 1996. Increased selective uptake in vivo and in vitro of oxidized cholesteryl esters from high-density lipoprotein by rat liver parenchymal cells. *Biochem. J.* **319:** 471–476.
16. Sattler, W., J. Christison, and R. Stocker. 1995. Cholesterylester hydroperoxide reducing activity associated with isolated high- and low-density lipoproteins. *Free Radic. Biol. Med.* **18:** 421–429.
17. Kalogerakis, G., A. M. Baker, S. Christov, K. G. Rowley, K. Dwyer, C. Winterbourn, J. D. Best, and A. J. Jenkins. 2005. Oxidative stress and high-density lipoprotein function in type 1 diabetes and end stage renal disease. *Clin. Sci.* **108:** 497–506.
18. Li, L. H., D. P. Liu, and C. C. Liang. 2003. Paraoxonase gene polymorphism, oxidative stress and disease. *J. Mol. Med.* **81:** 766–779.
19. Aviram, M., E. Hardak, J. Vaya, S. Mahmood, S. Milo, A. Hoffman, S. Billicke, D. Draganov, and M. Rosenblat. 2000. Human serum paraoxonase (PON1) Q and R selectivity decrease lipid peroxides in human coronary and carotid atherosclerotic lesions. *Circulation.* **101:** 2510–2517.
20. Mashima, R., Y. Yamamoto, and S. Yoshimura. 1998. Reduction of phosphatidylcholine hydroperoxide by apolipoprotein A-I: purification of the hydroperoxide-reducing proteins from human blood plasma. *J. Lipid Res.* **39:** 1133–1140.
21. Watson, A. D., J. A. Berliner, S. Y. Hama, B. N. La Du, K. F. Faull, A. M. Fogelman, and M. Navab. 1996. Protective effect of high density lipoprotein associated paraoxonase: inhibition of the biological activity of minimally oxidized low density lipoprotein. *J. Clin. Invest.* **96:** 2882–2891.
22. Ayub, A., M. I. Mackness, S. Arrol, B. Mackness, J. Patel, and P. N. Durrington. 1999. Serum paraoxonase after myocardial infarction. *Arterioscler. Thromb. Vasc. Biol.* **19:** 330–335.
23. Mackness, M. I., D. Harty, D. Bhatnagar, P. H. Winocour, S. Arrol, M. Ishola, and P. N. Durrington. 1991. Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. *Atherosclerosis.* **86:** 193–199.
24. Rosenblat, M., L. Gaidukov, O. Khersonsky, J. Vaya, R. Oren, D. S. Tawfik, and M. Aviram. 2006. The catalytic histidine dyad of HDL-associated serum paraoxonase 1 (PON1) is essential for PON1-mediated inhibition of LDL oxidation and stimulation of macrophage cholesterol efflux. *J. Biol. Chem.* **281:** 7657–7665.
25. Oram, J. F. 2003. HDL apolipoproteins and ABCA1: partners in the removal of excess cellular cholesterol. *Arterioscler. Thromb. Vasc. Biol.* **23:** 720–727.
26. Levine, R. L., L. Mosoni, B. S. Berlett, and E. R. Stadtman. 1996. Methionine residues as endogenous antioxidants in proteins. *Proc. Natl. Acad. Sci. USA.* **93:** 15036–15040.
27. Pankhurst, G., X. L. Wang, D. E. Wilcken, G. Baernthaler, U. Panzenbock, M. Raferty, and R. Stocker. 2003. Characterization of specifically oxidized apolipoproteins in mildly oxidized high density lipoprotein. *J. Lipid Res.* **44:** 349–355.
28. Garner, B., P. K. Witting, A. R. Waldeck, J. K. Christison, M. Raferty, and R. Stocker. 1998. Oxidation of high density lipoproteins: formation of methionine sulfoxide in apolipoproteins AI and AII

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is an early event that accompanies lipid peroxidation and can be enhanced by α-tocopherol. *J. Biol. Chem.* **273:** 6080–6087.
29. Garner, B., A. R. Waldeck, P. K. Witting, K. Rye, and R. Stocker. 1998. Oxidation of high density lipoproteins: evidence for direct reduction of lipid hydroperoxides by methionine residues of apolipoproteins AI and AII. *J. Biol. Chem.* **273:** 6088–6095.
30. Panzenbock, U., and R. Stocker. 2005. Formation of methionine sulfoxide-containing specific forms of oxidized high-density lipoproteins. *Biochim. Biophys. Acta.* **1703:** 171–181.
