Effect of Increased Concentration of D-Glucose or L-Fucose on Monocyte Adhesion to Endothelial Cell Monolayers and Activation of Nuclear Factor-κB

Mark A. Yorek and Joyce A. Dunlap

Increased adhesion of monocytes to endothelial cells has been linked to the development and progression of atherosclerosis in humans with diabetes. Previous studies have shown that increased concentration of glucose and subsequent generation of reactive oxygen species and the activation of the transcription factor nuclear factor-κB (NF-κB) may mediate this response. However, our studies have shown that in addition to glucose, other monosaccharides, such as L-fucose, which is increased in circulation in diabetes, may also contribute to the development of diabetic complications. In these studies, we examined the effect of an increased concentration of L-fucose on monocyte adhesion to cultured bovine aorta endothelial cells. Exposing cultured bovine aorta endothelial cells to an increased concentration of either glucose or L-fucose caused a concentration-dependent increase in adhesion of monocytes. The increase in monocyte adhesion induced by glucose or L-fucose was preceded by the activation to NF-κB and the generation of reactive oxygen species. The combination of glucose and L-fucose at a submaximal concentration did not appear to have an additive effect on the induction of monocyte adhesion. The addition of α-lipoic acid partially prevented the glucose and L-fucose–induced activation of NF-κB, generation of reactive oxygen species, and increase in monocyte adhesion. This suggests that the effect of an increased concentration of glucose or L-fucose on monocyte adhesion to endothelial cells is at least partially due to the production oxygen-derived free radicals. Furthermore, these studies provide evidence that monosaccharides other than glucose that are increased in the circulation of humans with diabetes may contribute to vascular defects in diabetes.

DIABETES MELLITUS IS associated with an increased risk of atherosclerosis. Endothelial dysfunction, which precedes the development of atherosclerotic lesions in diabetic patients, includes accelerated disappearance of capillary endothelium, weakening of intercellular junctions, altered protein synthesis, and the appearance of specific adhesive glycoproteins on endothelial cells, promoting local attachment of monocytes and leukocytes, as well as their transendothelial migration.[1,2] In diabetic patients, endothelial adhesion molecule expression is increased in the aorta, and increased plasma levels of vascular cell adhesion molecule-1 are considered to be a marker for vascular dysfunction and progressive vascular disease.[3-5] The mechanisms leading to the increase in expression of adhesion molecules and adherence of monocytes and leukocytes to the endothelium in diabetes is not well understood. Studies with cultured endothelial cells have demonstrated that elevated glucose levels promote adhesion molecule expression and monocyte adhesion.[2,6-10] Additional studies have suggested that the activation of the transcription factor nuclear factor-κB (NF-κB) and stimulation of protein kinase C may mediate the effects of glucose on endothelial cells.[10,11] The effects of glucose on endothelial cells may also be mediated by the generation of reactive oxygen species.[12] Tesfamariam and Cohen[12] have reported that free radicals mediate endothelial cell dysfunction caused by glucose. Mattia et al[13] reported that reduction of oxidative stress by oral administration of N-acetyl-L-cysteine reduced plasma soluble vascular cell adhesion molecule-1 in diabetic patients. Lastly, advanced glycation end product stimulation of expression of vascular cell adhesion molecule-1 and endothelial adhesion of human monocytes is reduced by α-lipoic acid.[14] Diabetes has also been shown to cause an activation of monocytes leading to increased binding to endothelial cells.[15,16] Diabetes/hyperglycemia-mediated activation of monocytes has been shown to be inhibited by α-tocopherol and possibly mediated by activation of protein kinase C.[15,16]

In addition to the effects of hyperglycemia, we have reported that the monosaccharide L-fucose, like glucose, alters endothelial cell function including collagen and proteoglycan synthesis.[17,18] L-fucose is the only levorotatory monosaccharide synthesized and utilized by mammalian tissues and is a constituent of the carbohydrate chains of glycoproteins and glycolipids.[19,20] Furthermore, Radhakrishnamurthy et al[21] have reported that unbound L-fucose levels in serum increases from 0.5 mg/dL in nondiabetics to as high as 4.0 mg/dL (mean, 2.7 mg/dL) in diabetic patients. Feeding rats a diet containing 10% to 20% L-fucose, which reproduced the same circulatory level of unbound L-fucose in rats that occurs in circulation of humans with diabetes while maintaining normal glycemia, caused a deficit in the myoinositol content of the sciatic nerve, a decrease in sciatic nerve Na^+/K^+ adenosine triphosphatase (ATPase) activity and a reduction in motor nerve conduction velocity (MNCV) that was similar to the deficits observed in streptozotocin-induced diabetic rats.[22,23] Nerve morphology has been shown to be altered in both rodent and human diabetic neuropathy.[24-26] In L-fucose–fed rats, following 24 weeks of a L-fucose–enriched diet, significant axonal atrophy, paranodal swelling, and paranodal demyelination in sural nerves was

From the Department of Internal Medicine, Diabetes-Endocrinology Research Center and Veterans Affairs Medical Center, University of Iowa, Iowa City, IA.

Submitted April 11, 2001; accepted July 20, 2001.

Supported by a Diabetes Center grant from the Veterans Affairs and International Juvenile Diabetes Foundation and by Grant No. DK-25295 to the Diabetes-Endocrinology Research Center grant from the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

Address reprint requests to Mark A. Yorek, PhD, 3 E 17 Veterans Affairs Medical Center, Iowa City, IA 52246.

0026-0495/02/5102-0001$35.00/0

doi:10.1053/meta.2002.29958

observed.[27] These studies suggest that an increased concentration of unbound L-fucose in diabetes may also contribute to diabetic complications affecting vascular or nerve tissue.

