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Glucose-Induced Phosphatidylinositol 3-Kinase and Mitogen-Activated Protein Kinase–Dependent Upregulation of the Platelet-Derived Growth Factor-β Receptor Potentiates Vascular Smooth Muscle Cell Chemotaxis

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The aim of this study was to investigate the effects of elevated D-glucose concentrations on vascular smooth muscle cell (VSMC) expression of the platelet-derived growth factor (PDGF) receptor and VSMC migratory behavior. Immunoprecipitation, immunofluorescent staining, and RT-PCR of human VSMCs showed that elevated D-glucose induced an increase in the PDGF receptor that was inhibited by phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathway inhibitors. Exposure to 25 mmol/l D-glucose (HG) induced increased phosphorylation of protein kinase B (PKB) and extracellular-regulated kinase (ERK). All HG chemotaxis assays (with either 10 days’ preincubation in HG or no preincubation) in a FCS or PDGF-BB gradient showed positive chemotaxis, whereas those in 5 mmol/l D-glucose did not. Assays were also run with concentrations ranging from 5 to 25 mmol/l D-glucose. Chemotaxis was induced at concentrations ≥9 mmol/l D-glucose. An anti-PDGFβ receptor antibody inhibited glucose-potentiated VSMC chemotaxis, as did the inhibitors for the PI3K and MAPK pathways. This study has shown that small increases in D-glucose concentration, for a short period, increase VSMC expression of the PDGF receptor and VSMC sensitivity to chemotactic factors in serum, leading to altered migratory behavior in vitro. It is probable that similar processes occur in vivo with glucose-enhanced chemotaxis of VSMCs, operating through PDGF receptor–operated pathways, contributing to the accelerated formation of atheroma in diabetes. Diabetes 52: 519–526, 2003

Vascular disease is responsible for much of the excess morbidity and mortality that affect patients with diabetes. The atherosclerotic plaque contains components derived from both the endothelial and muscle layers in addition to invading macrophages (1,2), as is the case for all causes of atheroma formation. However, clinically significant atherosclerosis occurs at an earlier age and is often more severe in patients who suffer from diabetes than in nondiabetic subjects (1).

Platelet-derived growth factor (PDGF), secreted by vascular cells, plays a central role in the development of atherosclerotic proliferation of vascular smooth muscle cells (VSMCs) (2). PDGF also stimulates chemotaxis (3). The effects of PDGF are mediated through binding to specific high-affinity surface receptors (4). Since PDGF receptors are found on VSMCs (5) and macrophages (6), this suggests that PDGF could influence atherosclerosis by regulating the functions of both macrophages and VSMCs.

Macrophages are present at all stages of development of the atherosclerotic plaque. Macrophages adhere to endothelial cells, move between these cells, and accumulate in the subendothelial space (7). Activated macrophages not only act as scavenger cells but also secrete several growth factors. Secretions of growth factors such as PDGF (8) and colony-stimulating factor (CSF)-1 (9) are increased when macrophages are activated. Glucose causes an upregulation of the CSF-1 receptor (c-fms) on macrophages (10), thereby allowing enhanced autocrine activation of the macrophage by CSF-1.

A high glucose environment causes the development of oxidative stress within VSMCs (11,12), and both this and/or the presence of increased amounts of chemotactic substances cause VSMCs to change their phenotype and migrate into the subendothelial space. VSMCs in the subendothelial space display a “secretory” phenotype expressing genes for a number of growth factors and their receptors such as c-fms (2). A range of factors, such as urokinase type plaminogen activator (13), thrombospondin-1 (14), bone morphogenetic protein-2 (BMP-2) (15), tumor necrosis factor-α (16), vascular endothelial growth factor (17), and IGF-1 (18), are believed to be
chemotactic for VSMCs. At present, PDGF is the best-characterized chemotactic agent for VSMCs (19).

The aims of this study were to determine whether high glucose levels per se induce alterations in the expression of the PDGFB receptor in primary human aortic VSMCs, to determine the signaling pathways involved, and to determine whether the chemotactic properties of VSMCs are altered by exposure to high glucose. High glucose concentrations cause VSMCs to divide. In this study, the movement of cells has been investigated using a Dunn chamber, which allows dividing and nondividing cells to be identified and treated separately. This assay is also more sensitive than the more commonly used transwell assay (20,21). Additionally, it allows accurate calculation of the speed of cell movement, which cannot be assessed in the transwell assay.

