High Levels of Dietary Advanced Glycation End Products Transform Low-Density Lipoprotein Into a Potent Redox-Sensitive Mitogen-Activated Protein Kinase Stimulant in Diabetic Patients

Weijing Cai, MD; John Cijiang He, MD; Li Zhu, MD; Melpomeni Peppa, MD; Changyong Lu, MD; Jaime Uribarri, MD; Helen Vlassara, MD

**Background**—LDL modification by endogenous advanced glycation end products (AGEs) is thought to contribute to cardiovascular disease of diabetes. It remains unclear, however, whether exogenous (diet-derived) AGEs influence glycoxidation and endothelial cell toxicity of diabetic LDL.

**Methods and Results**—Twenty-four diabetic subjects were randomized to either a standard diet (here called high-AGE, HAGE) or a diet 5-fold lower in AGE (LAGE diet) for 6 weeks. LDL pooled from patients on HAGE diet (Db-HAGE-LDL) was more glycated than LDL from the LAGE diet group (Db-LAGE-LDL) (192 versus 92 AGE U/mg apolipoprotein B) and more oxidized (5.7 versus 1.5 nmol malondialdehyde/mg lipoprotein). When added to human endothelial cells (ECV 304 or human umbilical vein endothelial cells), Db-HAGE-LDL promoted marked ERK1/2 phosphorylation (pERK1/2) (5.5- to 10-fold of control) in a time- and dose-dependent manner compared with Db-LAGE-LDL or native LDL. In addition, Db-HAGE-LDL stimulated NF-κB activity significantly in ECV 304 and human umbilical vein endothelial cells (2.3-fold above baseline) in a manner inhibitable by a MEK inhibitor PD98059 (10 μmol/L), the antioxidant N-acetyl-L-cysteine, NAC (30 mmol/L), and the NADPH oxidase inhibitor DPI (20 μmol/L). In contrast to Db-LAGE-LDL and native LDL, Db-HAGE-LDL induced significant soluble vascular cell adhesion molecule-1 production (2.3-fold), which was blocked by PD98059, NAC, and DPI.

**Conclusions**—Exposure to daily dietary glycoxidants enhances LDL-induced vascular toxicity via redox-sensitive mitogen-activated protein kinase activation. This can be prevented by dietary AGE restriction. (Circulation. 2004;110:285-291.)

Key Words: atherosclerosis ■ diabetes ■ endothelium ■ glycotoxins ■ glycation ■ kinases

Cardiovascular disease is the major cause of morbidity and mortality in diabetic patients. One of the key risk factors for cardiovascular disease in this population is increased LDL. Aside from the quantitative alterations, chemical changes of LDL, such as glycation or glycoxidation, can delay receptor-mediated LDL clearance, contributing to a range of abnormal vascular responses. LDL, whether from type 1 or type 2 diabetic patients, is found to exhibit proatherogenic properties. Major sources that cause diabetic LDL modification are endogenous reducing sugars. Several advanced glycoxidation end products (AGEs) and lipoxidation derivatives, such as N-(carboxymethyl)lysine (CML) and 4-hydroxy-nonenal (HNE), have been identified as having proatherogenic properties, involving cell surface AGE receptors, redox-dependent oxidant stress, and activation of mitogen-activated protein kinase (MAPK) signaling and nuclear factor (NF)-κB transcription factor pathways.

AGE formation can be vastly accelerated as a function of degree and time of exposure to heat and can be introduced into the body with heat-processed foods. Two thirds of the absorbed AGEs (≈10% of ingested) are integrated within tissue and blood components, including LDL. The impact of this large influx of diet-derived AGEs has come under investigation as a potentially important modulator of normal gene and protein function. Recently, AGE-restricted dietary intervention has been reported to be an effective inhibitor of atherogenic processes in hyperlipidemic apolipoprotein E–deficient mice, as in postinjury restenosis or diabetes-related severe atherosclerosis, despite persistent hyperglycemia. The mechanisms involved have not been defined, however.