In mammals, L-fucose is produced from D-glucose or by the conversion of guanosine 5′-(D-mannosyl diphosphate) (GDP-mannose) to guanosine 5′-(L-fucosyl diphosphate) (GDP-fucose).[20,28] Catabolically, an oxidative pathway for L-fucose metabolism has been described in ovine liver.[29] In this pathway, L-fucose is enzymatically converted to L-lactate. However, when administered orally, little L-fucose is oxidized to CO_2.[30] In rats following parenteral administration of ^{14}C-L-fucose, about 30% of the radioactivity appeared in the urine as free sugar and less than 2% was recovered as $^{14}CO_2$ after 10 hours.[31,32] The majority of the remaining radioactivity was found to be associated with glycoproteins or as free sugar in circulation. Both endogenous and parenterally administered free L-fucose can be incorporated into glycoproteins with no label appearing in other sugars.[33] L-fucose is found at the terminal or preterminal positions of many cell-surface oligosaccharide ligands that mediate cell-recognition and adhesion-signaling pathways. These include such normal events as early embryologic development and blood group recognition and pathologic processes, including inflammation, infectious disease recognition, and neoplastic progression. A recent study indicates that L-fucose provides the essential structure that enables carbohydrate ligands to bind to selectins and thereby may alter cellular homeostasis.[34] Therefore, L-fucose is a unique sugar that has a variety of important roles in mammals. In these studies, we sought to determine the effect of an increased concentration of unbound L-fucose on cultured endothelial cells. The hypothesis being addressed is that unbound L-fucose at a concentration that occurs in the circulation of diabetic patients induces oxidative stress independent of hyperglycemia and may alter endothelial cell properties and thereby contribute to vascular dysfunction.

MATERIALS AND METHODS

Materials

Chemicals were from Sigma (St Louis, MO) unless otherwise noted. Corning 75-cm^2 flasks and Falcon 6-well plates were from Fisher Scientific (Fair Lawn, NJ). [α-^{32}P] deoxyadenosine triphosphate (dATP) and ^{51}Cr were from Amersham (Arlington Heights, IL). Safety-Solve and scintillation vials were from RPI (Mount Prospect, IL). Dihydrorhodamine 123 was obtained from Molecular Probes (Eugene, OR). Tumor necrosis factor (TNF)-α was purchased from Intergen (Purchase, NY). Earle's balanced salt solution was obtained from GibcoRRL (Grand Island, NY). Media were obtained from the Diabetes Endocrinology Research Center, University of Iowa (Iowa City, IA).

Cell Culture

Bovine aortic endothelial cells (BAE) originated from freshly slaughtered steers and were grown in Dulbecco's Minimal Essential Medium supplemented with 10% heat inactivated fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 294 μg/mL glutamine as previously described.[35] The cells were propagated in Corning 75 cm^2 flasks in an incubator maintained at 37°C with 5% CO_2 in humidified air. Cells were passed weekly at a dilution of 1:10 and fed 3 times/week by replacing the medium. The medium used to culture the cells was obtained from the University of Iowa Diabetes Endocrinology Research Center. The water used to make the medium and buffers used in these studies was tested weekly using the *Limulus* amebocyte lysate test and was known to be free of endotoxins. The cells used in these studies were between passages 6 to 12. For monocyte adhesion and determination of reactive oxygen species, the cells were grown in 6-well plates and studies conducted in triplicate. These studies were conducted over a period of time and a minimum of 3 different cultures of cells was used.

Monocyte Adhesion

To characterize the effect of hyperglycemia or increased concentration of L-fucose on monocyte adhesion to cultured BAE cells, human monocytic U937 cells were labeled with ^{51}Cr and the adhesion assay conducted as described by DiCorleto and de la Motte.[36] Briefly, cultured BAE cells were grown to near confluency and then incubated overnight in Earle's balanced salt solution containing 1% fetal bovine serum and 5 to 25 mmol/L glucose and/or 0 to 1 mmol/L L-fucose with or without 1 mmol/L α-lipoic acid. As a positive control, cells were also treated overnight with 10 ng/mL TNF-α. Studies using raffinose (25 mmol/L) as an osmotic control demonstrated no effect on monocyte adhesion or activation of NF-κB (data not shown). Afterwards, the cells were washed and monocyte adhesion determined as described.[36] Data for adhesion was calculated as the number of monocytes attached/10^4 BAE cells and reported as percent of control. Cultured BAE cells were 1 of the types of endothelial cells used by DiCorleto and de la Motte[36] to characterize human monocyte adhesion to endothelial cells.

Electrophoretic Mobility Shift Assay

BAE cells were incubated in Earle's balanced salt solution containing 1% fetal bovine serum and 5 to 25 mmol/L glucose or 0 to 1 mmol/L L-fucose for 1 to 6 hours. For a positive control, cells were also incubated in this medium containing 10 ng/mL TNF-α. To determine the effect of reactive oxygen species on NF-κB activation by increased glucose of L-fucose concentration, cells were preincubated with 1 mmol/L α-lipoic acid for 1 hour prior to the addition of 10 mmol/L glucose or 0.5 mmol/L L-fucose to the medium. Following these incubations, cells were washed and harvested using phosphate-buffered saline at 4°C and low speed centrifugation. The cells were then resuspended in 1.5 mL of buffer A (10.0 mmol/L HEPES, pH 8.0, 1.5 mmol/L $MgCl_2$, 10.0 mmol/L KCl, 0.5 mmol/L dithiothreitol, 300 mmol/L sucrose, 0.1% Nonidet P-40, 1 μg/mL of pepstatin, antipain, chymostatin, and aprotinin, 0.1 μg/mL leupeptin, and 0.5 mmol/L phenylmethylsulfonyl fluoride) and incubated on ice for 5 minutes. The crude nuclear pellet was then collected by microcentrifugation for 2 minutes at 4°C. Afterwards, the pellet was quickly washed with buffer A and resuspended in buffer B (20 mmol/L HEPES, pH 8.0, 20% glycerol, 100 mmol/L KCl, 100 mmol/L NaCl, 0.2 mmol/L EDTA, 0.5 mmol/L phenylmethylsulfonyl fluoride, 0.5 mmol/L dithiothreitol, 1 μg/mL of pepstatin, antipain, chymostatin, and aprotinin, and 0.1 μg/mL leupeptin). The isolated nuclei were sonicated for 10 seconds at 4°C and clarified by microcentrifugation. Protein concentration of the extract was determined, and the extract was stored at -70°C until assayed. For gel mobility shift assays, annealed oligonucleotides containing the consensus sequence for NF-κB (5'-TTTCGCGGGGACTT-TCCCGCGC-3′; 5′-TTTGCGCGGGAAAGTCCCCGCG-3′) and the E-box of the adenovirus major late transcription factor promoter (5′-ATAGGTGTAGGCCACGTGACCGGGTGT-3′; 5′-ACACCCGGT-CACGTG-3′) were radiolabeled with [α-^{32}P]dATP and unlabeled deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), and deoxythymidine triphosphate (dTTP) using Klenow DNA polymerase and gel purified as previously described.[35] Fifteen micrograms of nuclear extract protein were preincubated for 10 minutes at 25°C with 1 μg poly (dIdC) under ionic conditions. Radiolabeled probe

