

# Stimulatory Effect of Glucose on Macrophage Lipoprotein Lipase Expression and Production

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Cardiovascular diseases are the leading cause of morbidity and mortality in diabetes. Lipoprotein lipase (LPL), a major secretory product of macrophages, has been suggested to play a key role in the development of atherosclerosis. In the present study, we evaluated the effect of high glucose on macrophage LPL mRNA expression and secretion. Exposure of murine J774 macrophages to high D-glucose concentrations (20–30 mmol/l) resulted in a dramatic upregulation of LPL mRNA expression and immunoreactive mass. This effect was not observed when these cells were incubated in the presence of L-glucose or mannitol. High glucose concentrations were also found to enhance LPL gene expression and immunoreactive mass in human monocyte-derived macrophages. J774 cells cultured in a high glucose environment expressed increased *c-fos* mRNA levels. Treatment of these cells with *c-fos* antisense DNA or protein kinase C inhibitor inhibited the stimulatory effect of glucose on LPL mRNA expression. In J774 cells exposed to high glucose concentrations, enhanced nuclear protein binding to the AP-1-responsive region of the murine LPL promoter was observed, while LPL mRNA stability remained unchanged. Overall, these results demonstrate that high glucose upregulates macrophage LPL gene expression and immunoreactive mass and that this effect involves transcriptional events. *Diabetes* 47:431–438, 1998

**A**therosclerosis occurs prematurely in diabetic patients and is considered to be a major complication of diabetes (1–5). Evidence has been provided that immune reactions are involved in the development of atherosclerosis. The early recruitment of monocytes to the arterial intima and their subsequent transformation into lipid-laden macrophages argue for a crucial role of these cells in the development and progression of atherosclerotic lesions (6–9). Macrophages constitutively synthesize lipoprotein lipase (LPL) (10–12), a key enzyme in

the catabolism of triglyceride-rich lipoproteins (13). Accumulating evidence suggests that LPL, by promoting the uptake of atherogenic lipoproteins by different vascular cell types, may contribute to lipid accumulation within the arterial wall, thereby promoting the atherogenic process (14,15). It has been shown that LPL activity is low in normal arteries, which have few macrophages, and increases during progression of the atherosclerotic plaque, which contains a large number of macrophage-like cells (16). Furthermore, macrophages have been documented to express LPL, both mRNA and protein, in atherosclerotic lesions in vivo (17–20).

Despite the high incidence of atherosclerosis in diabetic patients and the potential key role of macrophage LPL in the atherogenic process, the regulation of macrophage LPL expression in diabetes has not been investigated. Several studies suggest that macrophage LPL secretion could be enhanced in diabetes. High macrophage LPL expression and secretion have been previously documented in atherosclerosis- and diabetes-prone mice (21). In addition, incubation of human macrophages with hypertriglyceridemic VLDLs, which accumulate in the plasma of diabetic subjects, has been shown to result in enhanced LPL production by these cells (22).

The modulatory effect of a high glucose environment on macrophage function has been previously documented. It has been reported that high glucose concentrations increase the proliferation of macrophages (23) and enhance the growth response of these cells to colony-stimulating factor (CSF)-1 (24). Glucose and advanced glycation end products have also been shown to increase the production of interleukin (IL)-6 and tumor necrosis factor (TNF)- $\alpha$  by human monocytes (25,26). In the present study, we studied the effect of high glucose on macrophage LPL expression and secretion. In addition, based on previous data showing a stimulatory effect of high glucose on *c-fos* protein (27) and an involvement of this oncogene in the control of LPL expression (28), we evaluated the role of *c-fos* in the regulation of macrophage LPL by glucose.

## RESEARCH DESIGN AND METHODS

**Reagents.** Fetal calf serum (FCS) was purchased from Hyclone Laboratories (Logan, UT). Glucose-free Dulbecco's minimal essential medium (DMEM) and RPMI 1640 medium were obtained from ICN Biochemicals (Costa Mesa, CA) and Gibco BRL (Burlington, Ontario, Canada), respectively. D-glucose, L-glucose, mannitol, phorbol myristate acetate (PMA), actinomycin D, and heparin were purchased from Sigma (St. Louis, MO). Calphostin C was obtained from Calbiochem (La Jolla, CA).

**Murine and human macrophages.** The J774 murine macrophage cell line was obtained from the American Type Culture Collection (Rockville, MD). J774 cells were cultured in DMEM containing 10% FCS (FCS-DMEM) and 100  $\mu$ g/ml penicillin-streptomycin (Flow, McLean, VA). For experiments assessing the effect of increasing concentrations of glucose, we used a customized preparation of FCS-DMEM containing 5.6 mmol/l of glucose to which varying amounts of glucose were added to make up the desired final glucose concentrations.

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CSF, colony-stimulating factor; DEPC, diethyl pyrocarbonate; DMEM, Dulbecco's minimal essential medium; DTT, dithiothreitol; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; LPL, lipoprotein lipase; MDM, monocyte-derived macrophage; PCR, polymerase chain reaction; PDGF, platelet-derived growth factor; PKC, protein kinase C; PMA, phorbol myristate acetate; PMSF, phenylmethylsulfonyl fluoride; TNF, tumor necrosis factor.

