## Lipid advanced glycosylation: Pathway for lipid oxidation in vivo

(low density lipoprotein/atherosclerosis/aminoguanidine/lipofuscin)

Richard Bucala<sup>\*†</sup>, Zenji Makita<sup>\*</sup>, Theodor Koschinsky<sup>‡</sup>, Anthony Cerami<sup>\*</sup>, and Helen Vlassara<sup>\*</sup>

\*The Picower Institute for Medical Research, Manhasset, NY 11030; and ‡Diabetes Research Institute, Dusseldorf, Germany

Contributed by Anthony Cerami, March 18, 1993

To address potential mechanisms for oxida-ABSTRACT tive modification of lipids in vivo, we investigated the possibility that phospholipids react directly with glucose to form advanced glycosylation end products (AGEs) that then initiate lipid oxidation. Phospholipid-linked AGEs formed readily in vitro, mimicking the absorbance, fluorescence, and immunochemical properties of AGEs that result from advanced glycosylation of proteins. Oxidation of unsaturated fatty acid residues, as assessed by reactive aldehyde formation, occurred at a rate that paralleled the rate of lipid advanced glycosylation. Aminoguanidine, an agent that prevents protein advanced glycosylation, inhibited both lipid advanced glycosylation and oxidative modification. Incubation of low density lipoprotein (LDL) with glucose produced AGE moleties that were attached to both the lipid and the apoprotein components. Oxidized LDL formed concomitantly with AGE-modified LDL. Of significance, AGE ELISA analysis of LDL specimens isolated from diabetic individuals revealed increased levels of both apoprotein- and lipid-linked AGEs when compared to specimens obtained from normal, nondiabetic controls. Circulating levels of oxidized LDL were elevated in diabetic patients and correlated significantly with lipid AGE levels. These data support the concept that AGE oxidation plays an important and perhaps primary role in initiating lipid oxidation in vivo.

The oxidative modification of lipids *in vivo* has been proposed to play a central role in atherogenesis and to contribute to the diverse vascular sequelae of diabetes and aging (1). Oxidation of the lipid component of low density lipoprotein (LDL), for example, leads to the loss of LDL recognition by cellular LDL receptors and in the preferential uptake of oxidized LDL by macrophage scavenger receptors (2-4). The enhanced endocytosis of oxidized LDL by vascular wall macrophages transforms them into the lipid-laden foam cells that characterize early atherosclerotic lesions. This is followed progressively by the development of fatty streaks and the complex, proliferative lesions that ultimately cause arterial insufficiency and occlusion (1-5).

Despite increased investigation into the biological role of lipid oxidation, there has been little insight into the biochemical processes that initiate lipid oxidation *in vivo*. *In vitro* studies have demonstrated that metal-catalyzed peroxidation reactions occur readily at the unsaturated bonds within fatty acid residues. Polyunsaturated fatty acids are particularly sensitive to peroxidation because bisallylic hydrogens are more easily abstracted by free radical processes. Diene conjugation then occurs and hydroperoxides form. Once lipid oxidation is initiated, fatty acids decompose readily to a variety of reactive aldehydes that rapidly propagate oxidative reactions (6, 7).

It is important to note that in the absence of transition metals or free radicals, oxygen itself is a poor oxidant. The electronic structure of triplet (ground state) oxygen has two

unpaired electrons at the  $\pi$  antibonding level ( ${}^{3}\Sigma$ g). Thus, the reaction of oxygen with molecules of singlet multiplicity such as unsaturated fatty acids is spin forbidden. Singlet oxygen  $(^{1}\Delta g \text{ and } ^{1}\Sigma g)$  may add directly to unsaturated bonds to produce allylic hydroperoxides; however, this reaction does not initiate the abstraction of allylic hydrogens (6, 7). Furthermore, there is insufficient singlet oxygen available under normal, physiological conditions to initiate lipid peroxidation. Significant amounts of the superoxide anion  $(O_2^-)$  do form in vivo (0.1–1.0  $\mu$ M), but superoxide also is incapable of abstracting bisallylic hydrogens from unsaturated bonds. Under acidic conditions, superoxide may initiate oxidative modification by forming perhydroxyl radicals (HOO') or by reacting with transition metals to form reactive hydroxyl radicals (HO<sup>•</sup>) (8, 9). Nevertheless, low trace metal concentrations, the high availability of ligands that form tight coordination complexes with metals, and the abundant antioxidant capacity of plasma suggest that metal-catalyzed autoxidation and reactive oxygen species play little, if any, role in mediating lipid oxidation in vivo (10-12).