RESEARCH DESIGN AND METHODS

Chemicals and antibodies. D-Glucose and l-glucose were purchased from BDH (Poole, UK). PDGF-BB was purchased from Insight Biotechnology (Woburn, UK). Wortmannin and LY294002 were purchased from Sigma (Poole, UK). PD-98059 was from Calbiochem (La Jolla, CA). Trollox and α-tocopherol acid were from Sigma.

The following antibodies were used: anti-PDGFB receptor and anti-PDGF receptor antibodies (R&D Systems, Minneapolis, MN), rabbit polyclonal antibody to total extracellular-regulated kinase (ERK) (Santa Cruz), mouse polyclonal antibody to phosphorylated ERK (Santa Cruz), rabbit polyclonal antibody to Akt/protein kinase B (PKB) phosphorylated on serine 473 (Cell Signaling Technology), rabbit polyclonal antibody against Akt/PKB (Cell Signaling Technology), and horseradish peroxidase (HRP)-conjugated donkey anti-mouse IgG and HRP-conjugated donkey anti-rabbit IgG (Dako, Ely, UK).

Isolation of VSMCs. Porcine aorta was obtained from the local abattoir. VSMCs were isolated and grown as previously described (11,12). Human VSMCs were obtained from the thoracic aorta of transplant donors (written consent was obtained from the relevant family member, and the study was approved by the Ethical Committee of Queen’s University, Belfast). The VSMCs were isolated as previously described (11,12), except that the growth medium was RPMI-1640 (GibcoBRL, Life Technologies, Paisley, UK) instead of Dulbecco’s modified Eagle’s medium (GibcoBRL). Cells were confirmed as VSMCs by positive immunoﬂuorescence staining with a monoclonal antibody against smooth muscle α-actin. All VSMC experiments were carried out using cells of passage 4–7.

Chemotaxis assays. Chemotaxis was assessed by direct observation and recording of cell behavior in stable concentration gradients of FCS using the Dunn chamber, which cannot be assessed in the transwell assay. Additionally, it allows accurate calculation of the speed of cell movement, which cannot be assessed in the transwell assay.

RESULTS

Glucose induces increased growth factor receptor expression in VSMCs. To determine the effect of exposure to elevated β-glucose, immunofluorescent staining, immunoprecipitation, and RT-PCR were carried out for the PDGFB receptor.

Confocal laser microscopy demonstrated that human VSMCs express the PDGFβ receptor (Fig. 1A and B). Comparison of the cells cultured in 5 mmol/l (Fig. 1A) and 25 mmol/l β-glucose (Fig. 1B) suggested that the PDGFβ receptors were increased in 25 mmol/l β-glucose; quantitative image analysis revealed a 110% increase compared with 5 mmol/l β-glucose in staining for the PDGFβ receptor in 25 mmol/l β-glucose. Immunofluorescent staining also demonstrated that human VSMCs express the PDGFβ receptor; however, no change in expression was detected between 5 and 25 mmol/l β-glucose (data not shown).

Western blotting of human and porcine VSMC-immunoprecipitated lysates was carried out to gain a more quantitative comparison of the expression of the PDGFβ receptor between 5 and 25 mmol/l glucose. Densitometry showed that VSMCs exposed to 25 mmol/l β-glucose for 1 day had a 60% increase in the PDGFβ receptor compared with 5 mmol/l (Fig. 1C), whereas VSMCs exposed to 25...
mmol/l D-glucose for 10 days showed a 214% increase in the PDGF\(\beta\) receptor compared with 5 mmol/l D-glucose.

To determine how high the glucose concentration needed to be to cause an increase in PDGF\(\beta\) receptor expression, VSMCs exposed to a range of glucose concentrations from 5 to 25 mmol/l for 24 h were stained. Quantitative image analysis showed that with increasing glucose concentration up to 10 mmol/l, PDGF\(\beta\) receptor expression increased before leveling off at concentrations between 10 and 25 mmol/l (Fig. 1D).

RT-PCR showed that 24 h exposure to 25 mmol/l glucose induced a significant increase in PDGF\(\beta\) receptor mRNA (Fig. 1E).