Human monocytes were isolated as previously described (29). Peripheral blood mononuclear cells were isolated by density centrifugation using Ficoll, allowed to aggregate in the presence of FCS, then further purified by the rosetting technique. After density centrifugation, we recovered highly purified monocytes (85–90%), as assessed by flow cytometry (FACScan, Becton Dickinson, Rutherford, NJ), using phycoerythrin-conjugated anti-CD14 monoclonal antibody (Becton Dickinson). In some experiments, monocytes were positively selected according to CD14 expression by means of a FACSort (Becton Dickinson). Differentiation of monocytes into monocyte-derived macrophages (MDMs) was achieved by culturing the freshly isolated monocytes in RPMI 1640 medium supplemented with 20% (vol/vol) autologous serum. After 4 days in culture, MDMs were incubated for 1–5 days in fresh RPMI medium containing 5.6 or 30 mmol/l glucose and supplemented with 20% autologous serum. The low and high glucose RPMI media were prepared by adding to glucose-free RPMI medium (Gibco BRL) appropriate amounts of glucose to make up the desired final glucose concentrations.

**Analysis of mRNA expression.** Ten million J774 macrophages were plated in plastic petri dishes (100 × 20 mm) (Falcon, Lincoln Park, NJ). After treatment with appropriate agents, these cells were lysed with guanidine isothiocyanate. Total RNA was purified by centrifugation through a cesium chloride gradient as previously described in detail by Chirgwin et al. (30). Of the total RNA, 18 µg was separated in a 1.2% agarose gel containing 2.2 mol/l formaldehyde as previously described (31). The blots were prehybridized for 18 h in prehybridization buffer. The mRNA expression was analyzed by hybridization with [<sup>32</sup>P]dCTP-labeled LPL (specific activity ~3,000 Ci/mmol; Amersham, Arlington Heights, IL), *c-fos*, and S28 DNA inserts. Hybridization was detected by autoradiography with Kodak X-Omat-AR films (Rochester, NY). mRNA expression was quantified by high resolution optical densitometry (SciScan 5000, USB, Cleveland, OH).

Expression of the LPL gene in glucose-stimulated human MDMs was performed by the polymerase chain reaction (PCR) technique. Cytoplasmic RNA for use in the PCR reaction was extracted from human MDMs by the acid-phenol technique of Chomczynski (32), precipitated, and resuspended in diethyl pyrocarbonate water. cDNA was synthesized from RNA by incubating total cellular RNA with 0.1 µg oligodT (Pharmacia, Piscataway, NJ) for 5 min at 98°C then by incubating the mixture with reverse transcription buffer for 60 min at 37°C. The cDNA obtained was amplified by using 0.8 µmol/l of two synthetic primers specific for human LPL (5'-GAGATTTCTGTATGGCACC-3') (5'-CTGCAAATGAGACTTTTCTC-3') and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (5'-CCCTTCATTGACCTCAACTACATGG-3') (5'-AGTCTTCTGGGTGGCAGTGATGG-3') used as internal standard in the PCR reaction mixture. A 277-base pair human LPL cDNA fragment and a 456-base pair human GAPDH cDNA fragment were amplified enzymatically by 30 repeated cycles at 95°C for 60 s, 60°C for 40 s, and 72°C for 90 s. An aliquot of each reaction mixture was then subjected to electrophoresis on 4.2% PAGE gel followed by autoradiography with Kodak X-Omat-AR films. RNA expression was quantified by high resolution optical densitometry (SciScan 5000, USB).

**DNA binding assay.** The isolation of nuclei was performed as previously described (33). Briefly, 5 × 10<sup>7</sup> J774 cells were collected, washed with cold PBS, and lysed in 1 ml of ice-cold buffer A (15 mmol/l KCl, 2 mmol/l MgCl<sub>2</sub>, 10 mmol/l HEPES, 0.1% phenylmethylsulfonyl fluoride [PMSF], and 0.5% Nonidet P-40). After a 10-min incubation on ice, lysed cells were centrifuged and the nuclei were washed with buffer A without Nonidet P-40. The nuclei were then lysed in a buffer containing 2 mol/l KCl, 25 mmol/l HEPES, 0.1 mmol/l EDTA, and 1 mmol/l dithiothreitol (DTT). After a 15-min incubation period, a dialysis buffer (25 mmol/l HEPES, 1 mmol/l DTT, 0.1% PMSF, 2 µg/ml aprotinin, 0.1 mmol/l EDTA, and 11% glycerol) was added to the nuclei preparation. Nuclei were collected by centrifugation for 20 min at 13,000g. Fifty-microliter aliquots of the supernatants were frozen at -70°C, and protein concentration was determined. DNA retardation (mobility shift) electrophoresis assays were performed as previously described by Fried and Crothers (34). Briefly, 5-µg nuclear extracts were incubated for 15 min in the presence of 5× binding buffer (125 mmol/l HEPES, pH 7.5, 50% glycerol, 250 mmol/l NaCl, 0.25% Nonidet P-40, and 5 mmol/l DTT). End-labeled double-stranded consensus sequences of the LPL promoter AP-1-enhancing element (10,000 cpm per sample) were then added to the samples for 30 min. Samples were analyzed on a 4% nondenaturing polyacrylamide gel containing 0.01% Nonidet P-40. The specificity of the nuclear protein binding was assessed by incubating the nuclear proteins isolated from macrophages with labeled DNA probe in the presence of a 100-molar excess of unlabeled DNA probe.

