Diabetes is strongly associated with an accelerated rate of development of atherosclerosis. Epidemiological studies have reported that diabetes is an independent risk factor in atherosclerosis-associated morbidity and mortality and that hyperglycemia plays a significant role in atherogenesis. One of the key events in the early stages of atherosclerotic lesion formation is the recruitment of monocytes into the vessel wall. Gerrity has shown localization of monocytes and monocyte-macrophages in aortic lesions in swine. In vivo, rabbits with alloxan-induced diabetes show an accumulation of white blood cells on the endothelial cell surface. In addition, monocytes from individuals with diabetes mellitus show increased adhesiveness to endothelial cells in culture. However, the exact mechanisms mediating the action of glucose in monocyte adhesion to the vascular endothelium are not clearly understood.

Previous studies suggest that hyperglycemia may promote pathological effects on cell-cell interactions through several mechanisms, including glycation of lipoproteins, propagation of free radicals, and production of advanced glycation end products (AGEs). Glycation of lipoproteins leads to increased susceptibility to oxidation, which has been shown to stimulate atherosclerotic foam cell formation. In the presence of trace metals, glucose and its derivatives can auto-oxidize to form superoxide radicals, H$_2$O$_2$, and hydroxyl radicals. This increased oxidative environment may contribute to the formation of certain oxidized lipids that have been shown to stimulate monocyte attachment to the endothelium. Numerous studies show involvement of AGE products in the development of vascular diseases and their ability to promote monocyte transmigration through the endothelium. Our group has previously demonstrated in vitro that elevated glucose levels in culture medium (2 passages, 7 days) contributed to enhanced monocyte adhesion to human aortic endothelial cells (HAECs). Bucala et al. showed that significant formation of AGE products required an incubation period.

Abstract—The development of atherosclerosis is accelerated in individuals with type 2 diabetes. Adhesion of monocytes to the vascular endothelium is a key initial step in atherogenesis. We have previously shown that monocyte adhesion to human aortic endothelial cells (HAECs) cultured long-term in high-glucose medium (25 mmol/L, 2 passages) is increased compared with cells grown in normal glucose (5 mmol/L). One potential mechanism for increased monocyte adhesion to HAECs under hyperglycemic conditions is via the 12-lipoxygenase (12-LO) pathway. In this study, we demonstrated in HAECs that the major LO metabolite of arachidonic acid was the 12-LO product, 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE], which was increased several-fold in HAECs cultured under high-glucose conditions. Furthermore, treatment of HAECs with 12(S)-HETE induced monocyte, but not neutrophil, adhesion an average of 3-fold (range of 1.5- to 5-fold) compared with untreated cells (75±5 versus 26±1 monocytes per field, respectively, P<0.001). Expression of the adhesion molecules vascular cell adhesion molecule-1, E-selectin, and intercellular adhesion molecule-1 was not significantly increased. However, both glucose and 12(S)-HETE induced a 60% increase in HAEC surface expression of connecting segment-1 (ie, CS-1) fibronectin, a ligand for very late-acting antigen-4 (VLA-4). The antibodies used to block monocyte integrin VLA-4 and leukocyte function–related antigen-1, a monocytic counterreceptor for intercellular adhesion molecule-1, inhibited the ability of both 12-LO products and high glucose to induce monocyte adhesion. These results definitively demonstrate for the first time in HAECs that the 12-LO pathway can induce monocyte–endothelial cell interaction and that the effects of glucose may be mediated, at least in part, through this pathway. Thus, these results suggest that the 12-LO pathway may play a role in the increased susceptibility of diabetics to atherosclerosis.

Key Words: 12(S)-hydroxyeicosatetraenoic acid ■ endothelium ■ hyperglycemia ■ lipoxygenase ■ monocytes
period of at least 20 days after using much higher levels of glucose than employed by our group. Furthermore, the effects of glucose in our previous study occurred in the presence of low levels of LDL present in FBS (5% FBS in medium 199 [M199]). Thus, we have examined another possible and earlier mechanism of glucose action on monocyte–endothelial cell interactions.

