Monocyte and Neutrophil Adhesion Molecule Expression During Acute Hyperglycemia and After Antioxidant Treatment in Type 2 Diabetes and Control Patients

M.J. Sampson, I.R. Davies, J.C. Brown, K. Ivory, D.A. Hughes

Objective—We hypothesized that acute hyperglycemia (an independent cardiovascular risk factor) increases the expression of proatherogenic leukocyte adhesion molecule in type 2 diabetes and controls and that the expression of these adhesion molecules would be antioxidant sensitive.

Methods and Results—Twenty-three type 2 diabetes patients and 13 control patients underwent two oral glucose tolerance tests 14 days apart and took placebo or 800 IU daily of oral alpha tocopherol between tests. Monocyte and neutrophil expression of adhesion molecules Mac-1, LFA-1 and 3, ICAM-1, and VLA-4 were measured at 0, 120, and 240 minutes by using laser flow cytometry. Baseline adhesion molecule expression did not differ between groups, but there was a rapid, highly significant increase (P<0.0001) in the intensity of monocyte Mac-1 expression after a glucose load in both groups. Alpha-tocopherol supplementation reduced only Mac-1 expression in the diabetes group (P<0.03).

Conclusions—Acute glycemic excursions of any degree cause highly significant, rapid increases in monocyte Mac-1 expression in type 2 diabetes patients and controls. Mac-1 mediates leukocyte vascular infiltration and is prothrombotic. These data suggest a mechanism for the link between glycemic excursions and increased vascular event rates. (Arterioscler Thromb Vasc Biol. 2002;22:1111–1117.)

Key Words: type 2 diabetes ■ monocyte ■ adhesion molecule ■ hyperglycemia ■ antioxidants

Monocyte adhesion to the vascular endothelium is one of the necessary first steps in atherogenesis and is mediated by membrane adhesion molecules expressed on leukocytes such as leukocyte function antigen-1 (LFA-1 or CD11a), Mac-1 (CD 11b), and very late antigen 4 (VLA-4) and their respective vascular endothelial ligands, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and connective tissue components such as fibronectin.

The factors regulating expression of these leukocyte and endothelial adhesion molecules are increasingly well understood. In vitro, exposure to high glucose concentrations leads to the upregulation of some adhesion molecules on some cell lines, and this modulation may be antioxidant sensitive. Increased expression of some key adhesion molecules (such as Mac-1) on monocyte-macrophages and neutrophils can occur within minutes through mobilization from an intracellular vesicular storage compartment and may selectively increase in response to cytokines, proinflammatory mediators, or lipid oxidation products through antioxidant-sensitive mechanisms. This expression is of particular interest in type 2 diabetes, which is associated with increased rates of macrovascular disease and vascular events and in which acute or postprandial hyperglycemia may be an independent risk for vascular disease as it is in individuals without diabetes.

We hypothesized that acute hyperglycemia in individuals with type 2 diabetes leads to the increased expression of proatherogenic leukocyte adhesion molecules and that expression of these adhesion molecules during acute hyperglycemia would be influenced by antioxidant supplementation. We conducted a randomized placebo-controlled trial to examine the effects of antioxidant supplementation and acute hyperglycemia on the expression of these adhesion molecules.

Methods

Following local ethical committee approval and after obtaining written informed consent, we studied 23 patients with type 2 diabetes and 13 control patients without type 2 diabetes. (Clinical details on the diabetes and control groups are shown in Table 1 and online at http://atvb.ahajournals.org.)

Patients with type 2 diabetes were nonsmokers between 40 and 70 years of age who were treated either by diet or with oral hypoglycemics. All type 2 patients who were managed by diet alone had a fasting venous plasma glucose >7.0 mmol/L. Patients who had clinically expressed evidence of coronary artery disease (a history of previous myocardial infarction or angiia) or who were receiving
TABLE 1. Clinical Details of the Control and Type 2 Diabetes Groups