**DNA probes.** The probe for detection of murine LPL was prepared by PCR. cDNA was obtained from total RNA using a reverse transcription reaction. Two synthetic primers spanning bases 255–287 and 1,117–1,149 of the LPL cDNA were used to enzymatically amplify a 894-base pair region of the LPL probe. The cDNA probes for murine *c-fos* and S28 were purchased from American Type Culture Collection. A 20-mer double-stranded oligonucleotide (5'-GGGCACCTGACTAAGGCCAG-3'; 5'-TGTGCTGGCCTTAGTCAGGT-3') containing the consensus sequence for

the AP-1 responsive element of the murine LPL gene promoter (35,36) was synthesized with the aid of an automated DNA synthesizer. After annealing, the double-strand oligonucleotide was labeled with [<sup>32</sup>P]ATP by using the Boehringer Mannheim (Indianapolis, IN) 5'-end-labeling kit.

***c-fos* oligonucleotide synthesis.** Phosphorothioate-modified oligodeoxynucleotides specific to the 5'-end of murine *c-fos* were synthesized in both the sense (*fos*) and antisense (*sof*) orientation by means of an automatic DNA synthesizer. The sequences were 5'-ATGATGTTCTCGGGTTTC-3' for *fos* and 5'-GAAACCCGAGAATCATCAT-3' for *sof*. After synthesis, oligomers were purified by Guard-Pak C18 cartridges (Waters, Milford, MA), precipitated with 2-propanol, dissolved in water, and quantitated by spectrophotometry.

**Determination of murine and human LPL immunoreactive mass and activity.** Two million J774 cells or human MDMs were incubated for 1–5 days in culture medium containing 5.6 or 30 mmol/l glucose. At 1 h before the end of the incubation period, 0.5 U/ml heparin was added to the medium. The amounts of murine and human LPL immunoreactive mass were measured by enzyme-linked immunosorbent assay (ELISA) as previously described (37,38) and were expressed per milligrams protein per milliliter. Medium and intracellular LPL activity of J774 cells exposed for 5 days to 5.6 or 30 mmol/l glucose was determined using the confluolip kit (Progen, Heidelberg, Germany) (39).

**Determination of TNF-α immunoreactive mass.** J774 cells were cultured for 1–5 days in the presence of 5.6 or 30 mmol/l glucose. At the end of the incubation periods, the quantity of TNF-α immunoreactive mass released in the culture medium of the cells was determined by ELISA (40) and expressed per milligrams protein per milliliter.

**Determination of protein concentrations.** Total protein content was estimated according to the Bradford method (41) by using a colorimetric assay (Bio-Rad, Mississauga, Ontario, Canada).

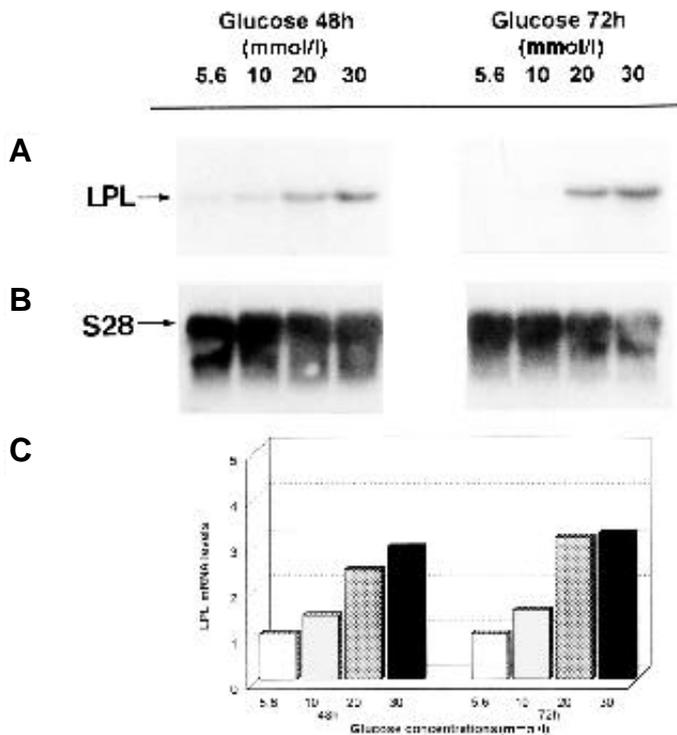
**Statistical analysis.** All values were expressed as the mean ± SE. Data were analyzed by Student's *t* test for single comparisons and by Student-Newman-Keuls test for multiple comparisons.

## RESULTS

**Effect of D-glucose on LPL expression, production, and activity.** Incubation of J774 cells with increasing D-glucose concentrations (5.6, 10, 20, and 30 mmol/l) increased, in a dose-dependent manner, the LPL mRNA expression by these cells (Fig. 1A). Maximal effect was observed after a 48-h incubation period in the presence of 30 mmol/l glucose concentration. Under these experimental conditions, no modulation of the mRNA expression of S28 was observed (Fig. 1B). LPL mRNA levels normalized to the levels of S28 mRNA are illustrated in Fig. 1C.