In the present study, we investigated the possibility that glucose-mediated advanced glycosylation reactions initiate oxidative modification in vivo. Advanced glycosylation is a major pathway for the posttranslational modification of tissue proteins and begins with the nonenzymatic addition of sugars such as glucose to the primary amino groups of proteins (13, 14). These early glucose-derived Schiff base and Amadori products then undergo a series of inter- and intramolecular rearrangement, dehydration, and oxidation-reduction reactions to produce the "late" products termed advanced glycosylation end products (AGEs). Excessive accumulation of AGEs on tissue proteins has been implicated in the pathogenesis of many of the sequelae of diabetes and normal aging (13-15). Protein-linked AGEs act to crosslink connective tissue collagen (16) and to chemically inactivate nitric oxide activity (17); they also act as recognition signals for AGE receptor systems that are present on diverse cell types (18-20).

The presence of reactive, primary amino groups on phospholipids such as phosphatidylethanolamine or phosphatidylserine led us to consider the hypothesis that glucose also reacts with lipids to initiate advanced glycosylation. Intermolecular oxidation-reduction reactions might then occur to oxidize fatty acid residues—in the absence of exogenous, free radical-generating systems.

## **MATERIALS AND METHODS**

**Reagents.** Glucose, EDTA, butylated hydroxytoluene (2,6di-*t*-butyl-*p*-cresol) (BHT),  $L-\alpha$ -phosphatidylethanolamine,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AGE, advanced glycosylation end product; LDL, low density lipoprotein; MDA, malonaldehyde bis(diethyl acetal); PC, phosphatidylcholine; PE, phosphatidylethanolamine; TBA, thiobarbituric acid; BHT, butylated hydroxytoluene.

<sup>&</sup>lt;sup>†</sup>To whom reprint requests should be addressed at: The Picower Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030.

dioleoyl {1,2-di[(cis)-9-octadecenoyl]-sn-glycero-3-phosphoethanolamine} (PE), and L- $\alpha$ -phosphatidylcholine, dioleoyl {1,2-di[(cis)-9-octadecenoyl]-sn-glycero-3-phosphocholine} (PC) were obtained from Sigma. Malonaldehyde bis(diethyl acetal) (MDA) was purchased from Aldrich and aminoguanidine-HCl was from Alteon (Northvale, NJ).

Glucose-Lipid Incubations. PE or PC dissolved in chloroform/methanol (1:1) was aliquoted into sterile scintillation vials and the solvent was evaporated under nitrogen. One milliliter of deaerated buffer containing 0.1 M sodium phosphate (pH 7.4), 1 mM EDTA, and various concentrations of glucose and test reagents then was added under nitrogen. The vials were sonicated in an ice water bath for 30 min to produce lipid suspensions and these were incubated in the dark at 37°C (21). At different time intervals (0-50 days), 1 ml of chloroform/methanol (2:1) was added to extract the lipid-soluble products from unreacted glucose. The vials were gently rocked for 10 min, the interface was cleared by centrifugation, and the extraction was then repeated two additional times. In various experiments, the content of lipid-soluble material was quantitated by weighing 1-ml aliquots that were evaporated in preweighed microcentrifuge tubes, by reaction with 1.6-diphenyl-1,3,5-hexatriene (21), and by visualization with  $I_2$  after thin-layer chromatography on silica gel GH (22).

Human LDL was purified as described below. Samples (5 mg) were incubated under nitrogen in 2 ml of sterile, deaerated buffer containing 0.2 M sodium phosphate (pH 7.4) and 1 mM EDTA together with glucose (100-200 mM), aminoguanidine (300 mM), or BHT (0.02-0.2 mM) as indicated. Incubations were terminated by dialysis at 4°C against phosphate-buffered saline containing 1 mM EDTA. To analyze the content of lipid AGEs and apoprotein AGEs, lipid-soluble material first was extracted with chloroform/methanol (2:1). Denatured apoproteins were pelleted by microcentrifugation, washed three times with ddH<sub>2</sub>O, and digested with proteinase K prior to analysis by a competitive AGE ELISA. For proteolysis, 1 mg of proteinase K (Boehringer Mannheim) was added to 2-4 mg of apoprotein and incubated for 18 hr at 37°C. Aliquots then were subjected to AGE-specific ELISA (23).