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Type 2 Diabetes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>61.5 (5)</td>
<td>60.7 (8.2)</td>
<td>0.6</td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>5:8</td>
<td>10:13</td>
<td></td>
</tr>
<tr>
<td>Diabetes duration, y</td>
<td>–</td>
<td>5.3 (4.1)</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.2 (3.2)</td>
<td>29.1 (3.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.86 (0.1)</td>
<td>0.91 (0.1)</td>
<td>0.07</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>122 (19)</td>
<td>145 (18)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>78 (10)</td>
<td>72 (10)</td>
<td>0.05</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>4.2 (0.8)</td>
<td>8.4 (3.7)</td>
<td>0.0002</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.1 (0.5)</td>
<td>7.4 (1.7)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.4 (0.8)</td>
<td>5.1 (1.2)</td>
<td>0.4</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.4 (0.7)</td>
<td>3.0 (1.1)</td>
<td>0.31</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.2 (0.4)</td>
<td>2.0 (1.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.5 (0.3)</td>
<td>1.1 (0.3)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

All data shown as mean (SD).

hormone replacement therapy, insulin, aspirin, angiotensin-converting enzyme inhibitors, or 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase inhibitors were excluded. Patients with or macroproteinuria were excluded. Medication was withheld on the morning of testing, and all subjects fasted for 15 hours before the testing. Control patients without diabetes were recruited from the general population, fulfilled the same criteria, and all had a fasting plasma glucose <6.1 mmol/L. All subjects were nonsmokers, were nonvegetarian, and took no antioxidant or vitamin supplements.

Study Design

All subjects underwent two glucose tolerance tests 14 days apart (GTT 1 and GTT 2) with 75 g of oral anhydrous glucose at 8:00 AM. Peripheral venous blood samples were taken at 0, 60, 120, and 240 minutes after glucose loading from an indwelling venous cannula. Measurements of monocyte and neutrophil adhesion molecule expression and soluble plasma adhesion molecule concentrations were made at 0, 120, and 240 minutes during each test. (Please see study design details online at http://atvb.ahajournals.org.) The 120-minute time point was chosen because the 2-hour blood glucose concentration after a glucose load is associated with adverse cardiovascular outcomes in type 2 diabetes and control patients, and we therefore were interested in adhesion molecule expression at this epidemiologically important time point. The 240-minute time point was chosen because stimulated adhesion molecule expression in vitro can take several hours to upregulate. Finally, to the best of our knowledge, there are no previous in vivo data on adhesion molecule expression in response to hyperglycemia that could inform selection of time points; also, the 120- and 240-minute time points chosen were reasonable choices biologically. After GTT 1 was completed, patients were randomized to take either oral 800 IU of alpha-tocopherol (AT; Henkel Corporation) or a matched gelatin placebo that contained no AT. (Please see study further details online at http://atvb.ahajournals.org.) This dose of AT was chosen because it is twice the dose we have shown to be insufficient to influence DNA or LDL oxidizability in type 2 diabetes and is the higher dose used in a secondary prevention coronary trial of AT that resulted in some vascular benefit. Twelve type 2 diabetes patients and 7 control patients were randomized to AT, and the remaining subjects were given a placebo. Compliance was assessed by weighed pill returns and by plasma AT concentrations.

Monoclonal Antibodies

Unless otherwise stated, all antibodies were purchased from BD Pharmingen (please see methods online at http://atvb.ahajournals.org). Fluorescein isothiocyanate (FITC)-conjugated CD14 antibody was used to identify monocytes. Phycoerythrin (PE)-conjugated antibodies to CD54 (ICAM-1), CD11a (LFA-1), CD58 (LFA-3), CD49d (VLA-4), and CD11b PE (C3bi) were used to investigate monocyte surface antigen expression. FITC-conjugated mouse IgG1, IgG2a, and IgG2b were used as isotype controls.

Immunofluorescence Staining

Monocytes in whole blood were stained with antibodies to the various surface molecules by a direct immunofluorescence technique (please see methods online at http://atvb.ahajournals.org). Briefly, 50 μL of appropriately diluted monoclonal antibody combinations (CD14 + CD11a, CD11b, CD54, or CD58) were incubated with 50 μL of blood for 15 minutes at room temperature in the dark. Erythrocytes were allowed to lyse for 10 minutes after addition of 2 mL of FACSlyse (diluted 1:10 with distilled water; Becton Dickenson). At the end of this period, further lysis was prevented by adding 2.1 mL of PBS at twice-normal strength. After centrifugation at 1000 rpm for 5 minutes, cells were washed once with PBS containing 1% sodium azide and 1% bovine serum albumin. Stained cells were then fixed with 1% paraformaldehyde and stored at 4°C until data acquisition by flow cytometer.