Many studies have shown changes in cellular eicosanoid production under hyperglycemic conditions (within 2 passages), and there is evidence that metabolites of lipoxigenases (LOs) play a role in many of the steps involved in inflammation, especially in modulating cell-cell interactions. A potential candidate for generating such eicosanoids is the leukocyte-type 12-LO, which incorporates molecular oxygen to arachidonic acid to form primarily 12(S)-hydroxyeicosatetraenoic acid (HETE) and, to a lesser extent, 15(S)-HETE. In vitro, studies have shown that macrophage attachment to isolated rat glomeruli is facilitated by 12(S)-HETE and that inhibitors of the LO pathway have blocked this adhesion. 12(S)-HETE has also been shown to have chemokinetic activity and to promote diapedesis of leukocytes. In addition, several studies have implicated a role for LOs in diabetes and atherogenesis. Elevated levels of LO products have been measured in different cell types cultured for 2 passages under hyperglycemic conditions, and the increase has been detected as early as 24 hours after glucose treatment. How-ever, the effects of glucose on cells are classically studied after 2 passages (7 days) in culture, and as a consequence, we have conducted our studies with the 2-passage treatment of cells in vitro. We have previously identified a leukocyte-type of 12-LO in HAECs, smooth muscle cells, and monocytes. However, the role of the 12-LO pathway in mediating glucose-induced monocyte adhesion to HAECs has never been examined. Although there are a number of ways in which glucose may elevate monocyte adhesion, we focused on the 12-LO pathway. Therefore, in the current study, we sought to directly test the role of 12(S)-HETE in the activation of monocyte-endothelial interactions to investigate whether hyperglycemia may act through the 12-LO pathway in mediating early events of atherogenesis.

Methods

Cell Preparation and Culture

HAECs were isolated by collagenase dispersion from aortic tissues obtained from cardiac transplant donors at the University of California at Los Angeles. Cells were grown in M199 containing 20% FBS with 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin, 20 mg/mL endothelial cell growth supplement, and 90 μg/mL heparin. HAECs were used between passages 4 and 8. For high-glucose exposure, HAECs were grown for 2 passages (7 days) in 25 mmol/L glucose–supplemented medium as previously described. For osmolality control, HAECs were grown in 5 mmol/L glucose plus 20 mmol/L mannitol for 2 passages (7 days). Human monocytes were isolated by the Recaldel method. 22 Human neutrophils were freshly isolated by Ficoll-Hypaque separation followed by red blood cell lysis. 23

Quantitation of Arachidonic Acid Metabolites

Confluent HAECs were incubated with 5 μCi/mL [3H]arachidonic acid for 24 hours. Cells were rinsed 3 times with 0.05% fatty acid–free BSA in M199 and incubated for 2 hours at 37°C in 10% FBS/M199 to ensure that most of the arachidonic acid was esterified. Previous studies have shown that most of the arachidonic acid is incorporated into phospholipids. 14 Cells were incubated in 10% FBS/M199 containing normal glucose, high glucose, or mannitol for 4 hours. The medium was collected, and the cells were washed with PBS containing Ca²⁺ and Mg²⁺, scraped, and harvested. A small aliquot was set aside for protein determination. Lipids in the medium were acidified, cellular lipids were hydrolyzed in methanolic NaOH, and both were extracted on C18 Bond-Elut columns and analyzed by high-performance liquid chromatography on a reverse-phase C18 column, as described earlier. 15 Radioactive metabolites were identified by comigration with authentic cold and tritiated standards (12(S)-, 15(S)-, and 5(S)-HETE).

12(S)- and 15(S)-HETE Detection by Radioimmunoassay

HAECs grown in 100-mm dishes in normal (5 mmol/L) or high-glucose (25 mmol/L) medium were incubated for 30 minutes in M199 with 0.2% fatty acid–free BSA. The dishes were placed on ice, the medium was collected and acidified, and the lipids were extracted with ethanol. Cells were washed once with ice-cold PBS containing 100 μmol/L EDTA, scraped, and then pelleted. The cell lipids were decylated with methanolic NaOH for 1 hour and extracted on C18 Bond-Elut columns. 12(S)-HETE levels in the supernatant and cell extracts were quantitated by a specific radioimmunoassay (Advanced Magnetics, Inc) as previously described. 24

Adhesion Assay

HAECs were grown to 100% confluence in 48-well dishes and treated at 37°C for various times and with various concentrations of 12(S)-HETE (Cayman Chemical). Cells were also either treated for 4 hours at 37°C with 1 nmol/L 12(R)-HETE, 5(S)-HETE, or 15(S)-HETE (BioMol Research Laboratories), ethanol (at the same dilution as the HETEs), or lipopolysaccharide (LPS, 2 ng/mL) or left untreated. The cells were then washed twice with 5% FBS/M199, and 2x10⁴ monocytes or freshly isolated neutrophils were added to each well. After a 15-minute incubation at 37°C, unbound cells were washed off, and bound cells were fixed with 1% glutaraldehyde. Bound monocytes or neutrophils were counted by phase-contrast microscopy. A minimum of 8 fields were counted for each experiment.