Analyses. Lipid samples were diluted in methanol (125-0.5 mg/ml) and absorbance and fluorescence spectra were recorded as described (23).

Lipid oxidation was assessed by formation of thiobarbituric acid (TBA)-reactive substances (24). After reaction with TBA, samples (1 ml) were extracted into 1 ml of 1-butanol prior to fluorescence measurement (emission at 553 nm upon excitation at 515 nm) (25). TBA-reactive substances were quantitated by comparison of duplicate samples to a MDA standard curve (expressed as pmol of MDA equivalents per  $\mu g$  of lipid).

Protein-linked AGEs were measured by competitive ELISA (23). One unit of AGE activity was defined as the amount of antibody-reactive material equivalent to 1.0  $\mu$ g of an AGE bovine serum albumin (BSA) standard (23). Lipid AGEs were measured in a direct, noncompetitive ELISA. Triplicate 100-µl aliquots of lipid-soluble material (dissolved in methanol) were added to round-bottomed, 96-well plates and the solvent was evaporated. The wells then were washed three times with phosphate-buffered saline (PBS)/0.05% Tween 20. Antiserum (final dilution, 1:1000) was added, the plates were incubated for 1 hr at room temperature, and the wells were washed and developed in the same manner as the competitive ELISA (23). Control samples were developed with preimmune serum in place of anti-AGE antiserum. Results were quantitated with reference to a standard curve that was obtained by assaying dilutions of an AGE BSA standard that were absorbed to wells in a concentration range from 0.3 ng/ml to 3  $\mu$ g/ml.

**Patient Analyses.** Plasma LDL (d = 1.025-1.063 g/ml) was isolated from healthy, nonhyperglycemic individuals and patients with diabetes mellitus by sequential ultracentrifugation (26), using 2.7 mM EDTA. The isolated and recentrifuged LDL was dialyzed extensively against PBS containing 2.7 mM EDTA and 0.2 mM BHT. The nondiabetic patient group (n = 8) had a mean age of 34.6 ± 9.6 years. The diabetic group (n = 16) consisted of 5 patients with type I diabetes and



FIG. 1. (A) Ultraviolet and visible absorbance spectra of lipid-soluble reaction products formed by incubation of 14 mM PE with 500 mM glucose for increasing time intervals (0-50 days as shown). Incubations performed with PE alone, PC with glucose (500 mM), and PC alone showed absorbance changes at 50 days that were equivalent to the day 0 spectrum shown. (B) Fluorescence excitation and emission spectra for lipid-soluble reaction products formed by reaction of PE with glucose. No significant fluorescence changes were observed in control incubations that contained PE alone, PC and glucose, or PC alone.

11 patients with type II diabetes. The mean age was  $55.5 \pm 16.3$  years and the mean duration of diabetes was  $11.9 \pm 5.6$  years. The mean hemoglobin A<sub>1c</sub> level was  $10.0\% \pm 1.7\%$ . P values were calculated by the unpaired Student's t test statistic for comparison between groups.

## RESULTS

Two model phospholipids were incubated with glucose at physiological pH and temperature for increasing time intervals. PE contains a free amino group that can react with glucose-derived carbonyls to form Schiff base and Amadori products. PC was used as a control. In contrast to PE, PC contains a blocked, tertiary amine that cannot react with glucose to form the initial Schiff base. Anaerobic, buffered suspensions of PE or PC (14 mM) were prepared and incubated at 37°C with glucose (500 mM) and EDTA (1 mM). PE but not PC was observed to react with glucose to form products with the absorbance and the fluorescence properties of AGEs (Figs. 1 and 2). The lipid-derived fluorophores showed an excitation maximum of 360 nm and an emission maximum of 440 nm. Progress curves for the development of lipid-derived AGEs are shown in Fig. 2. The time-dependent formation of lipid-derived AGEs was confirmed by immunoassay with a specific anti-AGE antibody (Fig. 2C) (23). Immunoreactive AGEs formed in incubation mixtures that contained PE and glucose, but not PE alone, PC and glucose, or PC alone.