Flow Cytometric Data Acquisitions and Analysis

Monocyte and neutrophil adhesion molecule expression were measured by flow cytometric analysis and are expressed as mean fluorescence intensity (MFI) values as indirect measures of antigen density (please see methods online at http://atvb.ahajournals.org). Data were acquired on a Beckman Coulter Ultra flow cytometer equipped with a 488-nm ion argon air-cooled laser. 525BP and 575BP interference filters were fitted for collection of FITC and PE emission, respectively. Instrument optical alignment and fluidics were verified by using Flow-Check Fluorospheres (Beckman Coulter) whereas day-to-day variability in instrument settings was monitored and adjusted with the aid of Flow-Set Fluorospheres (Beckman Coulter). For each sample, 10³ events were collected as list mode data at a flow rate of 50 events/second. During analysis, monocytes were identified by their reactivity with CD14-FITC, whereas neutrophils were selected by their distinctive forward and orthogonal light-scatter profile. Membrane adhesion molecule expression was monitored by using the described cell selection strategy. MFI values derived for cells stained with isotype control antibodies were taken as indicators of autofluorescence, non-specific antibody binding, or instrument noise and were subtracted from MFI values obtained through staining for adhesion molecules.
TABLE 2. MFI After a Glucose Load in 13 Controls and 23 Subjects With Type 2 Diabetes

<table>
<thead>
<tr>
<th>Type 2 diabetes</th>
<th>0</th>
<th>120</th>
<th>240</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFA-1</td>
<td>682 (41)</td>
<td>686 (41)</td>
<td>687 (39)</td>
<td>0.84</td>
<td>0.43</td>
</tr>
<tr>
<td>Mac-1</td>
<td>692 (48)</td>
<td>721 (35)</td>
<td>718 (36)</td>
<td>17.1</td>
<td>0.00001</td>
</tr>
<tr>
<td>VLA-4</td>
<td>525 (37)</td>
<td>523 (34)</td>
<td>525 (34)</td>
<td>0.08</td>
<td>0.92</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>514 (24)</td>
<td>518 (27)</td>
<td>517 (28)</td>
<td>1.22</td>
<td>0.30</td>
</tr>
<tr>
<td>LFA-3</td>
<td>546 (33)</td>
<td>548 (37)</td>
<td>543 (36)</td>
<td>0.57</td>
<td>0.56</td>
</tr>
<tr>
<td>Plasma AT</td>
<td>4.2 (0.4)</td>
<td>5.2 (1.2)</td>
<td>3.4 (0.4)</td>
<td>18.0</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

All data are shown as a mean (SD). Adhesion molecule data are shown as relative mean fluorescence intensity values (MFI). F and P values are for repeated measures ANOVA within group.

Plasma AT

Plasma concentrations of AT were measured by using a modified high-performance liquid chromatography (HPLC) method at baseline of GTT 1 and GTT 2. AT concentrations were corrected for plasma total cholesterol.

Plasma-Soluble Adhesion Molecule Concentrations

Plasma-soluble ICAM-1 (s-ICAM), soluble VCAM-1 (s-VCAM), and soluble E-selectin (s-E-selectin) were measured during each GTT at 0, 120, and 240 minutes after glucose loading. Concentrations of each soluble adhesion molecule were quantified by using commercially available ELISA kits (R&D Systems).

Plasma Interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α)

The plasma concentrations of IL-6 and TNF-α were quantified by using commercially available ELISA kits (Quantikine kits, R&D Systems) at 0 and 240 minutes.

Glycemic Control

HbA1c was assessed by using a commercially available kit (Roche Diagnostic systems) on an automated biochemistry analyzer (COBAS MIRA, Roche Diagnostic systems) with the normal range quoted as 4.5% to 5.7%.

Measurement of Plasma Lipid Profiles

Plasma lipid profiles were measured at baseline during each GTT. Lipid profiles were assessed by using commercially available kits (Roche) on an automated biochemistry analyzer (COBAS MIRA, Roche) with estimation of LDL cholesterol.