Adhesion Molecule Detection by ELISA

HAECs were grown in 96-well dishes to 100% confluence. Cells were then treated with LPS (2 ng/mL), 12(S)-HETE (1 nmol/L), or 15(S)-HETE (1 nmol/L) or were left untreated for 4 hours at 37°C. The wells were washed 3 times with 5% FBS/M199, placed on ice, and fixed with 1% paraformaldehyde. For detection of E-selectin and human vascular cell adhesion molecule-1 (VCAM-1), cells were washed twice in Tris-buffered saline containing 140 mmol/L glycine followed by 3 washes in PBS. Cells were then incubated on ice overnight at 4°C with 1 μg/mL monoclonal antibodies to VCAM-1 (BioSource) and E-selectin (BioSource/Biodesign). For detection of constant B-selectin segment-1 (CS-1), an alternatively spliced form of fibronectin, cells were fixed with 4% paraformaldehyde in Ca²⁺- and Mg²⁺-containing PBS before being blocked with 3% BSA in PBS for 1 hour at room temperature; then the cells were incubated with 0.5 μg/mL monoclonal CS-1 antibody overnight at 4°C. After the incubation period, unbound antibodies for all adhesion molecules were rinsed off with 3 washes in 5% FBS/M199 or with 0.1% BSA in PBS. For intercellular adhesion molecule-1 (ICAM-1) antibody wells, cells were fixed with 1% paraformaldehyde (after the incubation period and rinses). Wells were then rinsed 3 times in PBS and blocked for 20 minutes at room temperature in PBS containing 3% BSA and a 1:100 dilution of secondary antibody host serum. Cells were then
incubated with a secondary antibody conjugated to horseradish peroxidase for 1 hour. Cells were washed in 0.1% BSA in PBS followed by 3 washes in distilled water. The peroxidase substrate o-phenylenediamine dihydrochloride (Sigma) was added to the cells and the absorbance read at 450 nm for CS-1 or 490 nm for VCAM-1, ICAM-1, or E-selectin. (Spectra Max 250, Molecular Devices). Monoclonal anti-CS-1 antibody was a generous gift from Dr Mariano J. Elices of Cytel Corp, San Diego, Calif. An irrelevant IgM antibody, CD20 (Biosource), was used as a negative control.

**Immunofluorescence of CS-1 Fibronectin on HAECs**

HAECs were cultured onto glass coverslips that had been treated with Cell Tak (Collaborative Biomed No. 40240) followed by Vitrogen 100 (Collagen Corp No. 0701-1N) to ensure proper attachment of the cells without altering their characteristics. HAECs were either left untreated or treated with 25 mmol/L glucose (2 passages) or 12(S)-HETE (1 mmol/L, 4 hours), and the confluent monolayer of cells was then fixed with 4% paraformaldehyde. Incubation with antibody and wash conditions were identical to those in the ELISA method except that fluorescent CY3- (red) conjugated secondary antibody (Jackson ImmunoResearch No. 115-166-020) was used. The coverslips were mounted onto a microscope slide and then visualized by fluorescence or phase microscopy.

**Blocking Antibody Studies**

Monoclonal antibodies against either α4 or β2 integrins were incubated with monocytes at 5 μg/mL in 5% FBS/M199 for 10 minutes at room temperature before the monocyte adhesion assays. Monocyte adhesion assays were performed as described above. Antibodies to α4 (CD49d) block VCAM-1 and CS-1 fibronectin binding through VLA-4 (very late–acting antigen, or α4β1), and antibodies to β2 (TS1-18) block ICAM-1 binding through LFA-1 (lymphocyte function–related antigen, or αLβ2) on monocytes. Anti-α4 antibody was obtained from Becton Dickinson (No. 550019), and TS1-18 was obtained from American Type Culture Collection. A nonspecific mouse IgG1 (negative control antibody from Dako, No. X931) was also used.

**Statistical Analysis**

Data are represented as mean ± SE. Statistical analysis was performed by using unpaired Student’s t test. When multiple comparisons were performed, a 1-way factor ANOVA was employed. For the ANOVA, a Fisher exact test was used to determine 95% confidence intervals.