To assess the contribution of advanced glycosylation reactions to lipid oxidation, the PE and PC used in these experiments contained esterified oleic acid, a monounsaturated fatty acid. Monounsaturated residues are 10- to 30-fold less susceptible to oxidation than polyunsaturated fatty acids (7) but, once oxidized, can undergo  $\beta$ -scission to form reactive aldehydes that are representative of initiation reactions. Polyunsaturated fatty acids, although more susceptible to oxidation, readily undergo complex rearrangement and free radical propagation reactions (6, 27–29). Diene conjugation is a frequently measured early parameter of polyunsaturated fatty acid oxidation (6, 7). However, this analysis cannot be performed in glucose-containing incubations because of ultraviolet absorbance changes that result from advanced glycosylation.

Buffered phospholipid incubation mixtures were extracted with chloroform/methanol to remove glucose and the lipidsoluble material was then assayed for the presence of reactive, fatty acid-derived aldehydes by reaction with TBA. Under defined experimental conditions, TBA condenses with a variety of reactive aldehydes to form addition products that can be quantitated by reference to a standard solution of MDA (24, 25). As shown in Fig. 2D, lipid oxidation products formed at a rate that was slightly delayed but parallel to the rate of AGE formation. Control incubation mixtures that contained either PE alone, PC and glucose, or PC alone did not produce significant lipid oxidation. Of note, the inclusion of lysine (50 mM) in aqueous suspensions of PC and glucose did not lead to the formation of lipid oxidation products, pointing to the necessity for proximity of AGEs to unsaturated residues (data not shown).

Aminoguanidine interferes with the formation of proteinbound AGEs and was found to also inhibit the formation of AGEs that result from the reaction of glucose with phospholipid. Aminoguanidine appeared to inhibit only partially the formation of AGE-associated chromophores (Fig. 2A). This may be explained by the formation of aminoguanidine AGE addition products, which themselves absorb in the near ultraviolet (30). Inhibition by aminoguanidine of lipid advanced glycosylation also resulted in inhibition of lipid oxidation. Aminoguanidine inhibited lipid oxidation in a fashion analogous to the lipophilic antioxidant, BHT. BHT did not



FIG. 2. Time course for production of lipid-bound chromophores (A), fluorophores (B), AGE immunoreactive products (units of AGE per  $\mu g$  of lipid) (C), and lipid oxidation products (pmol of MDA equivalents per  $\mu g$  of lipid) (D). •, PE (14 mM) incubated with glucose (500 mM); O. PE (14 mM) incubated alone (same as PC incubated alone); ▲, PE (14 mM) incubated with glucose (500 mM) and aminoguanidine (100 mM); ▼, PE (14 mM) incubated with glucose (500 mM) and BHT (0.2 mM); D, PC (14 mM) incubated with glucose (500 mM). Values shown are representative of two or three independently performed experiments.

prevent formation of AGEs, however, indicating that it prevents oxidative modification of lipid independent of any effect on advanced glycosylation.

AGE formation generally follows complex, higher-order reaction kinetics that reflect in part the generation of reactive intermediates, which produce AGEs at a faster rate than the starting reactant, glucose (13, 14). Curvilinear, time- and concentration-dependent increases in advanced glycosylation occur when glucose reacts with protein or amino acids. Similar curvilinear increases in the formation of AGEs were observed by incubating glucose with PE (Fig. 2). Logarithmic transformation of measurements that were obtained with PE and increasing glucose concentrations demonstrated that four measured reaction products: chromophores, fluoro-



FIG. 3. Concentration-dependent reaction between glucose (5-500 mM) and PE (14 mM). AGE is expressed as unit per  $\mu$ g of lipid; MDA is expressed as pmol of MDA equivalents per  $\mu$ g of lipid.



FIG. 4. Time-dependent reaction of human LDL (2.5 mg/ml) with glucose (200 mM). Samples were dialyzed and portions separated into apoprotein (unit of AGE per  $\mu$ g of apoprotein) (A) and lipid (unit of AGE per  $\mu$ g of lipid) (B) components for AGE determination or assayed for the presence of oxidized LDL (pmol of MDA equivalents per  $\mu$ g of LDL) (C).  $\Delta$ , LDL incubated with 200 mM glucose;  $\blacktriangle$ , LDL incubated with 200 mM glucose;  $\bigcirc$ , LDL incubated alone;  $\bigcirc$ , LDL incubated with aminoguanidine. Values shown are means of duplicate determinations.

phores, AGE epitopes, and oxidized lipids appeared to follow similar, nonlinear reaction kinetics (Fig. 3).