**Glucose induces increased PDGF receptor expression via PI3K and MAPK pathways.** Pharmacological agents were used to determine which major second messenger systems regulate glucose-induced increases in PDGF\(\beta\) receptor expression. VSMCs were placed in 25 mmol/l glucose in the presence of standard concentrations of wortmannin (10 nmol/l), LY294002 (10 \(\mu\)mol/l) (PI3K inhibitors), or PD-98059 (10 \(\mu\)mol/l) (MAPK inhibitor) for 24 h. Immunoprecipitation and RT-PCR were carried out for the PDGF\(\beta\) receptor. Treatment with wortmannin or LY294002 led to significant reduction of the PDGF\(\beta\) receptor protein, as did treatment with PD-98059 (Fig. 2A). These agents were dissolved in DMSO, necessitating a DMSO control. To determine whether this inhibition effect was specific to the effects of high glucose, LY294002 and PD-98059 were also tested in 5 mmol/l glucose conditions. In 5 mmol/l glucose, both LY294002 and PD-98059 caused a slight reduction in expression of the receptor. RT-PCR showed that both LY294002 and PD-98059 caused significant inhibition of the PDGF\(\beta\) receptor mRNA levels compared with the 25 mmol/l glucose/DMSO control (Fig. 2B).

These results indicate that the glucose-induced upregulation of the PDGF\(\beta\) receptor is PI3K and MAPK dependent and that there is a low level of PI3K and MAPK pathway activity in 5 mmol/l D-glucose.

**Glucose leads to increased phosphorylation of PKB and ERK.** The effects of glucose on the PI3K pathway was assessed by investigation of protein kinase B (PKB or Akt) a downstream target for PI3K. Immunoprecipitation was carried out for total PKB and phosphorylated PKB. Densitometry revealed that levels of total PKB were not altered by exposure to 25 mmol/l glucose (Fig. 3A), but that after 24 h exposure to 25 mmol/l glucose, there was an increase...
of phosphorylated PKB compared with 5 mmol/l glucose (Fig. 3B), and that after 10 days’ exposure to 25 mmol/l glucose, the levels of phosphorylated PKB were further increased (Fig. 3B). Exposure to 5 mmol/l D-glucose plus 20 mmol/l L-glucose had no effect on either total PKB expression or the amount of phosphorylated PKB.

To further investigate the role of the MAPK pathway, immunoprecipitation was carried out for total ERK 1/2 (Fig. 3C) and phosphorylated ERK 1/2 (Fig. 3D). No significant increase in the expression of ERK 1/2 was detected (Fig. 3C); however, measurement of phosphorylated ERK showed a significant increase in ERK 1/2 phosphorylation after 24-h exposure to 25 mmol/l glucose, which increased further after 10 days’ exposure (Fig. 3D). Exposure to 5 mmol/l D-glucose plus 20 mmol/l L-glucose had no effect on either total ERK 1/2 expression or the amount of phosphorylated ERK 1/2.

To address the possibility that the changes in PKB and ERK activity was caused by increased oxidative stress, PKB and ERK were measured in cells exposed to the antioxidants a-lipoic acid (50 μmol/l) and Trolox (200 μmol/l) in 25 mmol/l D-glucose. No effect was noted (data not shown).

Glucose sensitizes VSMCs to PDGF and serum factors. To test the effects of increased glucose concentrations on human VSMC migration, cells were exposed to a gradient of FCS in either 5 or 25 mmol/l D-glucose using the Dunn chemotaxis chamber. The 25 mmol/l D-glucose–treated cells had been preincubated in 25 mmol/l D-glucose for 10 days before the experiment. In 5 mmol/l D-glucose, the human VSMCs moved randomly (Fig. 4A). By contrast, in 25 mmol/l D-glucose, the cells showed chemotaxis toward the source of the FCS (Fig. 4B). This experiment was repeated using porcine VSMCs; again in 5 mmol/l D-glucose, the cells moved randomly in the FCS gradient but chemotaxed in 25 mmol/l D-glucose (data not shown).