Statistical Analysis

Data are shown as a mean and one standard deviation, and all variables were normally distributed except for IL-6, which is shown as a median and interquartile range. Differences in individual variables measured more than twice during GTTs were analyzed by repeated-measures one-way ANOVA followed by a paired t test when a significant change was detected on ANOVA. (For details of statistical analysis please see details online at http://atvb.ahajournals.org.) Otherwise, paired t tests or unpaired t tests were used as appropriate, and Mann-Whitney U tests or Wilcoxon signed rank tests were used for nonparametric variables. The impact of AT on repeatedly measured variables during GTT 2 was assessed by using two-way repeated-measures ANOVA (AT versus placebo). Relationships between variables were analyzed by simple linear regression or stepped multiple regression analysis with entry at P<0.1. Data were analyzed on commercially available statistical software (Statview 4.0 for Windows; Adept Scientific).

Results

Baseline Adhesion Molecule Expression and Soluble Adhesion Molecule Concentrations

Between 98% and 100% of all monocyte and neutrophil populations expressed all the selected adhesion molecules, and this did not differ between groups (Tables 2 and 3). The intensity (MFI) of monocyte and neutrophil adhesion molecule expression did not differ significantly between groups at baseline. Monocyte LFA-3 expression was significantly lower in the diabetes group (464 [33] versus 571 [23]; P=0.015) as was neutrophil LFA-3 expression (450 [31] versus 474 [22]; P=0.01). Plasma s-ICAM-1 and s-VCAM-1 concentrations were significantly higher in the type 2 diabetes group (282 [60] ng/mL versus 237 [74] ng/mL, P=0.04; and 403 [84] ng/mL versus 333 [73] ng/mL, P=0.02, respectively), but there was no significant difference in plasma s-E-selectin (P=0.07; Table 3). Plasma IL-6 was significantly higher in the diabetes group at baseline (1.55 [1.7] pg/mL versus 0.94 [2.2] pg/mL; P=0.01), but TNF-α concentrations did not differ significantly between groups (P>0.1; Table 3). The only significant relationship between any soluble adhesion molecule concentration and any leukocyte adhesion molecule expression was between s-VCAM-1 and monocyte VLA-4 expression in the diabetes groups alone (r=0.45; P=0.03).

Plasma AT Concentrations

In the diabetic group, corrected plasma AT concentrations increased by 45% in the AT-supplemented subgroup (from 2208 [412] μmol/L to 3192 [644] μmol/L; P<0.0001) after 8
weeks of AT supplementation, when the latter value was significantly higher than in the matched placebo group (2245 [663] μmol/L; \( P = 0.0018 \)). In the control group, plasma AT concentrations increased by 58% in the AT-supplemented subgroup (from 1825 [202] μmol/L to 2874 [333] μmol/L; \( P < 0.0001 \)) after 8 weeks of AT supplementation, whereas the latter value was significantly higher than in the matched placebo group (2025 [238] μmol/L; \( P = 0.0004 \)).

Monocyte and Neutrophil Adhesion Molecule Expression After a Glucose Load
In the diabetes group, there was a highly significant (\( P < 0.00001 \)) and rapid increase in the MFI of monocyte Mac-1 expression after a glucose load (Table 2). In the control group, there was a highly significant (\( P < 0.0001 \)) and rapid increase in monocyte Mac-1 expression and a borderline-significant increase in monocyte LFA-1 and ICAM-1 intensity of expression (both \( P = 0.044 \)). There was no significant change in neutrophil MFI of any adhesion molecule after a glucose load (not shown). These highly significant changes in Mac-1 expression also occurred in the placebo-treated subgroup during GTT 2 in both the control patients (\( F = 6.2; \ P = 0.01, n = 6 \)) and in the type 2 diabetes groups (\( F = 11.0; \ P = 0.0003; n = 11 \)). There was no significant relationship between Mac-1 MFI at 120 minutes or change in Mac-1 MFI and plasma glucose concentrations at or increment in plasma glucose (all \( r > 0.2; \ P > 0.5 \)). Similarly, there were no significant relationships between Mac-1 MFI at 120 minutes or change in Mac-1 MFI and plasma triglycerides, TNF-α, or IL-6 at any time point.