(5×10^4 cpm, $\approx$ 2 ng) was added to each 20-μL reaction and incubated for 15 minutes at 37°C. Samples were analyzed on a 5% nondenaturing polyacrylamide gel in 0.5X tris(hydroxymethyl)aminomethane-borate-EDTA (45 mmol/L tris (hydroxymethyl)aminomethane-borate, 1 mmol/L EDTA, pH 8.0) and electrophoresed at 115 V for 3 hours at 25°C. Gels were then dried, and autoradiographs were exposed for the appropriate period at -70°C with intensifying screens.

Determination of Reactive Oxygen Species Generation Using Dihydrorhodamine 123

Increased intracellular generation of reactive oxygen species by the vascular endothelium has been proposed as a mechanism of tissue injury in a variety of pathologic processes including inflammation, ischemia-reperfusion injury, and hyperoxia.[37] Hydrogen peroxide is an important reactive oxygen species in this regard. To determine whether hyperglycemia or an increased concentration of L-fucose may be causing the generation of reactive oxygen species in cultured endothelial cells, we used dihydrorhodamine 123 to measure intracellular hydrogen peroxide.[38] BAE cells were grown in 6-well plates to confluence. Afterwards, the cells were preincubated with 5 μmol/L dihydrorhodamine 123 in serum-free and phenol red-free Medium 199 for 1 hour. Stock solutions of dihydrorhodamine, 28.9 mmol/L, were prepared in dimethylformamide. Following this incubation, the cells were washed with 10 mmol/L HEPES buffer and incubated for 1 hour in Earle's balance salt solution containing 1% fetal bovine serum and 15 mmol/L glucose (final concentration) or 0.5 mmol/L L-fucose. Afterwards, the medium was removed, the cell monolayer washed with HEPES buffer, and 2 mL of sonication buffer (50 mmol/L potassium phosphate, 0.1 mmol/L EDTA, and 0.1% (3-[(3-cholamidopropyl)-dimethyl-ammonia]-1-propane sulfanate) [CHAPS], pH 7.0) added, and the cells collected by scraping and lysed by sonication. Cells incubated for 1 hour in Earle's balanced salt solution with no additions (basal glucose concentration 5 mmol/L) were used to determine basal levels of rhodamine 123. For comparison, some cells were incubated in medium containing 10 ng/mL TNF-α. The cell lysate concentration of rhodamine 123 was determined by measuring the peak excitation and emission wavelengths at 500 and 536 nm, respectively. Background fluorescence and basal rhodamine 123 levels expressed in arbitrary fluorescence units were subtracted from the values obtained with cells incubated in normal or experimental medium. Generation of reactive oxygen species was expressed as nanomole/milligram protein.

Data Analysis

Significance of differences was determined by analysis of variance (ANOVA) and unpaired Student's *t* test and Dunnett's procedure.

RESULTS

Effect of D-Glucose or L-Fucose on NF-κB Activation and Monocyte Adhesion

To determine the effect of an increased concentration of D-glucose or L-fucose on NF-κB activation, BAE cells were incubated for 1 to 6 hours in Earle's balanced salt solution (basal glucose concentration, 5 mmol/L) containing 1% fetal bovine serum and 15 mmol/L D-glucose (final concentration) or 0.5 mmol/L L-fucose. For a positive control, cells were also incubated in medium containing 10 ng/mL TNF-α. Data in Fig