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**Results**

**HAEC Exposure to High Glucose Enhances 12(S)-HETE Production**

HAECs cultured in 25 mmol/L glucose for 2 passages (7 days) produced 12(S)-HETE as the major LO metabolite of arachidonic acid. HAECs either untreated or glucose treated were incubated with [3H]arachidonic acid for 24 hours, and high-performance liquid chromatography analysis revealed that the major arachidonate metabolite of the LO pathway was 12-HETE (data not shown). This result is consistent with findings by others. As shown in Figure 1, there was a 2- to 4-fold increase in the amount of 12(S)-HETE released into the medium, as measured by radioimmunoassay, when cells were cultured in high glucose (25 mmol/L) compared with cells cultured in normal (5 mmol/L) glucose (302.6 ± 87.9 versus 714.2 ± 149.5 pg/mL · mg protein⁻¹, P < 0.05). In the same experiments, there was also an ∼2- to 6-fold increase in cell-associated 12(S)-HETE in cells cultured in high glucose compared with cells cultured in normal glucose (164.1 ± 32.9 versus 852.6 ± 229.6 pg/mg protein, P < 0.05). Three different HAEC donors were used for these studies, which likely accounts for the variation in 12(S)-HETE levels that were measured. All results were normalized to total cell protein levels. These data suggest that chronic exposure of endothelial cells to elevated glucose conditions upregulates the 12-LO pathway.
We next examined whether the products of 12-LO could influence monocyte adhesion to the endothelium. We first determined the concentration and time dependence of 12(S)-HETE treatment on HAECs in monocyte adhesion assays. As shown in Figure 2A, 12(S)-HETE treatment of HAECs induced monocyte–endothelial cell interactions at concentrations as low as 0.1 nmol/L ($P < 0.001$). Higher concentrations of 12(S)-HETE (up to 1 mmol/L) did not result in greater induction of monocyte adhesion, suggesting a saturation effect of 12(S)-HETE.

Furthermore, a time-course study of 12(S)-HETE (1 nmol/L) incubation with HAECs between 30 minutes and 8 hours showed that the earliest significant increase in monocyte adhesion to HAECs occurred at 4 hours and remained elevated at 8 hours (Figure 2B, $P < 0.001$). Thus, all subsequent experiments were carried out with a concentration of 1 nmol/L 12(S)-HETE and a 4-hour incubation time.

As shown in Figure 3A, treatment of HAECs with either 12(S)- or 15(S)-HETE significantly induced monocyte adhesion by an average of 3-fold (a range of 1.5- to 5-fold over a series of 10 experiments: 75 ± 5 and 75 ± 3 versus 26 ± 1 monocytes, respectively, $P < 0.0001$). Incubation with 1 nmol/L 12(R)-HETE, which is the stereoisomer of 12(S)-HETE and not a product of the 12-LO pathway, did not have any effect on monocyte adhesion to HAECs. There was also no effect observed with 5(S)-HETE treatment, a metabolite of the 5-LO pathway. Owing to variability between donors, these monocyte adhesion assays were performed with 7 different monocyte and HAEC donors. These data indicate that products of 12-LO could significantly increase the interaction of monocytes with the endothelium. A similar

12-LO Products and 12(S)- and 15(S)-HETE Increase Monocyte Adhesion to HAECs

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Figure 6. Immunofluorescence of CS-1 fibronectin on HAECs by high glucose and 12(S)-HETE. HAECs were grown in normal glucose (A), cultured in 25 mmol/L glucose (B) for 2 passages, or treated with 1 nmol/L 12(S)-HETE (C) for 4 hours at 37°C. Cells were fixed and incubated with CS-1 antibody followed by fluorescent CY3 (red) conjugated secondary antibody as described in Methods. Glucose and 12(S)-HETE treatment increased the filamentous CS-1 fibronectin patches on the endothelial surface. Views of the images are of a confluent monolayer of HAECs and are all at the same ×573 magnification. There is ~1 CS-1 fibronectin patch per cell. A representative phase image of HAEC at the same ×573 magnification (D).
adhesion assay was performed with freshly isolated human neutrophils to determine whether HETEs stimulate monocyte binding specifically. HAECs treated with 12(S)- or 15(S)-HETE did not induce neutrophil adhesion to HAECs (Figure 3B). We have previously shown that HAECs cultured in high glucose also did not elicit neutrophil binding.12 Ethanol, a solvent for HETEs, did not induce monocyte or neutrophil adhesion (data not shown). LPS was used as a positive control in all of the adhesion assays. Thus, cell adhesion to HAECs induced by 12(S)- and 15(S)-HETE was specific for monocytes.