31. Panzenbock, U., L. Kritharides, M. Raftery, K. Rye, and R. Stocker. 2000. Oxidation of methionine residues to methionine sulfoxide does not decrease potential antiatherogenic properties of apolipoprotein A-I. *J. Biol. Chem.* **275:** 19536–19544.
32. Glaser, C. B., L. Karic, S. Pamelee, B. R. Premachandra, D. Hinkston, and W. R. Abrams. 1987. Studies on the turnover of methionine oxidized alpha-1-protease inhibitor in rats. *Am. Rev. Respir. Dis.* **136:** 857–861.
33. The DCCT Research Group. 1987. Feasibility of centralized measurements of glycated hemoglobin in the Diabetes Control and Complications Trial: a multicenter study. *Clin. Chem.* **33:** 2267–2271.
34. Jenkins, A. J., T. J. Lyons, D. Zheng, J. D. Otvos, D. T. Lackland, D. McGee, W. T. Garvey, and R. L. Klein, for the DCCT/EDIC Research Group. 2003. Lipoproteins in the DCCT/EDIC cohort: associations with diabetic nephropathy. *Kidney Int.* **64:** 817–828.
35. Jenkins, A. J., T. J. Lyons, D. Zheng, J. D. Otvos, D. T. Lackland, D. McGee, W. T. Garvey, and R. L. Klein, for the DCCT/EDIC Research Group. 2003. Serum lipoproteins in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications cohort: associations with gender and glycemia. *Diabetes Care.* **26:** 810–818.
36. Lyons, T. J., A. J. Jenkins, D. Zheng, R. L. Klein, J. D. Otvos, Y. Yu, D. T. Lackland, D. McGee, M. B. McHenry, M. Lopes-Virella, et al., for the DCCT/EDIC Research Group. 2006. Nuclear magnetic resonance-determined lipoprotein subclass profile in the DCCT/EDIC cohort: associations with carotid intima-media thickness. *Diabet. Med.* **23:** 955–966.
37. Jenkins, A. J., M. Rothen, R. L. Klein, K. Moller, L. Eldridge, D. Zheng, R. Durazo-Arvizu, D. McGee, D. Lackland, S. R. Thorpe, et al., for the DCCT/EDIC Research Group. 2007. Cross-sectional associations of C-reactive protein with vascular risk factors and vascular complications in the DCCT/EDIC cohort. *J. Diabetes Complications*, In press.
38. Cohen, J., A. J. Jenkins, C. Karschimkus, S. Qing, C. T. Lee, K. O'Dea, J. D. Best, and K. G. Rowley. 2002. Paraoxonase and other coronary risk factors in a community-based cohort. *Redox Rep.* **7:** 304–307.
39. Yu, Y., S. R. Thorpe, A. J. Jenkins, J. N. Shaw, M. A. Sochaski, D. McGee, C. E. Aston, T. J. Orchard, N. Silvers, Y. G. Peng, et al., for the DCCT/EDIC Research Group. 2006. Advanced glycation end-products and methionine sulphoxide in skin collagen of patients with type 1 diabetes. *Diabetologia.* **49:** 2488–2498.
40. Klein, R. L., M. B. McHenry, K. H. Lok, S. J. Hunter, N. A. Le, A. J. Jenkins, D. Zheng, A. J. Semler, W. V. Brown, T. J. Lyons, et al., for the DCCT/EDIC Research Group. 2004. Apolipoprotein C-III protein concentrations and gene polymorphisms in type 1 diabetes: associations with lipoprotein subclasses. *Metabolism.* **53:** 1296–1304.
41. Brock, J. W. C., D. J. S. Hinton, W. E. Cotham, T. O. Metz, S. R. Thorpe, J. W. Baynes, and J. M. Ames. 2003. Proteomic analysis of the site specificity of glycation and carboxymethylation of ribonuclease. *J. Proteome Res.* **2:** 506–513.
42. Von Eckardstein, A., M. Walter, H. Holz, A. Benninghoven, and G. Assmann. 1991. Site-specific methionine sulfoxide formation is the structural basis of chromatographic heterogeneity of apolipoproteins A-I, C-II, and C-III. *J. Lipid Res.* **32:** 1465–1476.
43. Shao, B., M. N. Oda, C. Bergt, X. Fu, P. S. Green, N. Brot, J. F. Oram, and J. W. Heinecke. 2006. Myeloperoxidase impairs ABCA1-dependent cholesterol efflux through methionine oxidation and site-specific tyrosine chlorination of apolipoprotein A-I. *J. Biol. Chem.* **281:** 9001–9004.