Recovery of enhanced amounts of LPL immunoreactive mass from the culture media containing high glucose concentrations reflected the increase in LPL mRNA expression of J774 cells exposed to a high glucose environment (Fig. 2A). Maximal levels of LPL mass were observed in J774 cells treated for 4 days with 20 mmol/l glucose. Enhanced TNF-α release by macrophages was also observed after culture of these cells in a high glucose concentration medium. Although significantly elevated after a 24-h and a 48-h incubation period in the presence of 30 mmol/l glucose (data not shown), maximal levels of TNF-α mass were observed after culture of the cells in the presence of 20–30 mmol/l glucose for 3–5 days (Fig. 2B). In J774 cells exposed to high glucose concentrations, a 1.25-fold increase in the levels of medium LPL activity was observed. In contrast, the levels of intracellular LPL activity in these cells remained unchanged (data not shown).

**Effect of D-glucose on human MDM LPL expression and secretion.** To ascertain the physiological relevance of our findings in J774 cells, LPL gene expression and secretion by human MDMs cultured in the presence of 5.6 or 30 mmol/l glucose concentrations were next measured. The stimulatory effect of high glucose concentrations on MDM LPL mRNA levels and immunoreactive mass was observed



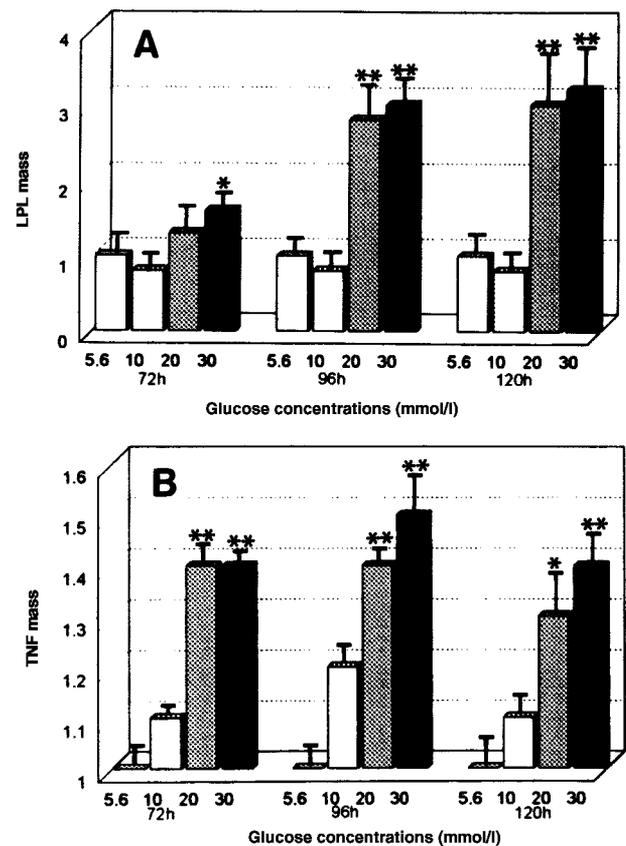
**FIG. 1.** Effect of high glucose concentrations on macrophage LPL mRNA levels. J774 cells were cultured for 48 and 72 h in the presence of increasing D-glucose concentrations. At the end of the incubation period, cells were lysed and total RNAs were extracted and analyzed by Northern blot analysis for LPL mRNA (A) and S28 mRNA (B) expression. C: LPL mRNA levels normalized to the levels of S28 mRNA. Data represent the results of one representative experiment out of six.

after a 5-day exposure of these cells to 30 mmol/l glucose concentration (Fig. 3).

**Effect of L-glucose and mannitol on murine macrophage LPL mRNA expression.** To control for osmolality, J774 cells were incubated for 48 h in the presence of 5.6 or 30 mmol/l L-glucose or mannitol. No demonstrable stimulatory effect of both L-glucose and mannitol was observed on macrophage LPL mRNA expression, thereby indicating a specific effect of D-glucose on macrophage LPL gene expression (LPL mRNA expression [fold increase over control values]: L-glucose, 1.2; mannitol, 1.1).

**Effect of high glucose concentration on basal and PMA-stimulated macrophage *c-fos* mRNA levels.** To evaluate the effects of high glucose concentrations on macrophage *c-fos* mRNA expression, J774 cells were cultured for 48 h in the presence of 5.6 or 30 mmol/l glucose. At the end of these incubation periods, cells were left unstimulated or were incubated in the presence of 50 nmol/l PMA for 30 min. Exposure of unstimulated macrophages to high glucose concentrations significantly enhances basal *c-fos* mRNA levels (*c-fos* mRNA expression [fold increase over control values]: 30 mmol/l glucose,  $2.1 \pm 0.1$ ,  $P < 0.005$ ) (Fig. 4A and C). A marked increase in *c-fos* mRNA levels was also observed in PMA-stimulated cells as compared with those observed in cells exposed to normal glucose concentrations (Fig. 4A and C).