Aminoguanidine prevents AGE formation by reacting with glucose-derived products that form at intermediate stages of the advanced glycosylation pathway. Early Amadori products, for example, rearrange to dideoxyosones that react with aminoguanidine to form triazine or dihydrazone products (30). Thus, aminoguanidine also might react directly with the aldehydes that form during fatty acid oxidation. In agreement with recent reports (31, 32), the addition of aminoguanidine to MDA inhibited the formation of TBA-reactive substances in a concentration-dependent manner (data not shown). These data suggest that aminoguanidine inhibits AGEmediated fatty acid oxidation by two mechanisms: inhibition of lipid advanced glycosylation and by direct reaction with reactive aldehydes that form during fatty acid oxidation.

The contribution of advanced glycosylation to the oxidative modification of LDL first was assessed by incubating LDL with glucose *in vitro* in the presence of EDTA. LDL was removed at intervals and analyzed for advanced glycosylation and oxidative modification. Portions of LDL were fractionated into the lipid and apoprotein (ApoB) components, and the AGEs were measured by an AGE-specific ELISA. Incubation of LDL with 200 mM glucose for 3 days resulted in the formation of readily measurable levels of AGEs on both lipid and apoprotein (Fig. 4). These measurements indicate that lipid-linked AGEs are present at a specific activity that is 100-fold greater than apoprotein-linked AGEs. Because of possible differences in the immunoreactivity of anti-AGE antibody with lipid AGEs versus apoprotein AGEs, it may not be possible to compare quantitatively AGE modification on lipid versus protein. Nevertheless, it can be concluded that in vitro there is a markedly higher rate of formation of AGEs on lipid than on apoprotein. Measurements of oxidative modification revealed that LDL was oxidized during the formation of AGEs. Lipid oxidation products formed at a more rapid rate than was observed during the incubation of PE and glucose (Fig. 2D) and this most likely is due to the presence in LDL of polyunsaturated fatty acids that rapidly propagate oxidative reactions. Aminoguanidine inhibited significantly both the advanced glycosylation and the oxidative modification of LDL.

To begin to define the relationship between advanced glycosylation and LDL oxidation in vivo, LDL was isolated from both nondiabetic and diabetic individuals and analyzed for the presence of lipid AGEs, apoprotein AGEs, and oxidative modification (Fig. 5). In agreement with prior studies (33), LDL from diabetic individuals showed significantly greater oxidative modification than the LDL from nondiabetic individuals [nondiabetics (n = 8),  $3.7 \pm 1.25$ pmol of MDA equivalents per  $\mu g$  of LDL; diabetics (n = 16),  $6.8 \pm 1.2$  pmol of MDA equivalents per  $\mu$ g of LDL (mean  $\pm$ SD); P < 0.0001]. Both the lipid- and the apoprotein-linked AGEs in the diabetic LDL specimens were found to be markedly elevated when compared to LDL obtained from nondiabetic individuals. Lipid AGE levels were elevated almost 4-fold in diabetic patients [nondiabetics (n = 8), 0.11  $\pm$  0.03 unit of AGE per  $\mu$ g of lipid; diabetics (n = 16), 0.41  $\pm$  0.25 unit of AGE per  $\mu$ g of lipid; P < 0.005)]. Apoprotein AGE levels were increased >2-fold in the diabetic samples [nondiabetics (n = 8), 0.0028 ± 0.0006 unit of AGE per  $\mu$ g of apoprotein; diabetics (n = 16), 0.0068 ± 0.004 unit of AGE per  $\mu g$  of apoprotein; P < 0.0001)]. These measurements revealed a similar quantitative ratio between LDL oxidation and the level of lipid AGEs and apoprotein AGEs that was observed during LDL advanced glycosylation in vitro (Fig. 4). There also appeared to be a marked increase in the level of lipid AGEs relative to the level of apoprotein-associated AGEs. Linear regression analysis of these data revealed a significant correlation between the level of AGE modification and LDL oxidation. For the measurement of AGE apoprotein versus LDL oxidation, this analysis showed a correlation coefficient of r = 0.52 and P < 0.01. For AGE lipid versus LDL oxidation, the corresponding values were r = 0.63 and P < 0.005.



FIG. 5. Measurement of AGE and oxidative modification of LDL from normoglycemic, nondiabetic individuals (NL) and patients with diabetes mellitus (DM). (A) AGE modification of LDL apoprotein (unit of AGE per  $\mu g$  of apoprotein). (B) AGE modification of LDL lipid (unit of AGE per  $\mu g$  of lipid). (C) Oxidative modification of LDL (pmol of MDA equivalents per  $\mu g$  of LDL).