To test whether a 10-day exposure to 25 mmol/l D-glucose was required to induce this alteration in behavior, human VSMCs cultured in 5 mmol/l D-glucose were exposed to 25 mmol/l D-glucose only when placed in the
Intermediate concentrations of D-glucose were tested. It was shown that in 15, 10, and 9 mmol/l D-glucose (without preincubation), there was chemotaxis (Fig. 4D, E, and F, respectively). However, in 8 mmol/l D-glucose, there was no chemotaxis (Fig. 4G). Because PDGF is known to be a chemotactic agent for VSMCs and because high glucose upregulates the PDGFβ receptor (Fig. 1), human VSMCs were preincubated for 5 h with either an antibody against the PDGFβ receptor (1 μg/ml) or the PDGFα receptor (1 μg/ml) in 25 mmol/l D-glucose before being exposed to a gradient of FCS, in order to determine whether PDGF plays a role in the chemotaxis to FCS in high glucose concentrations. The PDGFβ receptor antibody blocked chemotaxis in the FCS gradient (Fig. 4H); however, the PDGFα receptor antibody did not inhibit chemotaxis (Fig. 4I). Incubation with a nonrelevant IgG of the same species and class had no effect on chemotaxis (data not shown). This indicated that PDGF within the FCS played a key role in producing the chemotaxis shown in Fig. 4B. Control experiments were carried out to eliminate the possibility that the change in migratory behavior was caused by either osmosis or glycation. To achieve this, human VSMCs were exposed to 5 mmol/l D-glucose plus 20 mmol/l L-glucose for 10 days and did not show chemotaxis (Fig. 4J). A second control was carried out to eliminate the possibility that some characteristic of the Dunn chemotaxis chamber influenced the change in VSMC behavior. In these experiments, human VSMCs in 25 mmol/l D-glucose were placed in the chamber with 10% FCS in both the source and sink well, i.e., FCS throughout the chamber; in the absence of an FCS gradient, there was no chemotaxis (Fig. 4K).

The effects of glucose on PDGF-induced chemotaxis were tested. Human VSMCs were maintained in RPMI without FCS for 4 h before being placed in a gradient of PDGF-BB (10 ng/ml) in either 5 or 25 mmol/l D-glucose. No chemotaxis occurred in 5 mmol/l D-glucose (Fig. 5A), whereas it did occur in 25 mmol/l D-glucose (Fig. 5B). A very high concentration of PDGF-BB (30 ng/ml) did induce chemotaxis in 5 mmol/l glucose (Fig. 5C). If cells were preincubated with the anti-PDGFβ receptor antibody before being placed in a gradient of PDGF-BB in 25 mmol/l D-glucose, no chemotaxis was observed (Fig. 5D). Again, as a control, cells were also incubated with a nonrelevant antibody against PDGF-BB and 10 ng/ml PDGF-BB within the Dunn chamber did show chemotaxis (B). Human VSMCs exposed to 5 mmol/l D-glucose in a stronger gradient (30 ng/ml) of PDGF-BB showed chemotaxis (C). Human VSMCs exposed to 25 mmol/l D-glucose preincubated for 5 h with a neutralizing anti-PDGFβ receptor antibody did not show chemotaxis in a gradient of PDGF-BB (D). All assays were run for 18 h; n represents the number of cells tracked, and P represents Rayleigh test probability. All graphs represent the combined results of at least four independent experiments.

FIG. 4. VSMC migration in a gradient of FCS. Circular histograms show the proportion of human VSMCs migrating in each direction, with the source of FCS at the top. The Rayleigh test showed that cells exposed to 5 mmol/l glucose (A) did not show chemotaxis, while those exposed to 25 mmol/l D-glucose for 10 days did show chemotaxis to FCS (B). Human VSMCs exposed to 25 mmol/l D-glucose, only when placed within the Dunn chamber without a prior 10-day incubation, also showed chemotaxis (C), as did those exposed to 15 (D), 10 (E), and 9 (F) mmol/l glucose. However, human VSMCs exposed to 8 mmol/l D-glucose did not show chemotaxis to FCS (G). Human VSMCs exposed to 25 mmol/l D-glucose and preincubated for 5 h with a neutralizing anti-PDGFβ receptor antibody did not show chemotaxis in a gradient of FCS (H). Human VSMCs exposed to 25 mmol/l D-glucose and preincubated for 5 h with a neutralizing anti-PDGFα receptor antibody showed chemotaxis in a gradient of FCS (I). Human VSMCs exposed to 5 mmol/l D-glucose and 20 mmol/l L-glucose for 10 days did not show chemotaxis to FCS (J). Human VSMCs exposed to 25 mmol/l glucose (no 10-day preincubation) in isotonic FCS (with 10% FCS in both the source and sink well) move randomly (K). All experiments were run for 18 h; n represents the number of cells tracked, and P represents Rayleigh test probability. All graphs represent the combined results of at least four independent experiments.
showed that cells treated with 10 mM H9262 (i.e., in all concentrations of glucose in which chemotaxis to FCS occurs). 25*, cells exposed to 25 mM D-glucose for 10 days (all other cells were maintained in 5 mM glucose before the experiment). *P < 0.05 vs. 5 mM glucose.