44. Jira, W., G. Spiteller, and A. Richter. 1997. Increased levels of lipid oxidation products in low density lipoproteins of patients suffering from rheumatoid arthritis. *Chem. Phys. Lipids.* **87:** 81–89.
45. Jira, W., G. Spiteller, W. Carson, and A. Schramm. 1998. Strong increase in hydroxyl fatty acids derived from linoleic acid in human low density lipoproteins of atherosclerotic patients. *Chem. Phys. Lipids.* **91:** 1–11.
46. Aljada, A., R. Garg, H. Ghanim, P. Mohanty, W. Hamouda, E. Assian, and P. Dandona. 2001. Nuclear factor-kappaB suppressive and inhibitor-kappaB stimulatory effects of troglitazone in obese patients with type 2 diabetes: evidence of an antiinflammatory action? *J. Clin. Endocrinol. Metab.* **86:** 3250–3256.
47. Ferderbar, S., E. C. Pereira, E. Apolinario, M. C. Bertolami, A. Faludi, O. Monte, L. E. Calliari, J. E. Sales, A. R. Gagliardi, H. T. Xavier, et al. 2007. Cholesterol oxides as biomarkers of oxidative stress in type 1 and type 2 diabetes mellitus. *Diabetes Metab. Res. Rev.* **23:** 35–42.
48. Martin-Gallan, P., A. Carrascosa, M. Gussinye, and C. Dominquez. 2003. Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. *Free Radic. Biol. Med.* **34:** 1563–1574.
49. Herbert, P. N., D. N. Bernier, E. M. Cullinane, L. Edelstein, M. A. Kantor, and P. D. Thompson. 1984. High-density lipoprotein metabolism in runners and sedentary men. *J. Am. Med. Assoc.* **252:** 1034–1037.
50. Baynes, J. W., and S. R. Thorpe. 1999. The role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes.* **48:** 1–9.
51. Fortuno, A., O. Beloqui, G. San Jose, M. U. Moreno, G. Zolba, and J. Diez. 2005. Increased phagocytic nicotinamide adenine dinucleotide phosphate oxidase-dependent superoxide production in patients with early chronic kidney disease. *Kidney Int. Suppl.* **99:** 71–75.
52. Fort, J. 2005. Chronic renal failure: a cardiovascular risk factor. *Kidney Int. Suppl.* **99:** 25–29.
53. Larsen, J., M. Brekke, L. Sandvik, H. Arnesen, K. F. Hanssen, and K. Dahl-Jorgensen. 2002. Silent coronary atheromatosis in type 1 diabetic patients and its relation to long-term glycemic control. *Diabetes.* **51:** 2637–2641.
54. Distiller, L. A., B. I. Joffe, V. Melville, T. Welman, and G. B. Distiller. 2006. Carotid artery intima-media complex thickening in patients with relatively long-surviving type 1 diabetes mellitus. *J. Diabetes Complications.* **20:** 280–284.
55. Atabek, M. E., O. Pirgon, S. Kurtoglu, and H. Imamoglu. 2006. Evidence for an association between childhood type 1 diabetes and premature carotid atherosclerosis in childhood. *Pediatr. Cardiol.* **27:** 428–433.
56. Bhan, B. V., S. Sola, W. B. Lauten, R. Natarajan, W. C. Hooper, R. G. Menon, S. Lerakis, and T. Helmy. 2004. Quinapril, an ACE inhibitor, reduces markers of oxidative stress in the metabolic syndrome. *Diabetes Care.* **27:** 1712–1715.
57. Izuhara, Y., M. Nangaku, R. Inagi, N. Tominaga, T. Aizawa, K. Kurokawa, C van Ypersele de Strihou, and T. Miyata. 2005. Renoprotective properties of angiotensin receptor blockers beyond blood pressure lowering. *J. Am. Soc. Nephrol.* **16:** 3631–3641.
58. Ogawa, S., T. Mori, K. Nako, T. Kato, K. Takeuchi, and S. Ito. 2006. Angiotensin II type 1 receptor blockers reduce urinary oxidative stress markers in hypertensive diabetic nephropathy. *Hypertension.* **47:** 699–705.
59. Rosenson, R. S. 2004. Statins in atherosclerosis: lipid-lowering agents with antioxidant capabilities. *Atherosclerosis.* **173:** 1–12.

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