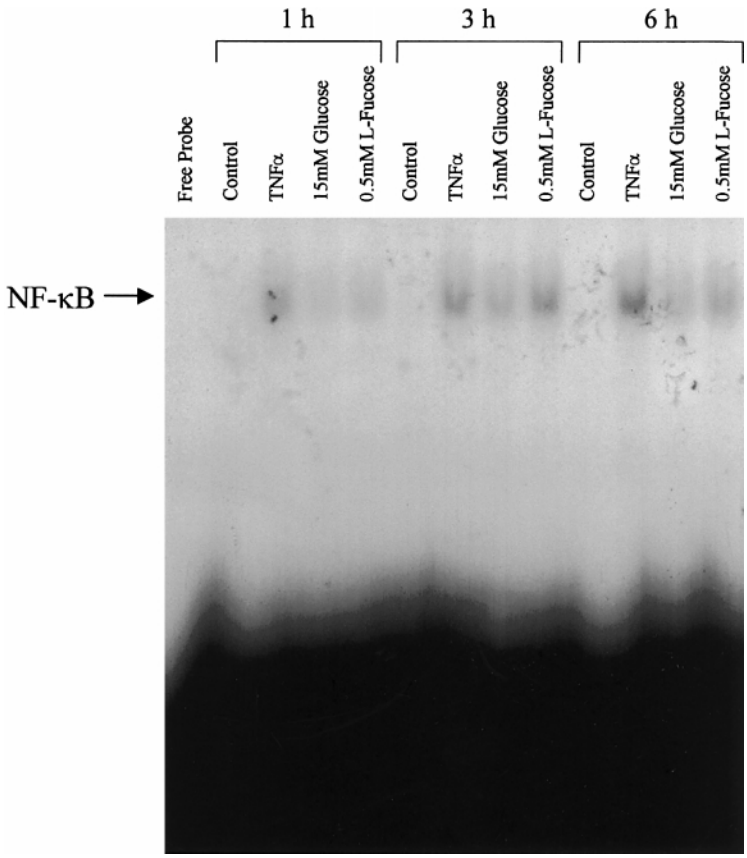

Fig 1. Effect of TNF-α, glucose, or L-fucose on NF-κB activation in cultured BAE cells. Cells were incubated in Earle's balanced salt solution for 1, 3, or 6 hours containing 1% fetal bovine serum plus 10 ng/mL TNF-α, 15 mmol/L glucose (final concentration) or 0.5 mmol/L L-fucose. After these incubations, nuclear extracts were prepared and gel mobility shift assays performed as described in Materials and Methods. The figure presents a representative autoradiograph from a single experiment that was repeated at least 4 times with similar results.

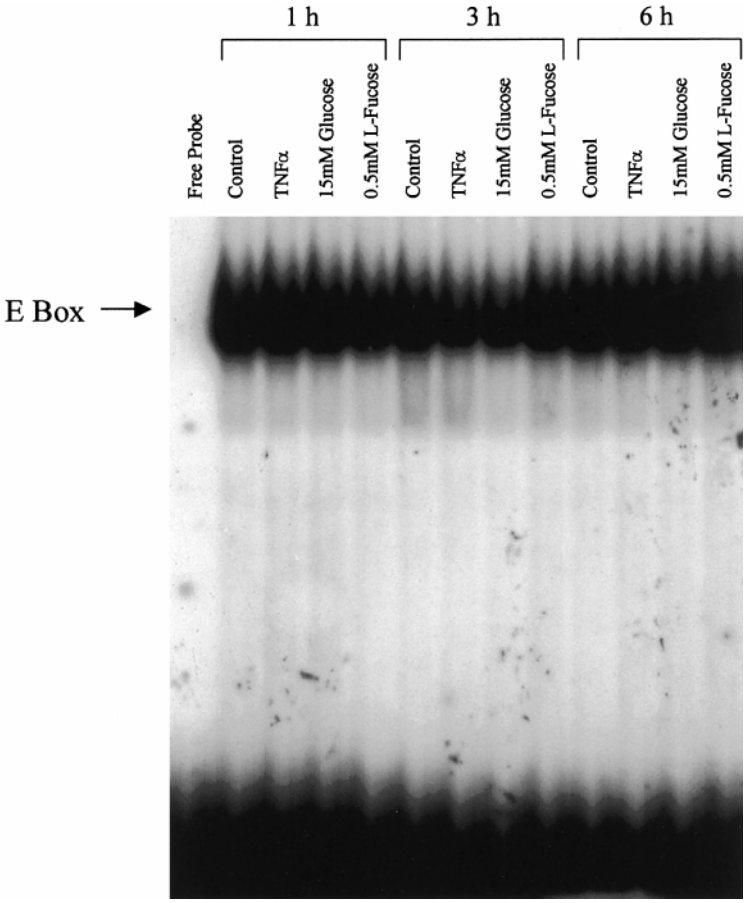

Fig 2. Effect of TNF-α, glucose, or L-fucose on the electrophoretic mobility shift assay for the adenovirus major late transcription factor promoter (E-box) in cultured BAE cells. Cells were incubated and nuclear extracts prepared as described in Fig 1. Afterwards, gel mobility shift assays for E-box were performed as described in Materials and Methods. The figure presents a representative autoradiograph from a single experiment that was repeated at least 4 times with similar results.

1 demonstrate that maximum activation of NF-κB is obtained following a 3-hour incubation. Data in Fig 2 demonstrate that DNA protein binding for an unrelated probe containing the consensus sequence for the E-box of the adenovirus major late transcription factor promoter was not affected by exposing the cells to 15 mmol/L glucose, 0.5 mmol/L L-fucose, or TNF-α. Based on these results, we decided to use a 3-hour incubation for examining NF-κB activation for the remaining studies.

To determine the effect of increased concentration of D-glucose or L-fucose on NF-κB activation and monocyte adhesion, BAE cells were incubated in Earle's balanced salt solution containing 1% fetal bovine serum and supplemented with 0.01 to 1.0 mmol/L L-fucose or 5 to 20 mmol/L D-glucose. As a positive control for the monocyte adhesion studies, cells were also incubated in medium containing 10 ng/mL TNF-α. Data in Fig 3 demonstrate that exposing cultured BAE cells to 0.5 mmol/L L-fucose or 15 mmol/L D-glucose (final concentration) for 3 hours causes a maximum increase in NF-κB activation. In addition, data in Fig 3 demonstrate that after a 16-hour incubation, increasing the concentration of L-fucose causes a concentration-dependent significant increase in monocyte adhesion. Incubating cultured BAE cells with 0.01 mmol/L L-fucose caused a 2-fold increase in monocyte adhesion. An 8-fold stimulation in monocyte adhesion was observed following an incubation with 1.0 mmol/L L-fucose. The stimulation of monocyte adhesion obtained with 1.0 mmol/L L-fucose was similar to the stimulation of monocyte adhesion observed with TNF-α. Exposing cultured BAE cells to 10, 15, or 25 mmol/L D-glucose also caused a concentration-dependent significant increase in monocyte adhesion compared with cells incubated in control medium containing 5 mmol/L D-glucose. However, the stimulation of monocyte adhesion obtained with hyperglycemia was less than observed following an incubation with L-fucose. In separate studies, exposing cells for 16 hours to 25 mmol/L raffinose as an osmotic control did not effect NF-κB activation or monocyte adhesion (data not shown).