On the basis of this evidence, we hypothesized that food-derived AGEs contribute to excessive chemical and functional modification of LDL, a change that would be expected to be most obvious in diabetic patients. To test this hypothesis, we compared plasma LDL extracted from diabetic patients who were exposed to diets differing only in AGE content and evaluated its effects on cultured endothelial cells.
Methods

Cell Culture and Preparation of Cell Lysates

Human endothelial cells (ECs) (ECV 304 cell line) from the European Collection of Cell Cultures (Wiltshire, UK) and human umbilical vein ECs (HUVECs) from American Type Culture Collection were used in these studies. ECV 304 is a human EC line that, like HUVECs, exhibits EC markers and receptors for native LDL (N-LDL) and modified LDL. Quiescent cells were stimulated by different LDL preparations with or without inhibitors. After stimulation, cells were lysed in a Tris buffer containing 1% Triton X-100 and protease inhibitor cocktail. Protein concentration in supernatants was determined by Bio-Rad assay kit (Bio-Rad Laboratories).

Subjects/Clinical Protocol

Twenty-four patients with known diabetes under good metabolic control (baseline HbA1c, 7.3±0.6%) and normal lipid profiles and renal function were recruited for a different study, after approval by the Mount Sinai School of Medicine Institutional Review Board. Four patients were treated with statins (Lipitor) and 2 with aspirin; these patients ended up equally divided between the 2 dietary groups. No patient was taking metformin. Two diets, differing in AGE content, were assigned to the patients. One diet was designed with a high AGE content (high-AGE, HAGE, or low-AGE, LAGE diet) to simulate a diet with high AGE content (10% of calories from AGE). The mean daily AGE intake was 139 and 120 mg for each group, respectively. The participants in both dietary groups received similar vitamin supplements. Average daily vitamin E intake was 7.8 and 8 mg and vitamin C intake was 139 and 120 mg for each group, respectively. The difference in AGE content of foods was achieved largely by modifying the cooking time and temperature. The mean daily AGE difference in AGE content of foods was achieved largely by modifying the cooking time and temperature. The mean daily AGE content was determined for each subject at the beginning and again at the end of 6 weeks. Fasting blood samples for LDL isolation were collected at both time points.

TABLE 1. Clinical Characteristics of Diabetic Donors of LDL Samples

<table>
<thead>
<tr>
<th></th>
<th>High-AGE Diet (n=11)</th>
<th>Low-AGE Diet (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 Weeks</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>6/5</td>
<td>5/8</td>
</tr>
<tr>
<td>Age, y</td>
<td>61±7</td>
<td>62±5</td>
</tr>
<tr>
<td>Diabetes type 1/type 2, n</td>
<td>2/9</td>
<td>4/9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>7.3±0.6</td>
<td>7.4±1.3</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>28.7±5.1</td>
<td>28.3±4</td>
</tr>
<tr>
<td>Serum AGE (CML), U/mL</td>
<td>13.1±2.6</td>
<td>18±1.7</td>
</tr>
<tr>
<td>Serum MG derivatives, nmol/mL</td>
<td>2.7±0.8</td>
<td>3.4±0.5</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>105.2±24.6</td>
<td>98.4±45.1</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>182.4±58.2</td>
<td>179.8±27.8</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>63.6±24.9</td>
<td>57.2±17.7</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>113.6±32.5</td>
<td>110.6±23.5</td>
</tr>
</tbody>
</table>

Data are shown as mean±SEM. Statistically significant difference between baseline and end of study: *P<0.05; †P<0.01.

Preparation and Characterization of Human LDL

LDL (1.019<d<1.063 g/mL) was isolated from EDTA-treated (1 mmol/L) fasting plasma samples collected from each subject (n=24) at the beginning and end of the study. Briefly, LDL was obtained by potassium bromide density gradient ultracentrifugation in a Beckman VTI 50 rotor at 110 000g for 24 hours at 4°C in the presence of EDTA. For certain experiments, in addition to 3 individual LDL preparations, samples from 5 diabetic subjects per diet group and from 3 normal subjects were pooled for cell signaling and activation studies. To generate in vitro oxidized LDL (ox-LDL), N-LDL (0.35 mg/mL) was incubated with 5.0 μmol/L CuSO4 for 24 hours at 37°C, and the reaction was terminated by addition of 1.0 mmol/L EDTA and 100 μmol/L BHT. All LDL preparations were rendered sterile by filtration (0.22-μm Millipore filter, Amicon) after endotoxin contamination had been excluded by the limulus amebocyte lysate (Bio-Whittaker). Peroxidation in all LDL preparations was determined on the basis of quantification of malondialdehyde (MDA) content. In vitro oxidized LDL (ox-LDL), N-LDL (0.35 mg/mL) was incubated with 5.0 μmol/L CuSO4 for 24 hours at 37°C, and the reaction was terminated by addition of 1.0 mmol/L EDTA and 100 μmol/L BHT. All LDL preparations were rendered sterile by filtration (0.22-μm Millipore filter, Amicon) after endotoxin contamination had been excluded by the limulus amebocyte lysate (Bio-Whittaker). Peroxidation in all LDL preparations was determined on the basis of quantification of malondialdehyde (MDA) content.