**Effects of *c-fos* antisense oligonucleotide on glucose-induced macrophage LPL gene expression.** To assess

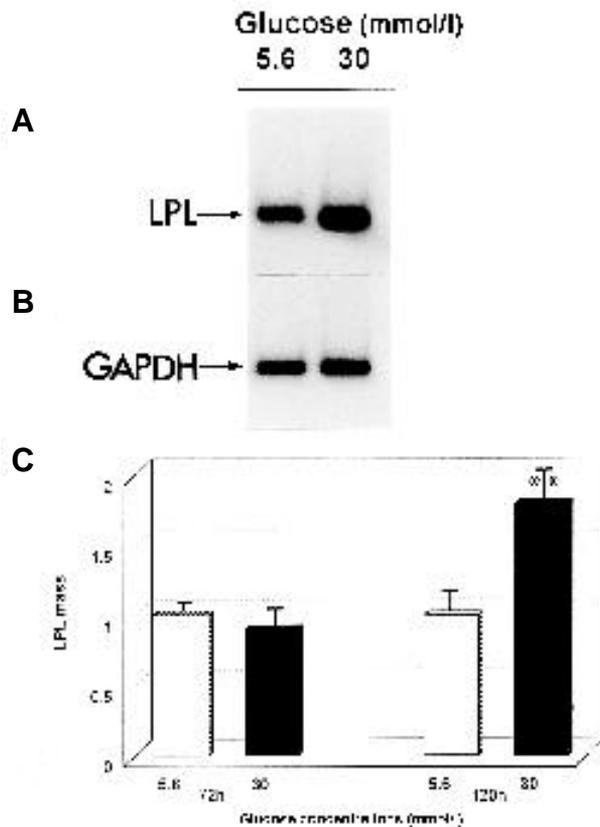


**FIG. 2.** Effect of high glucose concentrations on LPL and TNF- $\alpha$  immunoreactive masses. LPL and TNF- $\alpha$  immunoreactive masses were determined by ELISA in the culture medium of J774 cells exposed for 72, 96, and 120 h to increasing D-glucose concentrations. LPL (A) and TNF- $\alpha$  (B) productions are expressed as fold increase over basal values. Data represent the mean  $\pm$  SE of three experiments. \* $P < 0.05$ , \*\* $P < 0.01$  vs. controls.

the role of *c-fos* in the regulation of macrophage LPL expression by glucose, J774 cells were cultured for 48 h with 5.6 or 30 mmol/l glucose in the presence or absence of *c-fos* sense or antisense oligonucleotides. As shown in Fig. 5, addition of 10  $\mu$ mol/l *c-fos* antisense oligonucleotides to the macrophage culture medium led to a significant inhibition of glucose-stimulated macrophage LPL mRNA expression. Maximal effectiveness of the *sof* oligomer was observed between 5 and 10  $\mu$ mol/l (data not shown). No inhibitory effect of either *c-fos* sense (Fig. 5) or *c-myc* antisense oligonucleotide (data not shown) was observed on glucose-induced LPL expression.

**Effect of calphostin C on glucose-stimulated LPL gene expression.** To determine whether protein kinase C (PKC) may represent the signaling pathway involved in the upregulation of macrophage LPL gene expression by glucose, J774 cells were cultured for 48 h with 5.6 or 30 mmol/l glucose in the presence or absence of the PKC inhibitor calphostin C (0.1  $\mu$ g/ml). As shown in Fig. 6, incubation of the cells in the presence of calphostin C totally abolished the stimulatory effect of glucose on LPL mRNA levels.

**Effect of high glucose concentrations on the binding of nuclear proteins to the regulatory AP-1 sequence of the murine LPL gene promoter.** We next determined whether incubation of J774 cells in the presence of high glucose con-

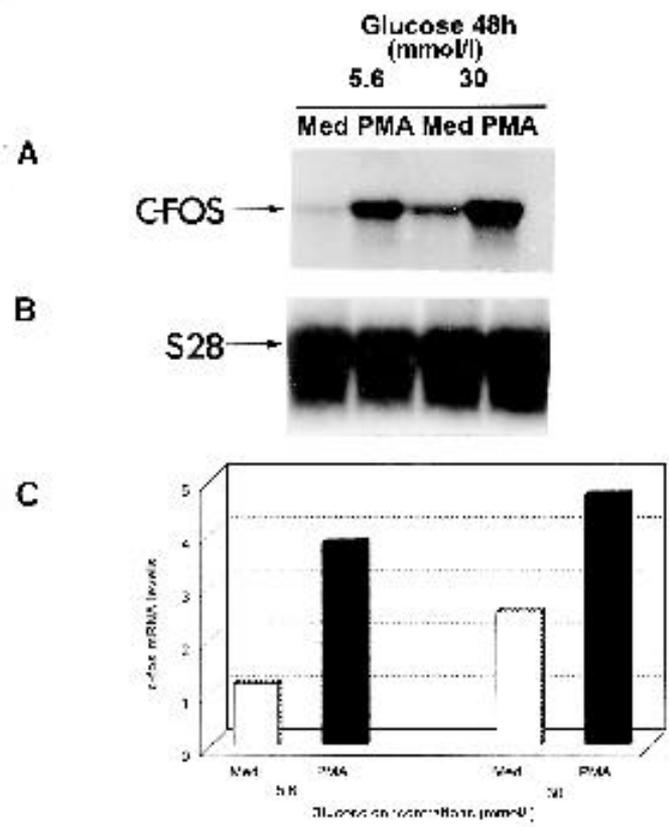


**FIG. 3.** Effect of high glucose concentrations on LPL gene expression and production by human MDMs. Human MDMs were cultured in the presence of 5.6 and 30 mmol/l glucose. After 5 days, cells were lysed and LPL and GAPDH mRNA expression were analyzed by reverse transcriptase-PCR (A and B). Human MDMs were cultured for 72 and 120 h in the presence of 5.6 and 30 mmol/l glucose. At the end of the incubation periods, supernatants were collected and assayed for LPL immunoreactive mass (C). Data represent the mean ± SE of three experiments. \*\**P* < 0.01 vs. controls.