Data in Fig 4 demonstrate that although there is a small increase in monocyte adhesion when cultured BAE cells were incubated in medium containing 0.01 and 10 mmol/L L-fucose and D-glucose, respectively, or 0.1 and 10 mmol/L L-fucose and D-glucose, respectively, the combined effects of L-fucose and D-glucose on monocyte adhesion do not appear to be additive under the conditions used for this study.

Effect of an Increased Concentration of D-Glucose or L-Fucose on the Generation of Reactive Oxygen Sspecies

To determine whether an increased concentration of D-glucose or L-fucose increases the accumulation of reactive oxygen species in BAE cells, we examined the oxidation of dihydror-

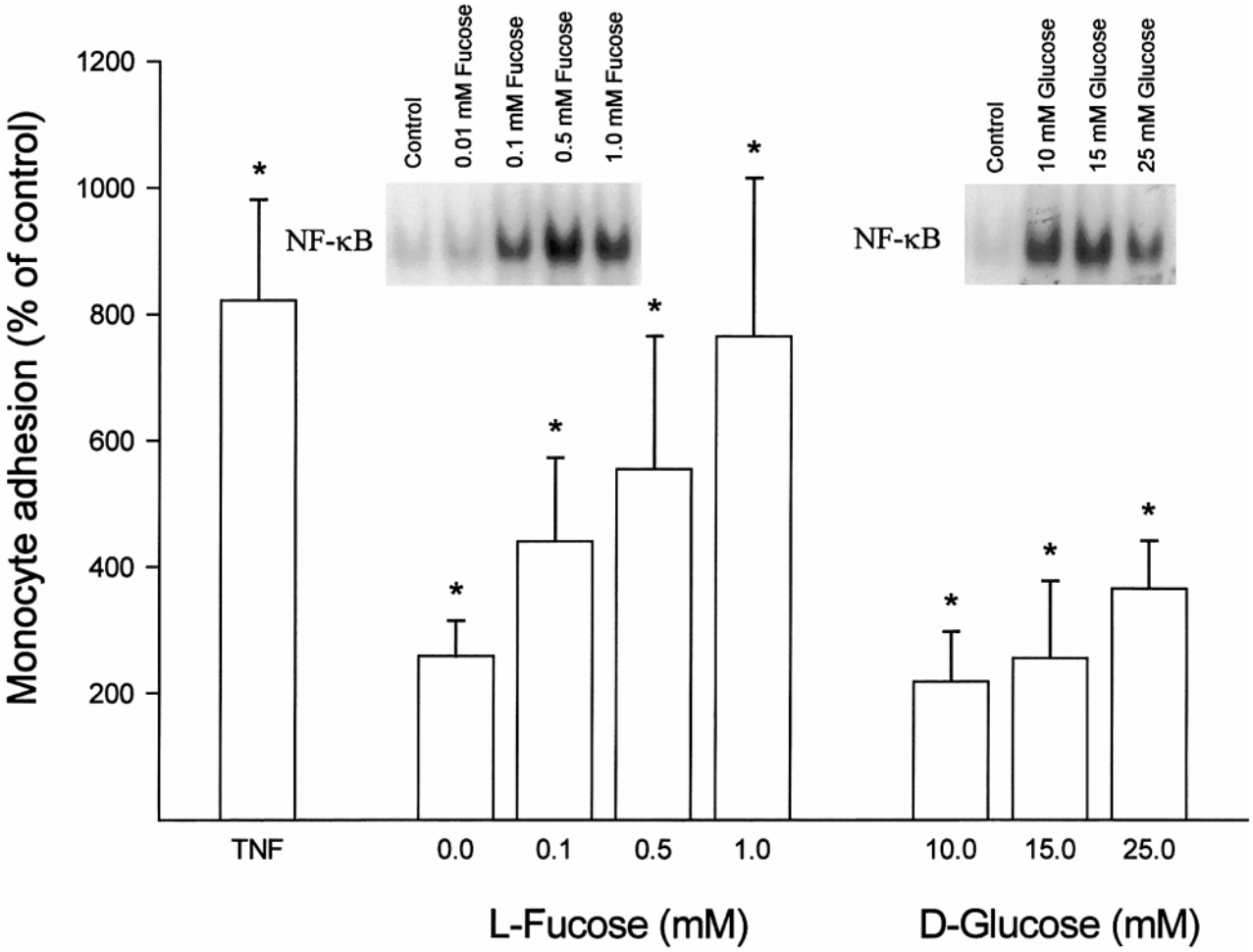

Fig 3. Effect of TNF-α, glucose, or L-fucose on monocyte adhesion and NF-κB activation in cultured BAE cells. For examination of the effect of TNF-α, glucose, or L-fucose on monocyte adhesion, cells were incubated in Earle's balanced salt solution for 16 hours containing 1% fetal bovine serum plus 10 ng/mL TNF-α, 10, 15, or 25 mmol/L glucose (final concentration) or 0.01, 0.1, 0.5, or 1.0 mmol/L L-fucose. Afterwards, monocyte adhesion to the endothelial cell monolayer was determined as described in Materials and Methods. Each value is the mean ± SEM of 9 separate determinations. The data for monocyte adhesion is presented as the percentage of control. The insert is a representative autoradiograph from a single experiment of the effect of 0.01 to 1.0 mmol/L L-fucose or 10 to 25 mmol/L glucose on the activation of NF-κB. For these studies, the cells were incubated in Earle's balanced salt solution for 3 hours containing 1% fetal bovine serum plus 10, 15, or 25 mmol/L glucose (final concentration) or 0.01, 0.1, 0.5, or 1.0 mmol/L L-fucose. After these incubations, nuclear extracts were prepared and gel mobility shift assays performed as described in Materials and Methods. The region of the autoradiograph showing the free probe has been removed for clarity. This experiment was repeated 3 times with similar results. *$P < .05$ compared with control.