Effect of Glucose and 12(S)- and 15(S)-HETE on Endothelial Expression of Adhesion Molecules

We had previously shown that VCAM-1, ICAM-1, and E-selectin on HAECs were not increased by high glucose.12 To examine the effect of 12(S)- and 15(S)-HETE on endothelial adhesion molecule expression, cell surface ELISAs were performed for known monocyte ligands. 12(S)- or 15(S)-HETE treatment of HAECs did not significantly induce VCAM-1, ICAM-1, or E-selectin (Figure 4). A slight increase of ICAM-1 was observed with 12(S)- and 15(S)-HETE treatment, although the result was not statistically significant. LPS increased expression of all 3 adhesion molecules. Previous studies have shown that CS-1 fibronectin can also act as a monocyte ligand26 and that its levels are increased in human atherosclerotic lesions.27 As determined by ELISA, treatment of HAECs with 12(S)-HETE or high glucose (25 mmol/L) significantly increased the endothelial cell surface expression of CS-1 fibronectin by ≈60% (Figure 5, P < 0.0001). Immunofluorescence studies demonstrated a dramatic increase in filamentous patches of CS-1 fibronectin deposits on endothelial cell surfaces after glucose or 12(S)-HETE treatment (Figure 6). These data suggest that glucose and 12-LO products stimulated monocyte–endothelial cell interaction through endothelial cell surface deposition of CS-1 fibronectin.

12(S)-HETE Treatment of Monocytes Also Increases Adhesion to HAECs

Because much of the 12(S)-HETE produced by endothelial cells is released, we next examined whether 12(S)-HETE could stimulate monocytes directly. Before the adhesion assay, monocytes were incubated for 12 minutes with 12(S)-HETE (1 nmol/L). 12(S)-HETE stimulated a modest (50% to 60%) increase in monocyte adhesion to HAEC compared with mock-treated monocytes (42.5 ± 2.6 versus 27.0 ± 1.8 bound monocytes, respectively, P < 0.0001). These results suggest that 12(S)-HETE can directly stimulate monocytes but that this effect only accounts for a part of the increase in adhesion to HAECs. Thus, 12(S)-HETE appears to influence both monocytes as well as endothelial cells in mediating adhesion events.

Blocking Antibodies Against Integrins on Monocytes Reduce Adhesion to HAECs

Because ICAM-1 is constitutively expressed on HAECs and since CS-1 fibronectin was induced by both glucose and 12(S)-HETE treatment, blocking antibodies to counterreceptors on monocytes were used to determine whether glucose- and 12(S)-HETE–mediated monocyte adhesion to HAECs could be abrogated. The mononuclear integrin VLA-4 (α4β1) is known to bind to CS-1 fibronectin and VCAM-1, and LFA-1 (αβ2) integrins are known to bind ICAM-1 and ICAM-2.28 As shown in Figure 7, preincubation of monocytes with blocking antibodies to either α4 or β2 inhibited both glucose- and 12(S)-HETE–mediated monocyte adhesion to HAECs (P < 0.005). The antibodies only partially reduced 15(S)-HETE–mediated monocyte adhesion to HAECs (P < 0.03, data not shown). Monocyte treatment with a nonspecific mouse IgG, negative control antibody did not inhibit monocyte adhesion (data not shown). These data suggest that both 12-LO products and glucose may influence monocyte-endothelial interactions through the monocyte integrin receptors α4 and β2.

Discussion

The present study suggests that one mechanism by which chronic glucose exposure stimulates monocyte–endothelial cell interactions is through the 12-LO pathway. We have demonstrated that HAECs conditioned in chronic high glucose produce elevated levels of 12(S)-HETE, the predominant LO metabolite of arachidonic acid in HAECs (Figure 1). Significantly less 15(S)-HETE was produced (data not shown). Our group, as well as others, has reported increased levels of 12(S)-HETE and 15(S)-HETE in glucose-cultured porcine aortic smooth muscle cells13 and endothelial cells,14 respectively. Furthermore, we have shown for the first time that the products of leukocyte-type 12-LO, 12(S)- and 15(S)-HETEs, can induce similar increases in monocyte adhesion to HAECs as seen with glucose alone (Figure 3A). The dose-response relationship of 12(S)- and 15(S)-HETE was similar; however, given the relative levels of the two HETEs in glucose-treated cells, 12(S)-HETE most likely represents the major HETE activator in HAECs. The induction of monocyte adhesion to HAECs by 12(S)- and 15(S)-HETE was specific
and was not a generalized effect of hydroxyeicosanoids, as evidenced by the lack of induction of monocyte adhesion to HAECS by 12(R)-HETE and 5(S)-HETE. Importantly, both glucose and 12(S)- and 15(S)-HETE, at relatively low concentrations, specifically stimulated the binding of monocytes but not neutrophils to HAECS (Figure 3B).