Western Analysis for ERK1/2

Aliquots (30 μg) of cell lysates as described above were used for Western blot analysis using a rabbit phospho-specific p44/42 MAPK polyclonal antibody (1:1000 dilution, New England Biolabs) or rabbit polyclonal antibody against total p44/42 MAPK (phosphorylation-independent) (New England Biolabs).

Cell Transfection and Luciferase Assay

NF-κB-luc reporter plasmid (0.2 μg NF-κB-luc reporter plasmid/24 g NF-κB-luc reporter plasmid/well) (Stratagene) was transferred by use of LipofectAMINE PLUS reagent (Gibco/BRL). pFC-MEKK (the catalytic domain of MEKK, Stratagene) was used as a positive control in NF-κB assays. Twenty-four hours after transfection, N-LDL, ox-LDL, DbHAGE-LDL, or Db-LAGE-LDL from 3 individuals/group and a pooled sample (n=5/group) were added, as indicated, for 3 hours, with or without inhibitors. After reporter lysis buffer (Packard Instrument Company) had been added to each well for 10 minutes, luminescence...
was detected in a scintillation TopCount microplate (Packard Instrument Company).

**Human Soluble VCAM-1 Assay**
ECV 304 cells were incubated in serum-free medium with different LDL preparations (50 μg/mL) for 6 hours at 37°C. Cell supernatants were concentrated by 10-fold by use of Centricon-10 filters (Amicon) and assayed for soluble human vascular cell adhesion molecule (sVCAM)-1 by an ELISA kit (R&D Systems, Inc) as described previously.27

**Statistical Analysis**
Data are expressed as mean±SD. Statistical significance between groups was determined by Student’s t test or 1-way ANOVA with Bonferroni correction, depending on the number of groups compared. A probability value of P<0.05 was considered significant.

**Results**

**LDL From Diabetic Patients on an HAGE Diet Is More Extensively Modified Than if Patients Consumed an LAGE Diet**
The clinical characteristics of the diabetic patients exposed to either a high- or a low-AGE diet are shown in Table 1. There were no significant differences between the baseline and end of the study within each group with regard to body mass index, HbA1c, fasting plasma glucose, and lipid profile, although significant changes were noted in total serum AGE levels between the baseline and end of the study (Table 1).18 LDL from diabetic patients on an HAGE diet (Db-HAGE-LDL), measured either individually or as a pooled sample, was significantly more glycated (≈4-fold, P<0.01) and oxidized (≈7-fold) compared with normal LDL (N-LDL, P<0.05) (Table 2).18 In contrast, LDL from diabetic patients who were fed a low-AGE diet (Db-LAGE-LDL) was less glycated (by 50%) and less oxidized (by ≈80%) compared with Db-HAGE-LDL. LAGE-LDL AGE and malondialdehyde content were ≈1.4- and 2.0-fold above N-LDL, respectively (Table 2).

**Diabetic LDL Is a Potent Activator of ERK1/2 When Derived From HAGE-Fed Patients but Not When Derived From LAGE-Fed Patients**
After incubation of ECV 304 cells with Db-HAGE-LDL for 10 minutes, a significant increase of ERK1/2 phosphorylation was noted (at 5 μg/mL of Db-HAGE-LDL), reaching maximal activation at 25 μg/mL (≈10-fold above baseline) (Figure 1, A and B). This was in marked contrast with Db-LAGE-LDL, which failed to activate ERK1/2 even at maximal concentrations (Figure 1, A and B), as did N-LDL. Of note, in vitro–prepared ox-LDL induced modest ERK1/2 phosphorylation: a significant response (≈6-fold of baseline) was observed only at doses 10 to 20 times greater (100 μg/mL) than those required for Db-HAGE-LDL (Figure 1, C and D). No changes in total ERK1/2 were noted during these studies (Figure 1E).