## DISCUSSION

Lipid AGEs form in a time- and concentration-dependent manner and display the absorbance, fluorescence, and immunochemical properties of protein-linked AGEs. Lipid advanced glycosylation is accompanied by the progressive oxidative modification of unsaturated fatty acid residues. Incubation of purified LDL with glucose produces AGE moieties that are attached to both the apoprotein and the lipid components. Under these model in vitro conditions, lipid AGEs form at a faster rate than apoprotein AGEs. LDL that is incubated with glucose also undergoes progressive, oxidative modification concomitantly with advanced glycosylation. Aminoguanidine, an inhibitor of advanced glycosylation, was observed to inhibit both processes: apoprotein and lipid advanced glycosylation and oxidative modification.

Oxidation of the lipid component of LDL has been proposed to play a central role in the pathogenesis of atherosclerosis. Nevertheless, the biochemical processes that initiate lipid oxidation in vivo remain poorly defined. The advanced glycosylation reaction between phospholipid and glucose described in this study provides a facile mechanism to explain the progressive, oxidative modification of lipids in vivo. Evidence in support of this pathway includes the direct relationship between advanced glycosylation and oxidative modification in vitro and the analysis of human LDL specimens, which shows a close association between the levels of lipid AGEs and lipid oxidation products in vivo. There are data to suggest that glucose or Amadori products themselves may enhance free radical formation (34, 35); however, the time course for formation of lipid oxidation products indicates that oxidative modification occurs only after more advanced glycosylation reactions have taken place. The precise interaction between AGEs and unsaturated fatty acid residues remains to be elucidated but presumably involves inter- and intramolecular oxidations-reductions and transient free radical formation that occur during advanced glycosylation (13, 14, 36, 37).

Lipid AGEs were found to be elevated almost 4-fold in diabetic patients, in contrast to apoprotein AGEs, which were elevated 2-fold when diabetic individuals were compared to normal controls. Measurement of lipid-linked AGEs may serve as a convenient, surrogate marker for lipid oxidation and prove useful in assessing the contribution of advanced glycosylation and lipid oxidation to the vascular sequelae of diabetes, chronic renal failure, and normal aging. It is noteworthy that there is close similarity between the spectral properties of lipid AGEs and the age-related pigment lipofuscin (38). The accumulation of lipofuscin and related lipophilic products within neurons, cardiac myocytes, and other long-lived cells may be due in part to the advanced glycosylation reactions between glucose and phospholipids.

In conclusion, amine-containing phospholipids react with glucose to initiate advanced glycosylation reactions, forming lipid-linked AGEs and promoting fatty acid oxidation. This process, termed AGE oxidation, appears to result from the inter- and intramolecular oxidation-reduction reactions that are an inherent feature of advanced glycosylation chemistry. Lipid-advanced glycosylation and oxidation increase during the chronic hyperglycemia of diabetes and support the concept that AGE oxidation plays an important and perhaps primary role in initiating lipid oxidation in vivo.

