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Extracellular Glucose Influences Osteoblast Differentiation and c–Jun Expression

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Abstract Insulin dependent diabetes mellitus, marked by high blood glucose levels and no insulin secretion, is associated with decreased bone mass and increased fracture rates. Analysis of bone histology suggests that osteoblast phenotype and function are influenced by diabetes. To determine if elevated extracellular glucose levels could directly influence osteoblast phenotype we treated mouse osteoblasts, MC3T3-E1 cells, with 22 mM glucose and analyzed osteoblast gene expression. Collagen I mRNA levels significantly increased while osteocalcin mRNA levels decreased 24 h after the addition of glucose. Expression of other genes, actin, osteopontin, and histone H4, was unaffected. Effects on collagen I expression were seen as early as 1 h after treatment. c-Jun, an AP-1 transcription factor involved in the regulation of osteoblast gene expression and growth, was also modulated by glucose. Specifically, an increase in *c-jun* expression was found at 1 h and maintained for 24 h following glucose treatment. Treatment of osteoblasts with an equal concentration of mannitol completely mimicked glucose treatment effects on collagen I and *c-jun* expression, demonstrating that osmotic stress rather than glucose metabolism is responsible for the effects on osteoblast gene expression and phenotype. Additional studies using staurosporine and Ro-31-8220 demonstrate that protein kinase C is required for the glucose up regulation of collagen I and *c-jun.* Taken together, our results demonstrate that osteoblasts respond to increasing extracellular glucose concentration through an osmotic response pathway that is dependent upon protein kinase C activity and results in upregulation of *c-jun* and modulation of collagen I and osteocalcin expression. J. Cell. Biochem. 79:301–310, 2000.

Key words: bone; AP-1; osteoblast; c-jun; diabetes

Insulin dependent diabetes mellitus (IDDM) is a chronic disease stemming from no insulin production and elevated blood glucose levels. IDDM manifests itself with many well-known complications such as vascular abnormalities, retinopathy, nephropathy, and neuropathy. Less well-known, but of major concern, is the association of IDDM with osteoporosis [Auwerx et al., 1988; Krakauer et al., 1995], decreased bone mass [Hui et al., 1985], and increased fracture rates [Bouillon et al., 1991]. Histochemical studies demonstrate a decreased number of early stage and mature osteoblasts in diabetic bone [Verhaeghe et al., 1990; Sasaki et al., 1991]. These changes cannot be accounted for by altered calcium and vitamin D metabolism [Shires et al., 1980]. These findings suggest that modulation of the regulation of osteoblast growth and differentiation is potentially a key component of the bone loss associated with diabetes.

Diabetes is diagnosed by a blood glucose level greater than 200 mg/dL (11 mM) at any random testing [Emancipator et al., 1999]. Elevated extracellular glucose has been shown to cause significant cellular effects including: 1) nonenzymatic glycosylation of intracellular and extracellular proteins and DNA [Brownlee et al., 1984; Bucala et al., 1984; Locatto et al., 1993; McCarthy et al., 1997; Katayama et al., 1996], 2) modulation of cellular redox state [Hunt et al., 1990; Wolf and Dean, 1987], 3) changes in cell metabolic pathways such as activation of the polyol pathway [Inaba et al., 1997; Gabbay et al., 1973; Larkins and Dunlop, 1992], and 4) activation of protein kinase C [Lee et al., 1989; Wolf et al., 1991; Craven et al., 1990; Ceolotto et al., 1999]. These changes and perhaps other unidentified changes in signaling pathways and transcription factor activities can directly affect cell growth, differenti-

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ation, and function. Only a few studies have addressed the influence of glucose on osteoblast function. All suggest that an elevation in extracellular glucose levels can influence osteoblast function. For example, Katayama et al. [1996] have shown that culturing of osteoblasts on glycosylated collagen I substrates can variably suppress alkaline phosphatase activity and osteocalcin secretion. In addition to matrix effects, direct effects of glucose on osteoblast responsiveness and gene expression have been suggested. When human osteoblasts (MG-63 cells) are exposed to elevated glucose levels, parathyroid hormone (PTH), vitamin D, and insulin-like growth factor (IGF-1) responsiveness are impaired [Yoshida et al., 1995; Inaba et al., 1995; Terada et al., 1998].