centrations might induce changes at the level of LPL gene promoter binding proteins. We found that a 48-h exposure of these cells to a high glucose environment resulted in a dramatic increase in the binding of nuclear proteins to the AP-1 consensus sequence of the LPL promoter (Fig. 7). This binding complex was specifically competed in the presence of a 100-fold molar excess of the unlabeled AP-1 oligonucleotide. **Regulation of macrophage LPL mRNA half-life by high glucose.** To investigate the possibility that high glucose concentrations might affect the mRNA half-life of macrophage LPL, the half-life of LPL mRNAs was measured in J774 cells cultured for 48 h in 5.6 or 30 mmol/l glucose after the addition of D-actinomycin. As shown in Fig. 8, no difference in the rate of decay of LPL mRNA was observed in cells treated with low or high glucose concentrations, the LPL mRNA half-life being approximated, under both experimental conditions, to 11 h.

**DISCUSSION**

Studies of human LPL regulation by nutritional factors have been limited to adipose tissue and skeletal muscle. With refeeding or glucose/insulin infusion, adipose LPL levels have been shown to increase, while skeletal muscle LPL lev-



**FIG. 4.** Effect of high glucose on macrophage *c-fos* mRNA levels. Macrophages were cultured for 48 h in the presence of 5.6 or 30 mmol/l glucose, then left unstimulated or stimulated with PMA for 30 min. At the end of the incubation period, cells were lysed, and *c-fos* mRNA (A) and S28 mRNA (B) levels were determined by Northern blot analysis. C: LPL mRNA levels normalized to the levels of S28 mRNA. Data represent the results of one representative experiment out of five.

els have been found to decrease (42–44). Apart from being secreted by parenchymal cells of various tissues (45–49), LPL is constitutively expressed and produced by macrophages (10–12). Glucose has been recently identified as a key regulator of macrophage function. A stimulatory effect of high glucose has been reported on macrophage proliferation (23) and production of several cytokines, including TNF-α and IL-6 (25,26). Furthermore, increased CSF-1 and platelet-derived growth factor (PDGF) receptor mRNA expression have been observed in macrophages exposed to a high glucose environment (24,50).

The present study demonstrates that macrophage LPL immunoreactive mass is enhanced by high glucose concentrations and that this effect requires the uptake of glucose by macrophages, since the nonmetabolized glucose isomer failed to produce a similar effect. The importance of this observation is underlined by our finding that high glucose concentrations also stimulate the secretion of LPL immunoreactive mass by human MDMs. Evidence has been provided that high glucose enhances human monocyte TNF-α secretion (26). In accordance with these data, we found that glucose-treated J774 cells and human MDMs (data not shown) also overproduce TNF-α. Our finding that TNF-α induction in these cells parallels that of LPL further supports

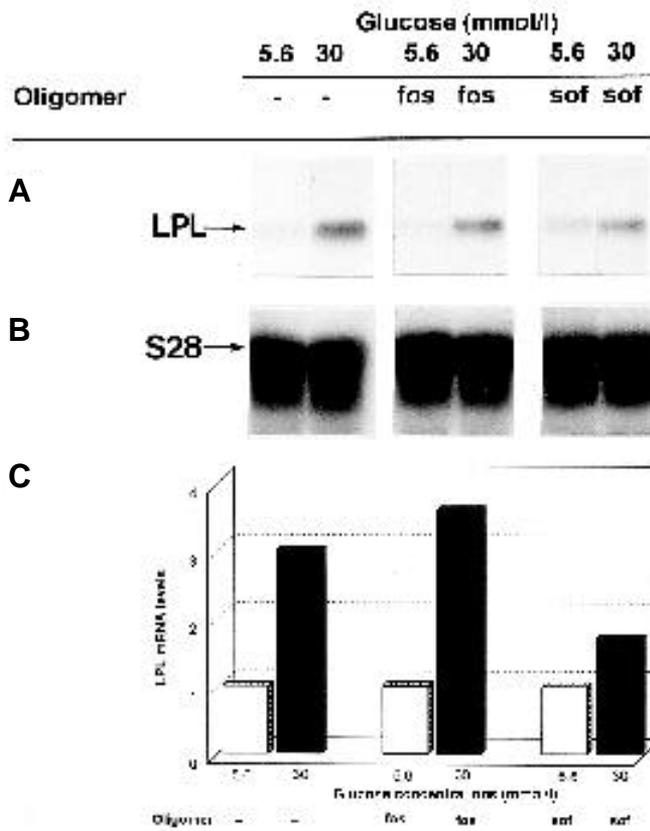


FIG. 5. Effect of *c-fos* antisense on glucose-induced macrophage LPL mRNA levels. J774 cells were cultured for 48 h with 5.6 or 30 mmol/l glucose in the absence or presence of 10  $\mu$ mol/l sense (*fos*) and antisense (*sof*) *c-fos* oligonucleotides. At the end of the incubation period, cells were lysed, and LPL mRNA (A) and S28 mRNA (B) levels were determined by Northern blot analysis. C: LPL mRNA levels normalized to the levels of S28 mRNA. Data represent the results of one representative experiment out of three.