Also, Db-HAGE-LDL (5 μg/mL) activated ERK1/2 in a time-dependent manner; ERK1/2 phosphorylation was evident within 1 minute, peaked at 5 to 10 minutes, and declined by 30 minutes (Figure 2A). To determine whether MAPK kinase (MEK) was involved in this response, cells were preincubated with the MEK inhibitor PD98059 (10 μmol/L, 30 minutes) and then stimulated with Db-HAGE-LDL (5 μg/mL, 10 minutes). ERK1/2 phosphorylation was completely inhibited, indicating that Db-HAGE-LDL–induced activation of ERK1/2 proceeds via a MEK-dependent pathway (Figure 2B).

**Diabetic HAGE-LDL–Induced Phosphorylation of ERK1/2 Is Prevented by Antioxidant NAC and NADPH Oxidase Inhibitor DPI**
ECV 304 cells were preincubated with the antioxidant NAC or the NADPH oxidase inhibitor DPI for 30 minutes and then stimulated with Db-HAGE-LDL (5 μg/mL) for 10 minutes. Both NAC and DPI effectively prevented HAGE-LDL–induced ERK1/2 activation (Figure 2C), suggesting that reactive oxygen species generation was involved.

**NF-κB Transcriptional Activation, via ERK1/2, Is Enhanced by Diabetic HAGE-LDL but Not by Diabetic LAGE-LDL**
NF-κB activity was tested in cells transiently transfected with a luciferase-coupled promoter plasmid driven by an NF-κB consensus sequence, after exposure to different LDL preparations. Transfected cells without any treatment were used as controls for basal luciferase activity. Stimulation with Db-

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**TABLE 2. Glycooxidative and Oxidative Modification of Plasma LDL From Diabetic Patients Exposed to Different Dietary AGE Regimens**

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. AGE-LDL, U/mg*</th>
<th>Ox-LDL, nmol/mg†</th>
</tr>
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</table>
| Diabetic high AGE diet LDL (Db-HAGE-LDL)‡ | 11 (5) 214±51 (192±25§) | 5.7±2.3]
| Diabetic low AGE diet LDL (Db-LAGE-LDL)‡ | 13 (5) 106±23 (92.4±14) | 1.5±0.5 |
| Normal LDL (N-LDL)‡                    | 5 (3) 64±15 (45±8) | 0.8±0.2 |
| Ox-LDL‡                               | 1 (1) 76±4 (76±5) | 10.3±2.8 |

All values represent the mean±SD of 3 independent measurements. Statistical analysis was performed using ANOVA.

*Based on competitive AGE ELISA (4G9 mAb).25
†Malondialdehyde equivalents by thiobarbituric acid–reactive substances.26
‡Denotes individual values of plasma LDL samples from diabetic patients on test diets. N-LDL was derived from nondiabetic subjects on regular (equivalent to HAGE) diet. 18 OxLDL was generated from diabetic patients on an HAGE diet (Db-HAGE-LDL). LAGE-LDL AGE and malondialdehyde (by 50%) and less oxidized (by 80%) compared with Db-HAGE-LDL. LAGE-LDL AGE and malondialdehyde content were ≈1.4- and 2.0-fold above N-LDL, respectively (Table 2).

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HAGE-LDL (5 μg/mL, 3 hours) caused a significant increase in relative luciferase activity above control values (1.8-fold), compared with the minimal response obtained from equal amounts of Db-LAGE-LDL (1.2-fold), N-LDL (1.15-fold), and ox-LDL (1.25-fold). The groups were significantly different, with a value of \( P < 0.005 \) (ANOVA) (Figure 3A).

Figure 1. Diabetic (Db)-HAGE-LDL but not LAGE-LDL stimulates EC ERK1/2 phosphorylation in a dose-dependent manner. ECV 304 cells were stimulated with different concentrations of Db-HAGE-LDL, Db-LAGE-LDL, N-LDL, and ox-LDL for 10 minutes before lysis. Cell proteins were separated by SDS-PAGE (10%) and immunoblotted by an anti-phosphorylated ERK1/2 (p-ERK1/2) or anti-total ERK1/2 (T-ERK1/2) antibody. A, p-ERK1/2 after incubation with diabetic HAGE-LDL and LAGE-LDL. B, Densitometry of data shown in A; C, p-ERK1/2 after incubation with N-LDL and ox-LDL. D, Densitometry of data shown in C; E, Total ERK1/2 after incubation with diabetic HAGE-LDL. Bars represent mean ± SD of increase (x-fold) above control values from 3 individual experiments. *\( P < 0.05 \), **\( P < 0.01 \) vs control cells (without stimulation).