IgG of the same species and class that had no effect on chemotaxis (data not shown).

Analysis of the speed of cell movement showed a significant drop in cell speed, at 9 mM glucose and higher, in gradients of FCS (Fig. 6).

**Glucose-potentiated chemotaxis is PI3K and MAPK dependent.** VSMCs were placed in a gradient of FCS in 25 mM glucose in the presence of 10 mM wortmannin or 10 μM LY294002 or PD-98059. The cells were exposed to each inhibitor within the chamber, and the experiments were carried out in a darkened room due to the light sensitivity of these inhibitors. It was found that the cells treated with the inhibitors wortmannin (Fig. 7A), LY294002 (Fig. 7B), and PD-98059 (Fig. 7C) did not show chemotaxis in the FCS gradient. As these agents are soluble in DMSO, a control experiment was run with 1 μl/ml DMSO present in 25 mM glucose, and the cells in this experiment showed positive chemotaxis (Fig. 7D).

Treatment with each of these inhibitors significantly reduced the speed of the cells compared with untreated cells in 25 mM glucose (data not shown). These results indicate that the n-glucose-potentiated chemotaxis in the FCS gradient was mediated via PI3K and MAPK.

**Glucose induces increased f-actin and filopodia formation in VSMCs.** The actin cytoskeleton is a major component of the cellular migratory apparatus (25). Porcine VSMCs were fixed and stained using TRITC-conjugated phalloidin and then visualized using a confocal laser-scanning microscope. This clearly showed an increase in the amount of f-actin within the VSMCs exposed to 25 mM n-glucose compared with 5 mM n-glucose (Fig. 8A and B).

Staining of sparsely plated porcine VSMCs allowed the peripheral actin structures, lamellipodia and filopodia, to be observed. Lamellipodia are broad sheet-like structures (Fig. 8C, arrow), while filopodia (also known as microspikes) are fine spike-like actin structures (25). The number of filopodia (Fig. 8D, arrows) was increased in 25 mM glucose (Fig. 8D) compared with 5 mM glucose (Fig. 8C). This finding is consistent with the chemotaxis results, as filopodia have been shown to be essential for chemotaxis (25).

**Discussion**

Because PDGF is suspected to play a central role in the proliferation and migration of VSMCs in atherosclerosis (2,3,26), the aim of this study was to investigate in detail the effects of exposure to elevated glucose concentrations of primary human aortic VSMCs on the expression of the PDGF receptors and how this altered expression affects chemotaxis of human aortic VSMCs. The study also aimed to determine whether PI3K- and MAPK-mediated pathways play a role in glucose-induced altered expression of the PDGF receptors.

Results obtained by immunofluorescent staining and immunoprecipitation showed that in high glucose, the expression of the PDGFβ receptor is increased. RT-PCR showed an increase in PDGFβ receptor mRNA, indicating that glucose alters transcription of the gene.

Treatment with inhibitors to PI3K and MAPK showed that glucose-induced upregulation of the PDGFβ receptor is dependent on both the PI3K and MAPK pathways. This is further supported by the finding that 25 mM glucose induced an increase in phosphorylation of both PKB (PI3K pathway) and ERK (MAPK pathway). In light of the fact that previous studies found that glucose-induced PDGFβ receptor expression was protein kinase C dependent (6,27), these findings indicate that the mechanisms by which high glucose alters PDGFβ receptor expression are complex.

Results obtained in this study demonstrated that raised concentrations of glucose were able to increase the sensitivity of VSMCs to serum factors and PDGF-BB, inducing chemotaxis in a gradient of FCS. It may seem surprising that in 5 mM n-glucose, VSMCs do not show chemotaxis to FCS or PDGF-BB. Many cells will show chemotaxis in a gradient of FCS (regardless of glucose

**FIG. 6. Speed of movement.** Bar graph showing the mean speeds (and standard errors) of human VSMCs exposed to various glucose concentrations within the Dunn chamber, in a gradient of FCS. Speed of cell movement was significantly reduced in glucose concentrations ≥9 mM (i.e., in all concentrations of glucose in which chemotaxis to FCS occurs). 25*, cells exposed to 25 mM D-glucose for 10 days (all other cells were maintained in 5 mM glucose before the experiment). *P < 0.05 vs. 5 mM glucose.