the notion that TNF- $\alpha$  does not suppress LPL secretion in J774 cells and human MDMs (51–52).

Our study also demonstrates that the effect of glucose on macrophage LPL secretion is exerted at the transcriptional level, as reflected by the dramatic increase in LPL mRNA levels after macrophage exposure to high glucose concentrations. It is well known that the regulation of LPL gene expression is controlled by several *cis*- and *trans*-acting factors surrounding the LPL transcriptional start site (53). Evidence of the involvement of *c-fos* in LPL regulation has been recently provided by the observation that antisense *c-fos* inhibits the expression of adipocyte LPL mRNA (28). Based on the finding that high glucose stimulates *c-fos* protein in mesangial cells (27) and on the characterization of an AP-1 site in the regulatory sequences of the murine LPL gene (35,36), we elaborated a tentative model in which *c-fos* protein could mediate the regulatory effect of glucose on macrophage LPL gene expression. Analysis of *c-fos* mRNA levels in macrophages cultured in a high glucose environment demonstrated that glucose enhances *c-fos* mRNA levels in macrophages. The similar kinetic pattern of induction of *c-fos* and LPL gene expression in macrophages exposed to high glucose sug-

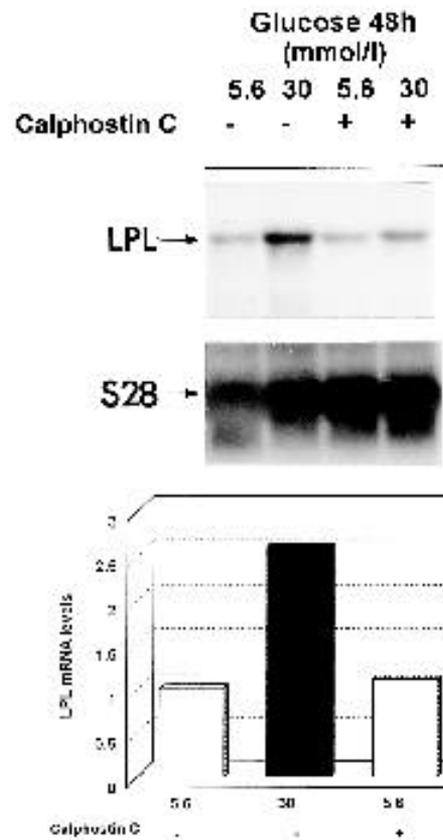


FIG. 6. Effect of calphostin C on glucose-stimulated macrophage LPL mRNA levels. J774 cells were cultured for 48 h with 5.6 or 30 mmol/l glucose in the presence or absence of calphostin C (0.1  $\mu$ g/ml). At the end of the incubation period, cells were lysed, and total RNAs were extracted and analyzed by Northern blot analysis for LPL mRNA (A) and S28 mRNA (B) expression. C: LPL mRNA levels normalized to the levels of S28 mRNA. Data represent the results of one representative experiment out of three.

gested a possible role of *c-fos* in the LPL regulation by glucose. Evidence of the involvement of *c-fos* in the stimulatory effect of glucose on macrophage LPL gene expression was provided by our finding that antisense *c-fos* oligonucleotides significantly inhibit glucose-induced macrophage LPL gene expression. In accordance with the results of Barcellini-Couget et al. (28), we did not observe, even with high oligomer concentration, a complete inhibition of the glucose-induced LPL mRNA levels. These data argue for the involvement of other regulatory pathways besides *c-fos* protein in the modulatory regulation of LPL gene expression by glucose.

Gene expression in eukaryotic cells is governed by nuclear transcription factors. These proteins interact with regulatory DNA elements and may accelerate or retard gene transcriptional rate. AP-1 has been identified as a family of related transcription factors, which frequently consist of either a *c-jun/c-fos* heterodimer or *c-jun/c-jun* homodimers. The presence of an AP-1 site in the regulatory sequence of the murine LPL gene suggests that *c-fos* could enhance the transcriptional rate of the macrophage LPL gene expression by binding to the AP-1 regulatory domain of the LPL gene. The enhanced nuclear protein binding to the AP-1 regulatory