hodamine 123 following a 1-hour incubation of the cells with 15 mmol/L D-glucose or 0.5 mmol/L L-fucose. Cells exposed to 10 ng/mL TNF-α were used for a positive control. Data in Table 1 demonstrate that incubating BAE cells for 1 hour in Earle's balanced salt solution containing 1% fetal bovine serum and either 15 mmol/L glucose (final concentration basal medium contains 5 mmol/L glucose) or 0.5 mmol/L L-fucose significantly increased the oxidation of dihydrorhodamine 123 by about 10-fold. A similar increase in the oxidation of dihydrorhodamine 123 was observed when the cells were incubated for 1 hour with 10 ng/mL TNF-α. Preincubating the cells with 1 mmol/L α-lipoic acid for 16 hours significantly decreased by about 50% the oxidation of dihydrorhodamine 123 caused by 15 mmol/L glucose or 0.5 mmol/L L-fucose. In contrast, coincubating the cells with 1 mmol/L α-lipoic acid and either 15 mmol/L glucose or 0.5 mmol/L L-fucose had no effect on the 15 mmol/L glucose- or 0.5 mmol/L L-fucose–induced increase in the oxidation of dihydrorhodamine 123 (data not shown). For these studies, we used 1 mmol/L α-lipoic acid because previously Bierhaus et al[39] demonstrated that 1 to 2 mmol/L α-lipoic acid suppressed the activation of NF-κB by advanced glycation end products in cultured BAE cells. Moreover, α-lipoic acid has been used as an antioxidant in animal studies of diabetic complications.[40-42] Incubating BAE cells for 16 hours in Earle's balanced salt solution containing 1% fetal bovine serum had no effect on the oxidation of dihydrorhodamine 123.

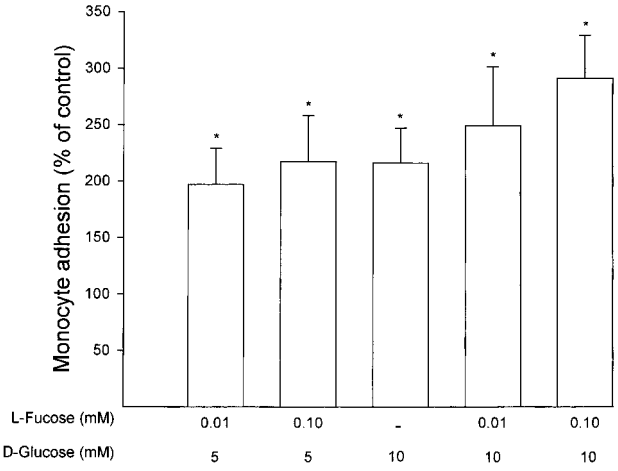

Fig 4. The combined effect of glucose and L-fucose on monocyte adhesion in cultured BAE cells. For these studies, the cells were incubated for 16 hours in Earle's balanced salt solution (basal glucose concentration, 5 mmol/L) containing 1% fetal bovine serum plus 0.01 or 0.1 mmol/L L-fucose, 10 mmol/L glucose, or 10 mmol/L glucose and 0.01 or 0.1 mmol/L L-fucose. Afterwards, monocyte adhesion to the endothelial cell monolayer was determined as described in Materials and Methods. Each value is the mean ± SEM of 6 separate determinations conducted in triplicate. The data for monocyte adhesion is presented as the percentage of control, which was assigned a value of 100%. *$P < .05$ compared with control.

Effect of α-Lipoic Acid on the Induction of NF-κB Activation and Monocyte Adhesion by D-Glucose and L-Fucose

Data in Fig 5 demonstrate that preincubating BAE cells with 1 mmol/L α-lipoic acid prevents the stimulation of NF-κB activation by D-glucose and L-fucose. Preincubating BAE cells with 1 mmol/L α-lipoic acid also prevented the stimulation of monocyte adhesion by D-glucose or L-fucose (Fig 6).

DISCUSSION

In these studies, we demonstrate that another sugar besides glucose that is increased in human diabetes, L-fucose, causes the generation of reactive oxygen species, activation of NF-κB, and an increase in adhesion of monocytes in cultured BAE cells. The concentration at which L-fucose induces these effects is similar to the concentration of unbound L-fucose reported to occur in circulation of humans with diabetes.[21] Previously, we have shown that exposing cultured neuroblastoma cells to an increased concentration of L-fucose causes a decrease in myoinositol metabolism, a decrease in Na^+/K^+ ATPase activity, and a reduction in the peak conductance of voltage-activated Na^+ channels.[43,44] Similar effects were observed when neuroblastoma cells were exposed to increased concentrations of glucose.[43,44] In cultured endothelial cells, we have previously reported that an increased concentration of L-fucose causes a decrease in myoinositol metabolism, cell proliferation, and proteoglycan and collagen production.[16,17] In a normoglycemic L-fucose–fed rat animal model designed to duplicate the circulatory concentration of unbound L-fucose that occurs in humans with diabetes, we have demonstrated that increased circulatory levels of L-fucose induces nerve functional and morphologic changes that are similar to diabetic rat models.[22,27] In L-fucose–fed rats, sciatic nerve Na^+/K^+ ATPase activity and myoinositol levels are decreased, motor nerve conduction velocity is reduced, and sural nerve axonal atrophy, paranodal swelling, and paranodal demyelination is increased.[22,23,27] These changes were partially to totally prevented by supplementing the diet with 1% myoinositol.[22,27] These studies suggest that in addition to an increased concentration of glucose, other changes in the diabetic circulation, such as an increased concentration of unbound L-fucose, may also contribute to the development of diabetic complications affecting the nerve and/or vascular tissue. This could be 1 reason for the complex etiology for the development of diabetic complications and emphasizes the importance of understanding the metabolic-related changes that occur in diabetes. In this regard, we have extended our study of the effect of an increased concentration of L-fucose on endothelial cell function. For these studies, we decided to use cultured BAE cells rather than a cultured human endothelial cell model. This was done for several reasons. First, we have previously reported that exposure to increased concentrations of L-fucose causes an alteration in proliferation and myoinositol metabolism in these cells.[17] Second, cultured BAE cells are a reproducible and stable endothelial cell line that represents an endothelial cell type that is impaired by diabetes and was used by DiCorleto and de la Motte[36] to develop endothelial cell monocyte adhesion studies. Third, we were concerned that the specialized growth conditions that are required to propagate many of the human cultured endothelial cell types could possibly interfere with our experimental conditions.