- 1. Witztum, J. L. & Steinberg, D. (1991) J. Clin. Invest. 88, 1785-1792.
- Goldstein, J. L., Ho, Y. K., Basu, S. K. & Brown, M. S. (1979) Proc. Natl. Acad. Sci. USA 76, 333-337. 2.
- Fogelman, A. M., Schecter, J. S., Hokom, M., Child, J. S. & 3. Edwards, P. A. (1980) Proc. Natl. Acad. Sci. USA 77, 2214-2218.
- 4. Sparrow, C. P., Parthasarathy, S. & Steinberg, D. (1989) J. Biol. Chem. 264, 2599-2604.
- 5. Ross, R. (1986) N. Engl. J. Med. 314, 488-500.
- Dix, T. A. & Aikens, J. (1993) Chem. Res. Toxicol. 6, 2-18. 6.
- 7. Kanner, J., German, J. B. & Kinsella, J. E. (1987) Crit. Rev. Food Sci. Nutr. 25, 317–364.
- Bielski, B. H. J., Cabelli, D. E., Ravindra, L. A. & Ross, 8. A. B. (1985) J. Phys. Chem. Ref. Data 14, 1041–1091. Bielski, B. H. J. & Cabelli, D. E. (1991) Int. J. Radiat. Biol. 59,
- 9. 291-319.
- 10. Klaassen, C. D. (1985) in Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds. Gilman, A. G., Good-man, L. S., Rall, T. W. & Murad, F. (Macmillan, New York), pp. 1605–1627. Frei, B., Yamamoto, Y., Niclas, D. & Ames, B. N. (1988)
- 11. Anal. Biochem. 175, 120-130.
- Frei, B., Stocker, R. & Ames, B. N. (1988) Proc. Natl. Acad. 12. Sci. USA 85, 9748-9752.
- Bucala, R. & Cerami, A. (1992) Adv. Pharmacol. 23, 1-34. 13.
- Njoroge, F. G. & Monnier, V. M. (1989) Prog. Clin. Biol. Res. 14. 304, 85-107.
- 15. Brownlee, M., Cerami, A. & Vlassara, H. (1988) N. Engl. J. Med. 318, 1315-1321.
- Monnier, V. M., Kohn, R. R. & Cerami, A. (1984) Proc. Natl. 16. Acad. Sci. USA 81, 583-587.
- 17. Bucala, R., Tracey, K. J. & Cerami, A. (1991) J. Clin. Invest. 87, 432-438.
- 18. Vlassara, H., Brownlee, M. & Cerami, A. (1985) Proc. Natl. Acad. Sci. USA 82, 5588-5592.
- 19. Esposito, C., Gerlach, H., Brett, J., Stern, D. & Vlassara, H. (1989) J. Exp. Med. 170, 1387–1407. Skolnik, E. Y., Yang, Z., Makita, Z., Radoff, S., Kirstein, M.
- 20. & Vlassara, H. (1991) J. Exp. Med. 174, 931-938.
- 21. London, E. & Feigenson, G. W. (1978) Anal. Biochem. 88, 203-211.
- 22. Jain, S. K. & Subrahmanyan, D. (1978) Ital. J. Biochem. 27, 11-18.
- 23. Makita, Z., Vlassara, H., Cerami, A. & Bucala, R. (1992) J. Biol. Chem. 267, 5133-5138.
- Kikugawa, K., Kojima, T., Yamaki, S. & Kosugi, H. (1992) 24. Anal. Biochem. 202, 249-255.
- 25. Ohkawa, H., Ohishi, N. & Yagi, K. (1979) Anal. Biochem. 95, 351-358.
- Havel, R. J., Eder, H. A. & Bragdon, J. H. (1955) J. Clin. 26. Invest. 34, 1345-1353.
- 27 Labeque, R. & Marnett, L. J. (1987) J. Am. Chem. Soc. 109, 2828-2829.
- 28. Porter, N. A. & Wujek, J. S. (1987) J. Org. Chem. 52, 5085-5089.
- Esterbauer, H., Schaur, R. J. & Zollner, H. (1991) Free Radical 29. Biol. Med. 11, 81-128.
- 30. Chen, H.-J. C. & Cerami, A. (1993) J. Carbohydr. Chem., in press.
- 31. Bucala, R. & Cerami, A. (1992) Diabetes 41, 23 (abstr.).
- Picard, S., Parthasarathy, S., Fruebis, J. & Witztum, J. L. (1992) Proc. Natl. Acad. Sci. USA 89, 6876-6880. 32.
- 33. Nishigaki, I., Hagihara, M., Tsunekawa, H., Maseki, M. & Yagi, K. (1981) Biochem. Med. 25, 373-378.
- 34. Hicks, M., Delbridge, L., Yue, D. K. & Reeve, T. S. (1988) Biochem. Biophys. Res. Commun. 151, 649-655
- Mullarkey, C. J., Edelstein, D. & Brownlee, M. (1990) Bio-35. chem. Biophys. Res. Commun. 173, 932-939.
- Ahmed, M. U., Dunn, J. A., Walla, M. D., Thorpe, S. R. & 36. Baynes, J. W. (1988) J. Biol. Chem. 263, 8816-8821.
- 37. Namiki, M. & Hayashi, T. (1981) Prog. Food Nutr. Sci. 5, 81-91.
- 38. Tsuchida, M., Miura, T. & Aibara, K. (1987) Chem. Phys. Lipids 44, 297-325.

We are grateful to Drs. Scott Grundy and Sharon Brunelle for helpful discussions. These studies were supported by the American Diabetes Association, the Brookdale Foundation, and National Institutes of Health Grants DK19655-15, AGO-9453, and AGO-6943.