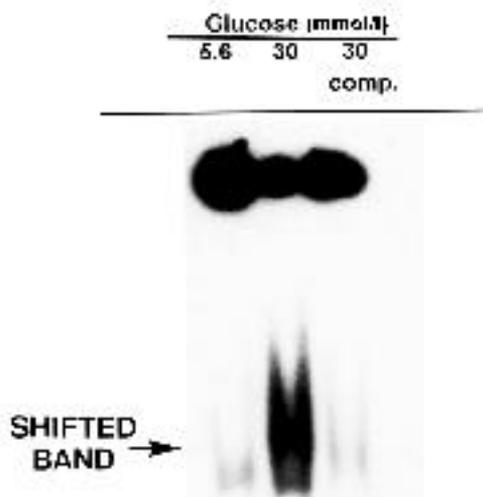


FIG. 7. Effect of high glucose concentrations on the binding of nuclear proteins extracted from J774 cells to the regulatory AP-1 sequence of the murine LPL gene promoter. J774 cells were exposed for 48 h to 5.6 or 30 mmol/l glucose. The nuclear proteins isolated from these cells were incubated with double-stranded AP-1 regulatory element of the LPL gene. Retardation was assessed by gel electrophoresis in 4% polyacrylamide gels. Data represent the results of one representative experiment out of three.

sequence of the LPL promoter that we observed supports this hypothesis. Based on the association between product of the *c-fos* gene and members of the *c-jun* family, an induction of *c-jun* transcript and protein in macrophages by high glucose concentrations can be assumed. The parallel induction of *c-fos* and *c-jun* observed in mesangial cells exposed to high glucose concentrations seems to support this possibility (27).

The activity of inducible transcription factors is regulated by various signal transduction pathways. Transcription of both *c-fos* and *c-jun* is rapidly induced by activators of PKC, leading to an increase in AP-1 activity. Because PKC is activated in several cells, including macrophages, in response to high glucose (28,54–56), it was tempting to postulate that the signal transduction pathway involved in the stimulatory effect of glucose on macrophage LPL mRNA levels could involve PKC. Our data, which show an abrogation of the stimulatory effect of glucose on macrophage LPL production in the presence of the PKC inhibitor calphostin C, support this possibility. From these observations, we propose that exposure of macrophages to high glucose sequentially induces PKC activation and its well-known targets, *c-fos* and *c-jun*, which by interacting with the AP-1 sequence may then increase the transcriptional rate of the LPL gene. Our finding that glucose modulates macrophage LPL mRNA levels is at variance with other data generated in adipocytes showing that high glucose concentrations enhance LPL synthetic rate without affecting LPL mRNA levels (57). While it is well known that LPL regulation is cell and tissue specific, these observations could indicate that the mechanisms responsible for LPL regulation may also markedly differ from one cell system to another.

It is well established that LPL gene expression is controlled at both the transcriptional and posttranscriptional levels. The earliest point where posttranscriptional regulation can occur

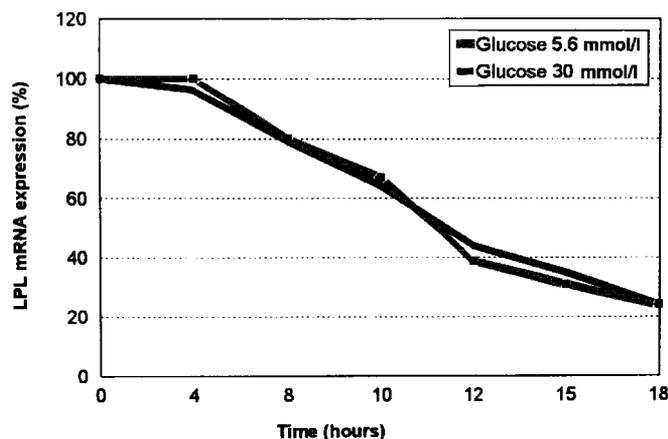


FIG. 8. Effect of high glucose concentrations on the half-life of macrophage LPL mRNA. J774 cells were cultured for 48 h in the presence of 5.6 or 30 mmol/l glucose. Levels of LPL mRNA expression after 4, 8, 10, 12, 15, and 18 h of actinomycin D treatment were calculated and plotted as a percentage expression compared with 100% of LPL mRNA extracted from cells exposed to 5.6 or 30 mmol/l glucose. Data represent the results of one representative experiment out of five.

is at the level of LPL mRNA stability. Our results, which demonstrate identical LPL mRNA half-life in J774 cells cultured under normo- or hyperglycemic conditions, indicate that glucose does not exert its stimulatory effect on macrophage LPL gene expression by enhancing LPL mRNA stability.

Data generated by the current study provide one important piece of information about the secretion of LPL immunoreactive mass by macrophages exposed to a high glucose milieu. Macrophage LPL has been implicated in the pathogenesis of one of the most important complications of diabetes, namely, the atherosclerotic plaque. It has been suggested that this enzyme may favor the uptake of atherogenic lipoproteins by vascular cells and their retention in the vascular wall (14,15). Although the regulation of macrophage LPL in diabetic patients has not yet been investigated, our data suggest that hyperglycemia may tend to increase the in vivo production of LPL by macrophages at vascular sites and that this alteration may contribute to the accelerated atherosclerosis associated with diabetes. This possibility is further supported by the observations that high glucose also increases macrophage receptors for CSF-1 and PDGF, two well-characterized enhancers of macrophage LPL production (58,59).

In conclusion, our data demonstrate that high glucose enhances macrophage LPL secretion. This effect is exerted at the transcriptional level and is, at least partly, mediated by *c-fos* oncogene.

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