Diabetes has been reported to cause an increase in the expression of adhesion molecules and the attachment of leukocytes and monocytes to the endothelium.[3-5] Both are considered to be risk factors in the development of atherosclerosis. The mechanism(s) responsible for the development of endothelial dysfunction in diabetes is not understood. The cytokine, TNF-α, has been shown to activate NF-κB and endothelial cell

Table 1. Effect of an Increased Concentration of Glucose or L-Fucose on the Generation of Reactive Oxygen Species

Conditions	Oxidation of Dihydrorhodamine 123 (nmol/mg protein)
Control	0.06 ± 0.01
Control + α-lipoic acid	0.05 ± 0.01
Glucose	0.44 ± 0.03*
Glucose + α-lipoic acid	0.23 ± 0.04*†
L-fucose	0.47 ± 0.04*
L-fucose + α-lipoic acid	0.22 ± 0.04*†

NOTE. Cells were incubated in Earle's balanced salt solution for 16 hours containing 1% fetal bovine serum with or without 1 mmol/L α-lipoic acid. Afterwards, all cells were prelabeled with 5 μmol/L dihydrorhodamine 123 for 1 hour. The cells were then incubated with or without 15 mmol/L glucose (final concentration), or 0.5 mmol/L L-fucose ± 1 mmol/L α-lipoic acid. After this incubation, samples were collected and analyzed for oxidation of dihydrorhodamine 123 as described in Materials and Methods. Data is expressed as the mean ± SEM from 19 separate observations conducted in triplicate.

*$P < .05$ compared with control.

†$P < .05$ compared with the paired untreated condition.

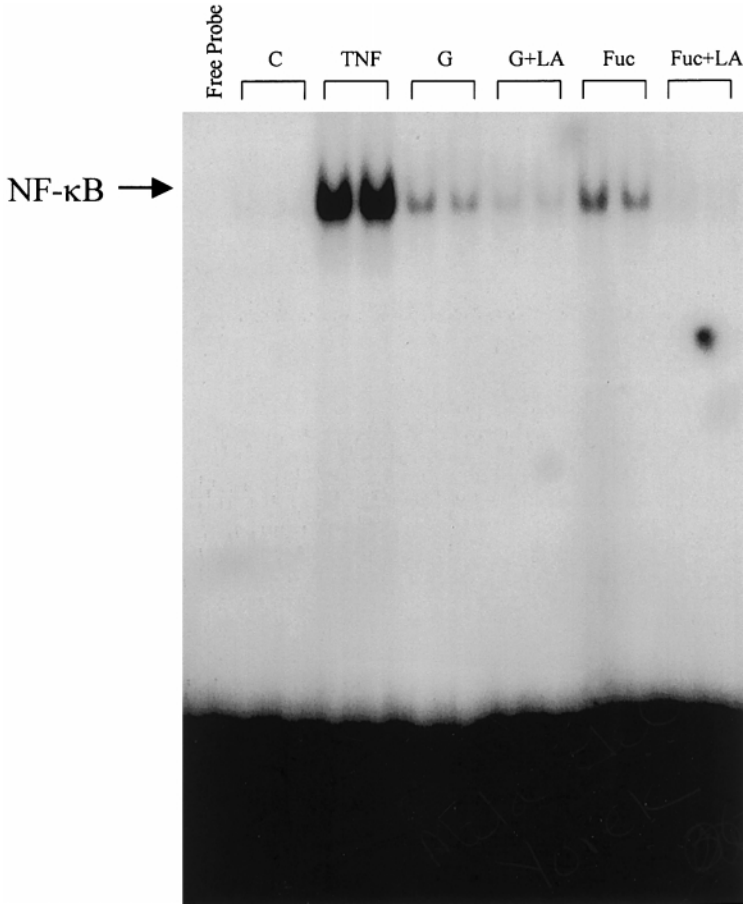

Fig 5. Effect of α-lipoic acid on the induction by glucose or L-fucose on NF-κB activation in cultured BAE cells. For these studies, some of the cells were preincubated for 1 hour in Earle's balanced salt solution containing 1% fetal bovine serum plus 1 mmol/L α-lipoic acid. Afterwards, cells were incubated for 3 hours in the same medium containing 1% fetal bovine serum plus 15 mmol/L glucose (final concentration) with or without 1 mmol/L α-lipoic acid or 0.5 mmol/L L-fucose with or without 1 mmol/L α-lipoic acid. For a positive control, cells were also incubated in medium containing 10 ng/mL TNF-α. The figure presents a representative autoradiograph from a single experiment that was repeated at least 3 times with similar results.