Figure 2. Db-HAGE-LDL-induced ERK1/2 phosphorylation is MAPK- and redox-dependent. A, Time course of ERK1/2 phosphorylation stimulated by Db-HAGE-LDL. ECV 304 cells were stimulated with Db-HAGE-LDL (5 μg/mL) for indicated periods of time, harvested, and lysed. p-ERK1/2 was assayed by immunoblotting cell lysates with anti-p-ERK1/2 antibody. Data are expressed as mean ± SD of 3 individual experiments. *\( P < 0.05 \), **\( P < 0.01 \) vs time 0. B and C, Cells were preincubated with MEK inhibitor PD 98059 (10 μmol/L), antioxidant NAC (20 and 30 mmol/L), or NADPH oxidase inhibitor DPI (10 and 20 μmol/L) for 30 minutes and then with Db-HAGE-LDL (5 μg/mL) for 10 minutes. p-ERK1/2 was determined by immunoblotting as in A. Densitometric analysis of data from 3 independent experiments is shown as mean ± SD of increase (x-fold) above control values (no stimulation). **\( P < 0.01 \) vs control cells (without stimulation). #\( P < 0.05 \), ##\( P < 0.01 \) vs Db-HAGE-LDL.
Db-HAGE-LDL–induced NF-κB activity was further assessed in cells pretreated with PD 98059 (10 μmol/L, 30 minutes), NAC (30 mmol/L, 30 minutes), or DPI (20 μmol/L, 30 minutes), respectively. Virtually all inducible NF-κB activity was inhibited by each inhibitor: PD 98059, \( P < 0.01 \); NAC, \( P < 0.05 \); and DPI, \( P < 0.01 \) (Figure 3B).

**Diabetic HAGE-LDL but Not Diabetic LAGE-LDL Stimulates EC Production of sVCAM-1**

Stimulation of ECV 304 cells with Db-HAGE-LDL (50 μg/mL, 6 hours) induced sVCAM-1 secretion in the supernatant (2.3-fold above control values) \( P < 0.01 \) on the basis of ELISA. In contrast, only a slight enhancement of sVCAM production was observed in response to Db-LAGE-LDL and ox-LDL and none to N-LDL (Figure 3C). Also, as in the previous experiments, sVCAM-1 production induced by Db-HAGE-LDL was inhibited by PD 98059 (10 μmol/L), NAC (30 mmol/L), or DPI (20 μmol/L) (Figure 3C).

**Diabetic HAGE-LDL Activates HUVECs via a Similar Redox-Dependent Pathway**

To assess the reproducibility of these findings in ECs derived from normal human vessel wall, similar experiments were performed in HUVECs exposed to the same LDL preparations as used above. Db-HAGE-LDL (5 μg/mL) but not LAGE-LDL, ox-LDL, or N-LDL induced marked ERK1/2 phosphorylation in HUVECs (Figure 4, A and B) at a similar concentration with an identical time course as with ECV 304 (maximum at 10 minutes and 5 μg/mL) (Figure 4C). As in ECV 304 cells, Db-HAGE-LDL triggered marked HUVEC NF-κB activity (2.3-fold above control values, \( P < 0.05 \)) and was suppressed in the presence of identical amounts of antioxidants (NAC, DPI) or of MEK inhibitor (Figure 5).

**Discussion**

The present report demonstrates that LDL isolated from diabetic patients exposed to a diet with a high glycoxidant content is more extensively altered than LDL from diabetic subjects fed a diet with reduced AGE, both in terms of degree of chemical modification and of cell-toxic properties. The combination of endogenous hyperglycemia because of diabetes with intake of large quantities of exogenous AGE appeared to give rise to a modified LDL particle significantly more potent as a promoter of abnormal EC gene activation than LDL exposed to diabetes combined with only a fraction \((\%) \) of the AGE present in regular diet.