Plasma-Soluble Adhesion Molecules, IL-6, and TNF-α Concentrations After a Glucose Load
There was a highly significant decrement in plasma s-ICAM-1 and s-VCAM-1 in the diabetes group and the control group (Table 3), although the change s-E-selectin concentrations was at borderline significance in the diabetes group and not significant in the control group (Table 3). TNF-α concentrations did not change significantly after a glucose load; however, IL-6 concentrations increased significantly in both type 2 diabetes and control groups after a glucose load (Table 3).

### AT Supplementation and Monocyte Adhesion Molecule Concentrations, Plasma TNF-α, and IL-6
After 8 weeks of AT supplementation, the only difference in adhesion molecule expression at baseline of GTT 2 was in the monocytes in the type 2 diabetes group, whereas the AT-supplemented subgroup had a significantly lower Mac-1 intensity of expression compared with the placebo-treated subgroup (688 [30] versus 715 [19]; \( P = 0.03 \)). AT- and placebo-treated subgroups did not otherwise differ at the baseline of GTT 2 in either group. Two-way repeated-measures ANOVA demonstrated no significant effect of AT compared with placebo in any adhesion molecule expression (all \( P > 0.1 \)) in either group after a glucose load, although there was a borderline effect in suppressing monocyte Mac-1 expression in the diabetes group alone (\( F = 3.57; \ P = 0.07 \)). AT supplementation had no significant effect on changes in soluble adhesion molecule concentrations during GTT 2 compared with controls (all \( P > 0.3 \)), and baseline values for soluble adhesion molecule concentrations did not differ after 8 weeks of AT supplementation (all \( P > 0.1 \); data not shown).

AT supplementation had no significant effect on changes in IL-6 concentrations during GTT 2 compared with placebo (all \( P > 0.3 \)); and baseline values for plasma IL-6 and TNF-α concentrations at GTT 2 did not differ after 8 weeks AT supplementation between AT- and placebo-treated groups (all \( P > 0.1 \); data not shown).

### TABLE 3. Change in Plasma Soluble Adhesion Molecule, Triglyceride, IL-6, and TNF-α Concentrations During GTT1 in 23 Subjects With Type 2 Diabetes and in13 Controls

<table>
<thead>
<tr>
<th>Type 2 diabetes</th>
<th>0</th>
<th>120</th>
<th>240</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1, ng/mL</td>
<td>282 (60)</td>
<td>242 (71)</td>
<td>265 (56)</td>
<td>0.0001</td>
</tr>
<tr>
<td>VCAM-1, ng/mL</td>
<td>403 (84)</td>
<td>366 (83)</td>
<td>372 (96)</td>
<td>0.003</td>
</tr>
<tr>
<td>E-Selectin, ng/mL</td>
<td>73 (38)</td>
<td>69 (35)</td>
<td>72 (36)</td>
<td>0.046</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>0.69 (0.6)</td>
<td>–</td>
<td>0.80 (0.7)</td>
<td>0.8</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>1.55 (1.7)</td>
<td>–</td>
<td>2.85 (3.3)</td>
<td>0.05</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.0 (1.2)</td>
<td>1.91 (0.9)</td>
<td>–</td>
<td>0.8</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICAM-1, ng/mL</td>
<td>237 (74)</td>
<td>213 (50)</td>
<td>231 (57)</td>
<td>0.03</td>
</tr>
<tr>
<td>VCAM-1, ng/mL</td>
<td>333 (73)</td>
<td>302 (63)</td>
<td>323 (84)</td>
<td>0.02</td>
</tr>
<tr>
<td>E-Selectin, ng/mL</td>
<td>53 (15)</td>
<td>51 (12)</td>
<td>52 (14)</td>
<td>0.15</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>0.64 (0.6)</td>
<td>–</td>
<td>0.53 (0.6)</td>
<td>0.8</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>0.94 (2.2)</td>
<td>–</td>
<td>1.56 (2.7)</td>
<td>0.03</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.24 (0.45)</td>
<td>–</td>
<td>1.13 (0.47)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