We examined whether monocyte–endothelial cell interactions stimulated by glucose and 12-LO products could be mediated through similar pathways. An important component of leukocyte adhesion is activation and upregulation of adhesion molecules on the endothelial cell surface. Our previous findings showed that chronic exposure of HAECS to glucose did not induce endothelial cell surface expression of VCAM-1, E-selectin, or ICAM-1, the major known adhesion molecules involved in tethering of monocytes to the endothelium.20 Similarly, we now report that 12(S)- and 15(S)-HETE also do not induce these endothelial cell adhesion molecules. This result differs from that of Sultana et al,20 who used human umbilical vein endothelial cells and found upregulation of all these adhesion molecules with 12(S)-HETE. The differences between our studies and those of Sultana et al may be due to the difference in endothelial cell types. Another molecule important in mediating monocyte-endothelial interactions is the fibronectin isoform containing CS-1.19 We showed that exposure of HAECS to high glucose or to 12(S)-HETE induced endothelial cell surface deposition of CS-1 fibronectin as measured by ELISA (Figure 5) and also demonstrated increased filamentous patches of CS-1 fibronectin by immunofluorescence (Figure 6). Studies have shown that CS-1 is a counterreceptor for bronectin by immunofluorescence (Figure 6). Studies have shown that CS-1 fibronectin deposition on HAECs is supported by α5β1 integrin activation on the endothelial surface and that binding of monocytes to HAECs is mediated by VLA-4 on monocytes and CS-1 on the endothelium. Thus, these integrins may also be involved in mediating the 12(S)-HETE effect on CS-1 fibronectin deposition.

We also observed a second mechanism by which 12(S)-HETE stimulated monocyte–endothelial cell interaction. When monocytes were incubated directly with 12(S)-HETE for 12 minutes before the adhesion assay, we found a modest increase (≈60%) in monocyte adhesion to HAECs. The 12-minute time point was chosen to mimic the time period of monocyte incubation with HAECs during the adhesion assay, the only time during which monocytes could possibly be exposed to 12(S)-HETE. These data suggest that part of the increase in 12(S)-HETE– and glucose-induced monocyte adhesion is through direct stimulation of monocytes by released 12(S)-HETE. It is possible that 12(S)-HETE may activate integrins, such as α4β1 or αLβ2, on monocytes to mediate binding to counterligands on the endothelium.

The exact mechanism of action of 12(S)-HETE in mediating monocyte–endothelial cell interactions will require additional studies. Wang et al37 have shown that HETEs can be readily taken up by cells, incorporated into phospholipids, and subsequently hydrolyzed. These data suggest that HETEs may function either as part of the endothelial lipid bilayer or in free form released into the medium. There are other studies that suggest a putative receptor for 12(S)-HETE. High-affinity binding sites have been observed on some cell types,38,39 suggesting a possible role for 12(S)-HETE as a signaling ligand.

Other products of the LO pathway may also be activators of monocyte adhesion. 12(R)-hydroxyeicosatetraenoic acid, a downstream metabolite of 12(S)-HETE,40 can also increase monocyte adhesion at concentrations of 10^{-10} and 10^{-11} mol/L (data not shown). 12(R)-Hydroxyeicosatetraenoic acid can be derived from metabolites of both the 12-LO and cytochrome P450 pathways.41 Furthermore, products of linoleic acid metabolism by leukocyte-type 12-LO may also play a role in mediating inflammatory processes.42 Additional studies will be needed to evaluate the most active lipids generated by the 12-LO pathway.

In summary, our data suggest that one mechanism by which glucose may mediate monocyte–endothelial cell interactions in the artery wall is via the 12-LO pathway. We hypothesize that glucose promotes production of 12(S)-HETE through the 12-LO pathway, which results in stimulation of monocytes and induction of CS-1 fibronectin on the apical surface of HAECS. These molecules interact to form clustered adhesion patches on the endothelial surface, allowing monocytes to firmly adhere to the endothelium. Because the binding of monocytes to the endothelium is a key early event in the development of atherosclerosis, upregulation of this process by vascular cells exposed to chronic elevations in glucose may be one explanation for the accelerated atherosclerosis observed in people with type 2 diabetes.
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