The structural and functional LDL changes found in the present studies were consistent with the differences in AGE content of the diet, with AGE levels in serum as reflected in CML and MG derivatives, but also with levels of the circulating inflammatory markers C-reactive protein, tumor necrosis factor-α, and VCAM-1 reported previously.18 These findings supported the postulated relationship between exogenous AGE and diabetic cardiovascular disease.18,28,29 and opened a new window into the mechanisms involved in these events.

For instance, a significant corollary of the excessive modification of Db-HAGE-LDL was the marked increases in
MAPK phosphorylation, NF-κB activity, and VCAM production triggered on interaction of this LDL preparation with ECs. These were attributable to the greater degree of modification of the LDL by ingested reactive AGE intermediates in those patients, because they were clearly absent when LDL from the low-AGE–fed diabetic patients was used. Thus, under similar levels of hyperglycemia, diet-enhanced glycoxidation of LDL can transform it to a powerful promoter of intracellular oxidant stress–dependent changes.

As a key component of the MAPK system, ERK1/2 or p44/42 plays an important role in cell proliferation and differentiation. Activation of this kinase system is triggered by multiple stimuli, including AGE. Induction of ERK phosphorylation by in vitro modified LDL preparations has been reported in studies of cultured smooth muscle cells, mesangial cells, and macrophages. The human diabetic LDL used in this study was modified while in the circulation and contained glycation and oxidation derivatives in proportion to the dietary AGE content and not to levels of glycemia.

These derivatives prompted greater EC changes than expected on the basis of glycemic control alone. Of note, LDL derived from diabetic patients exposed to a low-AGE regimen (Db-LAGE-LDL) proved strikingly incapable of mobilizing significant activation signals, despite similar ambient hyperglycemia.

In vitro–prepared AGE can indeed activate NADPH oxidase and generate intracellular reactive oxygen species, suggesting that glycoxidation derivatives can act as second messengers, leading to MAPK activation. The events set in motion by in vivo AGE-modified LDL, however, are less well defined. The present findings provide evidence that at least reactive oxygen species and NADPH could be involved in these steps, because both an antioxidant, NAC, and an NADPH oxidase inhibitor abolished the HAGE-LDL induced ERK1/2 phosphorylation and NF-κB activity.

The identity of pathogenic AGE or lipoxidation derivatives of LDL remains largely undefined. However, a reasonable choice for making valuable correlations possible was the measurement of CML, a common end product of glucose-protein– and glucose-lipid–derived glycoxidative reactions, occurring abundantly in vivo and in heat-processed foods. Also, CML-like AGE deposits are shown to correspond to the vascular pathology and to the subinflammatory state of diabetes. Of note, circulating CML also paralleled levels of ingested CML. Whether high or low in AGE content, the diets used were equal in nutrient composition and not rich in fats or carbohydrates. Glucose and lipid levels remained likewise unchanged over the course of the study. The different levels of AGE in the diets used here were achieved by modulating cooking temperature and time. That the mode of meal preparation alone could alter the properties of circulating LDL to the extent that this would, in turn, drastically affect vascular cell signaling, nuclear factor transcription activity, and key adhesion molecule expression (sVCAM-1) was an unanticipated yet intriguing finding.

Glycoxidation of LDL involves free radical generation and fatty acid oxidation, which, together with hyperglycemia, can promote vascular injury. Given the evidence presented above, limiting AGE–apolipoprotein B–LDL formation by way of reducing dietary AGE intake could prevent atherogenic events attributed to the LDL particle. Indeed, an AGE-restrictive intervention in animals led to significant changes in the activity of intracellular oxidant stress–dependent changes.
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protection against postinjury restenosis and diabetes-accelerated atherosclerosis in apolipoprotein E-negative mice. Moreover, in diabetic humans, manipulation of dietary AGE led to the suppression of inflammatory markers as well as of AGE-LDL. In this sense, the inflammatory state seen in diabetic patients could be attributed to LDL alterations caused by the high-AGE diets ingested combined with hyperglycemia. In conclusion, plasma LDL from diabetic patients can be transformed into a powerful trigger of EC redox-dependent activation. This may be largely a result of exogenous glycoxidants consumed with standard diets. The present study provides a new mechanistic basis and the rationale for clinical studies to establish potentially significant antiatherogenic benefits of AGE-restricted diets in humans that protect LDL particles from overt oxidative modification.

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References
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