All data are shown as a mean (SD). \( P \) values are for repeated measures ANOVA within group.
Discussion
Monocyte and neutrophil adhesion to the intact or damaged vascular endothelium is mediated by integrin receptors with a shared β2 subunit but a unique α subunit.1,2 On leukocytes, these include LFA-1 (CD11a) and Mac-1 (CD11b) and their endothelial ligand ICAM-1, although Mac-1 also has nonendothelial matrix ligands.1,2 In addition, VLA-4 is a monocyte ligand that promotes monocyte adherence to endothelial VCAM-1 and to connective tissue components, particularly the connecting segment part of fibronectin.23 The most significant finding in this study was a rapid and highly significant increase in the monocyte expression of the adhesion molecule Mac-1 in both type 2 diabetes patients and controls after an oral glucose load. Postprandial hyperglycemia (or glycemic excursions within the normal range) are independent predictors of cardiovascular events in groups with or without diabetes,12–14 and much of the controversy over the diagnostic classification of diabetes was based on the predictive power of postglucose load hyperglycemia as a marker for vascular risk.14 The present findings suggest one pathway for an independent association between glycemic excursions and vascular risk through increased monocyte Mac-1/endothelial cell ICAM-1 interactions in both type 2 diabetes patients and controls.

The mechanism of Mac-1 cell surface expression is unusual because it can increase rapidly as a result of the translocation of stored receptor to the cell surface,4–8 a process not available to other adhesion molecules, such as LFA-1,2 and that occurs in both monocytes and neutrophils.6 Our observation of a highly significant increase in the membrane expression of monocyte Mac-1 within 120 minutes during glycemic excursions would be compatible with increased translocation of stored Mac-1 to the cell surface rather than increased Mac-1 mRNA and protein synthesis. Mac-1 expression in vitro on a number of cell lines is increased in response to high glucose concentrations, oxidatively modified lipoproteins, fatty acid oxidation products such as 8-epi F2α isoprostane, cytokines and proinflammatory mediators such as interleukins, TNF-α and TNF-β, monocyte chemokines such as MCP-1, and Mn2+ ions,14–8,24–31 but it is unclear which mechanism(s) are responsible for the Mac-1 changes in both groups. The most obvious candidate contributing to these changes is changes in plasma glucose, and monocyte Mac-1 expression is increased in response to substantial hyperglycemia mediated through a protein kinase C pathway.32 The lack of a direct relationship between the degree of plasma glucose excursion and increased Mac-1 expression, in addition to the equivalent increase in Mac-1 expression in both diabetic and control groups, could be accounted for by a pattern of “all or none” Mac-1 translocation in response to any glycemic excursion irrespective of basal plasma glucose. There are little available data on acute changes in plasma interleukin, TNF-α, or MCP-1 after a glucose load in control or type 2 diabetes patients that would link these variables to Mac-1 expression, and as far as we aware, no data that indicate a role for changes in insulin, C-peptide, or proinsulin in promoting Mac-1 expression. However, the increase in IL-6 in both groups after glucose loading is of interest because IL-6 can promote Mac-1 expression in vitro in some cell lines,33 and the present finding could reflect increased monocyte activation in response to glycemic excursions, resulting in parallel increases in monocyte CD11b expression and IL-6 production or a direct effect of IL-6 (or other unmeasured cytokines) on CD11b expression. Others34 have shown that increased diabetic monocyte adherence to the endothelium is associated with increased monocyte cytokine production. We have shown in other studies that a glucose load in type 2 diabetes patients leads to increased free radical generation of the arachidonic acid oxidation product plasma 8-epi F2α isoprostane,35 which has been shown in vitro to directly and indirectly increase expression of leukocyte Mac-1.29