adhesion molecule expression and monocyte adhesion by a mechanism linked to the generation of reactive oxygen species.[45-48] Supplementation with α-tocopherol or N-acetylcysteine was shown to inhibit the effects of TNF-α on NF-κB activation and monocyte adhesion in endothelial cells.[49,50] In endothelial cells, TNF-α has also been shown to activate protein kinase C, as well as mitogen-activated protein kinases.[47,51] These pathways may also be involved in the activation of NF-κB by reactive oxygen species.[51,52] In endothelial cells, an increased concentration of glucose has been shown to activate some of the same pathways. High glucose levels have been demonstrated to activate NF-κB in both cultured endothelial and vascular smooth muscle cells.[10,11,53,54] In addition, the activation of NF-κB in cultured endothelial cells, as well as mesangial cells, by an increased glucose concentration has been linked to the generation of reactive oxygen species and increased expression of adhesion molecules and monocyte adhesion.[6,7,10,55] In cultured endothelial cells, an increased concentration of glucose has also been shown to activate mitogen-activated protein kinases.[56,57] The effects of increased glucose concentration on adhesion molecule expression and monocyte adhesion in endothelial cells can be prevented by inhibition of protein kinase C or antioxidants.[10,11] The effect of an increased concentration of glucose on the activation of NF-κB, the generation of reactive oxygen species, and induction of monocyte adhesion in cultured endothelial cells was confirmed in these studies. Furthermore, we demonstrated that the antioxidant α-lipoic acid partially inhibited the effects of hyperglycemia on these cells. α-Lipoic acid has been shown to prevent vascular and neural defects induced by diabetes in animal models.[58-63] In addition, α-lipoic acid has been shown to reduce expression of vascular cell adhesion molecule-1 and endothelial adhesion of monocytes after exposure to advanced glycation end products.[14] α-Lipoic acid treatment of patients with diabetic nephropathy was also shown to reduce the activation of NF-κB in ex vivo isolated peripheral blood mononuclear cells.[64]

These studies demonstrated for the first time that the monosaccharide L-fucose, whose unbound and bound circulatory concentration is increased in humans with diabetes, causes the generation of reactive species, activation of NF-κB, and an increase in monocyte adhesion in cultured endothelial cells. The concentration of L-fucose required to induce these effects was similar to the circulatory concentration of unbound L-fucose that occurs in humans with diabetes.[21] The effects of L-fucose on the activation of NF-κB and increase in monocyte adhesion in endothelial cells was greater than the effects of an increased concentration of glucose. Like the effects of hyperglycemia, the effect of L-fucose on NF-κB activation and monocyte adhesion was partially prevented by α-lipoic acid. This suggests that L-fucose may mediate some of its effects by

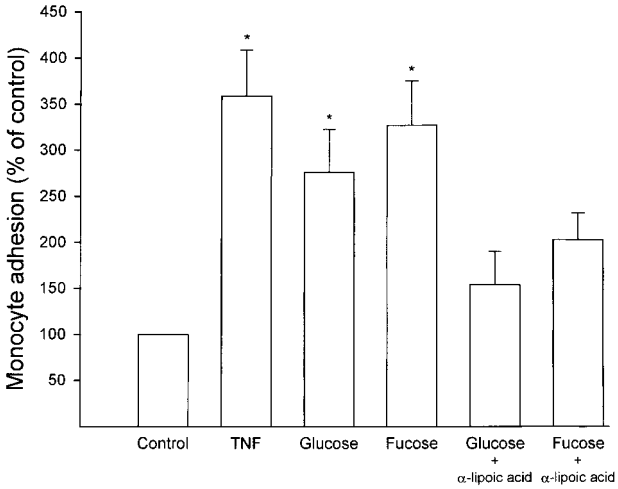

Fig 6. Effect of α-lipoic acid on the induction by glucose or L-fucose on monocyte adhesion in cultured BAE cells. For these studies, some of the cells were preincubated for 1 hour in Earle's balanced salt solution containing 1% fetal bovine serum plus 1 mmol/L α-lipoic acid. Afterwards, cells were incubated for 16 hours in the same medium containing 1% fetal bovine serum plus 15 mmol/L glucose (final concentration) with or without 1 mmol/L α-lipoic acid or 0.5 mmol/L L-fucose with or without 1 mmol/L α-lipoic acid. For a positive control, cells were also incubated in medium containing 10 ng/mL TNF-α. Afterwards, monocyte adhesion to the endothelial cell monolayer was determined as described in Materials and Methods. Each value is the mean ± SEM of 12 separate determinations conducted in triplicate. The data for monocyte adhesion is presented as the percentage of control, which was assigned a value of 100%. *$P < .05$ compared with control.

the generation of reactive oxygen species. However, unlike the effects of an increased glucose concentration, we have not been able to demonstrate that L-fucose increases the activation of protein kinase C or mitogen-activated protein kinases in cultured endothelial cells (data not shown). Therefore, the mechanism responsible for the generation of reactive species in endothelial cells by L-fucose may be different from hyperglycemia. Aortic tissue is capable of forming GDP-fucose a precursor for L-fucose synthesis.[65] It is unknown whether hyperglycemia promotes this activity in cultured endothelial cells or whether the effects of hyperglycemia described in these studies may be mediated, in part, by the formation of L-fucose. However, previously we have not been able to detect an increase in unbound L-fucose in cultured endothelial cells incubated with 30 mmol/L glucose (data not shown). Therefore, it is unlikely that an increased concentration of L-fucose produced from glucose is responsible for the effects of hyperglycemia in these studies. Additional studies are necessary to determine the mechanism by which L-fucose induces these defects in endothelial cells. However, it appears likely that the generation of reactive species is involved in the endothelial cell dysfunction induced by L-fucose.

In summary, these studies have demonstrated that sugars other than glucose, which are also increased in the circulation of humans with diabetes, may induce endothelial cell dysfunction and contribute to the development and/or progression of diabetic vascular disease. The contribution of circulatory factors other than glucose to the development of diabetic complications may help explain the complex nature of the etiology of diabetic complications in humans.

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