**FIG. 7. Glucose-potentiated VSMC chemotaxis is PI3K and MAPK dependent.** Circular histograms show the proportion of human VSMCs migrating in each direction, with the source of FCS at the top. The Rayleigh test showed that cells exposed to 25 mM glucose and incubated in 10 mM wortmannin (a PI3K inhibitor) failed to show chemotaxis (A), as did those exposed to 25 mM glucose and the 10 μM LY294002 (another PI3K inhibitor) (B). The Rayleigh test also showed that cells treated with 10 μM PD-98059, a MAPK inhibitor (C), and exposed to 25 mM n-glucose do not show chemotaxis. A control experiment was run with cells exposed to 25 mM glucose and DMSO (as wortmannin, LY294002 and PD-98059 are soluble in DMSO), which showed chemotaxis (D). All experiments were run for 18 h; n represents the number of cells tracked, and P represents Rayleigh test probability. Graphs show the combined results of three to four independent experiments.
concentration) if cultured in the absence of FCS for an extended period of time (which upregulates receptor expression); however, without this serum starvation, most cells will not respond to a gradient of FCS. Increasing the concentration of PDGF-BB to 30 ng/ml induced chemotaxis in 5 mmol/l glucose.

The change in chemotactic behavior occurred after short periods of exposure to relatively modest increases in the concentration of glucose. This was an interesting finding in light of the high-glucose–induced increase in PDGF/H9252 receptors. It is possible that this upregulation of receptors could explain the greater sensitivity of VSMCs to FCS and PDGF-BB in high glucose than in normal glucose; this conclusion was supported by the observation that treatment with an antibody against the PDGF/H9252 receptor prevented human VSMC chemotaxing in either the FCS or PDGF-BB gradients. Use of both PI3K and MAPK pathway inhibitors blocked glucose-potentiated VSMC chemotaxis, again showing the importance of these pathways in glucose-modified VSMC biology.

Morphological examination of the actin cytoskeleton showed both an increase in total f-actin and an increase in the number of filopodia in VSMCs exposed to raised concentrations of glucose. This is an interesting finding since it has been previously shown that filopodia play a crucial role in macrophage chemotaxis (25).

Glucose has been shown to directly stimulate cell migration in a human umbilical vein endothelial cell (HUVEC)-derived cell line (24). By contrast, high glucose concentrations have been shown to inhibit neural crest cell migration in rat embryos (28). It is also possible that other high-glucose–induced processes, such as polyol accumulation (29), nonenzymatic glycation of proteins (29–32), and altered redox potential (33), mediate this change in migratory behavior. Yasunari et al. (34), who preincubated cells in high glucose for 72 h, found that the aldose reductase inhibitor epalrestat did inhibit glucose potentiated VSMC chemotaxis to PDGF, suggesting a role for the polyol pathway. However, it is well established that in high glucose, it requires 2–3 days for the subsequent increase in diacylglycerol levels to rise in VSMCs (35–37); therefore, since the chemotactic behavior shown in the present study occurred without prior incubation in high glucose, it is unlikely that it is mediated by this mechanism. The receptor for advanced glycation end products has been shown to mediate rabbit smooth muscle cell chemotaxis (38). In the present study, chemotaxis occurred without prior incubation in glucose but did not occur when cells were incubated for 10 days with L-glucose, which, although not metabolized, can cause glycation. This suggests that advanced glycation end product–related phenomena were not responsible for the chemotactic behavior of VSMCs in high glucose seen in the present study.

It is perhaps surprising that in the present study, the speed of cell movement was significantly lower in concentrations of glucose that potentiate chemotaxis to FCS, i.e., 9 mmol/l D-glucose and higher. In macrophages, inhibition of Cdc42, which leads to loss of chemotaxis to CSF-1, also causes an increase in the speed of cell movement (25), suggesting that movement by chemotaxis is slower than random movement.

In conclusion, the results presented above show that exposure to even modest and short-lived increases in glucose concentration potentiate VSMC chemotaxis to serum factors by inducing an upregulation of PDGF receptors and possibly other growth factor receptors by PI3K
and MAPK signaling pathway–dependent processes. Taken together with our earlier finding that exposure to high glucose concentrations increases VSMC proliferation (11,12), these findings provide the cellular basis for one of the many mechanisms that contribute to atherosclerosis in diabetes.

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