Plasma LDL oxidizability, or LDL oxidation products, were not measured in this study, but we and others18,36 have shown that LDL oxidizability does not differ between controls and type 2 diabetes patients without macrovascular disease and that acute hyperglycemia after a glucose load does not increase plasma LDL oxidizability in type 2 diabetes patients or control patients.37 Although oxidatively modified LDL can promote Mac-1 expression in vitro from a variety of cell lines,38 changes in LDL oxidizability or oxidation after a glucose load would not account for the rapid increase in CD11b expression seen in the present study. Others9 have shown that Mac-1 expression on monocytes and neutrophils is reduced by AT treatment, although it is unclear whether this expression is mediated through an antioxidant effect of AT or through other pathways such as protein kinase C activation. Devaraj and Jialal39 have shown that monocytes from type 2 diabetes patients show reduced endothelial adhesion and also IL-1 and TNF-α release when exposed to 1200 IU AT for 3 months and that this dose also reduced plasma LDL oxidizability in type 2 diabetes patients. Lower doses of AT (between 400 and 800 IU) in this and other studies have not had these effects on LDL oxidizability, monocyte function, or plasma TNF-α and interleukin levels in plasma in type 2 diabetes patients,18,39 and dose-response studies may be necessary to determine the threshold for AT effects on these variables.38 Monocyte Mac-1 also functions as a link between cellular adhesion and thrombosis because monocyte Mac-1 can activate coagulant cascades through activation of factor X40 and by increased monocyte expression of the procoagulant tissue factor after binding to fibrinogen41; increased procoagulant activity during glycemic excursions resulting from acute increases in monocyte Mac-1 expression might also contribute to an association between acute hyperglycemia and vascular event rates and coronary restenosis after angioplasty.42–45

There is no evidence that Mac-1 expression is regulated differently in monocytes and neutrophils; however, Mac-1 expression did not increase in neutrophils. It is possible that Mac-1 expression increased in neutrophils more rapidly than in monocytes and that this was not detected at the first sampling point at 120 minutes. In addition, there is some limited evidence that neutrophil expression of Mac-1 can upregulate more rapidly than in monocytes46,47, and that neutrophils expressing increased Mac-1 are cleared more rapidly than monocytes,48 which could account for the present findings. There was a slight increase (P=0.044) in the
expression of monocyte LFA-1 and ICAM-1 in the controls after a glucose load, but it is unclear what mechanisms may account for this, and these adhesion molecules do not seem to be stored submembranously as is Mac-1.

Plasma-soluble adhesion molecules are derived by the cleavage of the membrane-bound adhesion molecule at the point of membrane insertion,49 and in this study there was no significant or consistent relationship between the intensity of leukocyte adhesion molecule expression and the plasma-soluble forms of these adhesion molecules or their ligands. We have shown in other studies in type 2 diabetes36 that acute hyperglycemia leads to a rapid decrement in plasma concentrations of s-ICAM-1, s-VCAM-1, and s-E-selectin (as occurred in the present study) and that this could be due to suppression of release mechanisms or increased hepatic clearance after a glucose load associated with increased liver blood flow.50,51 Our findings on AT and plasma-soluble adhesion molecule concentrations in this study differ from those of Jialal and Devaraj,38 who found that 1200 IU of AT did reduce plasma-soluble adhesion molecule concentrations; this may reflect the lower dose used in the present study and reinforces the need for a dose titration study of AT effects on the variables.

To the best of our knowledge, the lower LFA-3 expression in monocytes and neutrophils derived from subjects with type 2 diabetes has not been described previously. LFA-3 is a surface membrane glycoprotein involved in antigen-presenting functions and T cell-target cell interactions. Lower LFA-3 expression might contribute to poorer immunological surveillance, but it is unclear what mechanisms might underlie this observation. Finally, the type 2 diabetes group was characterized by a higher mean systolic blood pressure than the controls, which may reflect increased arterial stiffness in these patients and indicate increased prevalences of insulin resistance, endothelial dysfunction, and occult atherosclerotic disease52,53 so that the groups were unlikely to be identical in these variables at baseline, although this does not detract from the observation of increased Mac-1 expression after a glucose load in both groups.

We have shown that glycemic excursions in individuals with and without type 2 diabetes led to a rapid and highly significant increase in the expression of monocyte Mac-1, a key leukocyte adhesion molecule that promotes leukocyte adherence to normal and damaged vascular endothelium and is a link between cellular adhesion, atherogenesis, and thrombosis. These observations suggest a pathway between glycemic excursions and increased vascular event rates in people with and without type 2 diabetes.

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Monocyte and Neutrophil Adhesion Molecule Expression During Acute Hyperglycemia and After Antioxidant Treatment in Type 2 Diabetes and Control Patients

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