The AP-1 family of transcription factors has been shown to play an important role in the coupling of gene expression to changes in the environment [Morgan and Curran, 1991; Angel and Karin, 1991]. This family includes c-Fos, Fos B, Fra-1, Fra-2, c-Jun Jun B, and Jun D. Fos:Jun heterodimers and Jun:Jun homodimers bind to a DNA consensus response element designated as an AP-1 site (5′-TGAg/cTCA-3′) [Angel et al., 1991]. Changes in the level of AP-1 member expression and post-translational modifications play an important role in regulating AP-1 DNA binding and transactivation [McCabe et al., 1996; Gruda et al., 1994; Smeal et al., 1992; Yamamoto et al., 1992].

Osteogenic hormones, bone strain and fracture, and Fos/Jun over-expression stimulate both AP-1 member expression and bone formation implicating this family of transcription factors in the regulation of osteoblast growth and differentiation [McCabe et al., 1996; Hadman et al., 1993; Candeliere et al., 1991; Clohisy et al., 1992; Koe et al., 1997; Merriman et al., 1990; Breen et al., 1994; Machwate et al., 1995; Ohta et al., 1991, 1992; Mikuni-Takagaki, 1999]. This is not surprising seeing as osteoblasts must be highly responsive to extracellular changes such as bone strain and calcium and nutrient status within the body and must react immediately by modulating growth, differentiation, and gene expression. Exactly how osteoblasts respond to the stress of increased extracellular glucose is not known. Previous reports in other cell types suggest that protein kinase C activity, an activator of AP-1 expression, is elevated in response to glucose treatment [Lee et al., 1989; Wolf et al., 1991; Craven et al., 1990; Ceolotto et al., 1999]. Furthermore, Kreisberg et al. [1994] have shown that 1 h after glucose treatment mesangial cells have increased *c-fos* and *c-jun* expression, suggesting that AP-1 may play a role in cellular responsiveness to glucose. These findings led us to speculate that diabetes associated changes in osteoblast phenotype could at least in part be the result of altered AP-1 family member expression which ultimately leads to changes in gene expression. Our results support this hypothesis and demonstrate that osteoblasts are sensitive to changes in extracellular glucose and respond through an osmotic stress pathway involving protein kinase C, chronic induction of c-Jun expression, and altered osteoblast phenotype.

MATERIALS AND METHODS

Cell Culture System

MC3T3-E1 cells [Sudo et al., 1983] were plated at 100,000 cells per 100 mM dish and fed every 2 days with alpha MEM (Gibco; Grand Island, NY) containing 5.5 mM glucose (normal level) and supplemented with 10% fetal calf serum. The typical concentration of insulin in the culture media is obtained from the serum component and ranges between 1 and 5 picomole concentration, a level lower than physiologic (50–800 pM), but consistent with early development of IDDM. Eleven days after plating and 24 h after the last feeding, glucose or mannitol (0.5M stock) were added directly to the media in the tissue culture dish to yield the final concentrations of sugar noted for each experiment (7.5–22 mM). For kinase inhibition studies, 30 min prior to addition of sugar the cells were pre-treated with 50 nM of staurosporine or 200 nM of Ro-31-8220, concentrations lower than those previously reported to inhibit protein kinase C [Nose and Shibanuma, 1994; Beltman et al., 1996].

RNA Analysis

Twenty-four h after feeding, glucose or mannitol (0.5M stock) was added directly to the media in the tissue culture dish to yield a final monosaccharide concentration of 7.5 to 22 mM. Cells were washed and scraped in PBS at the indicated times following treatment. Cells were centrifuged at 800*g* for 5 min and quick frozen in liquid nitrogen. Total RNA was ex-

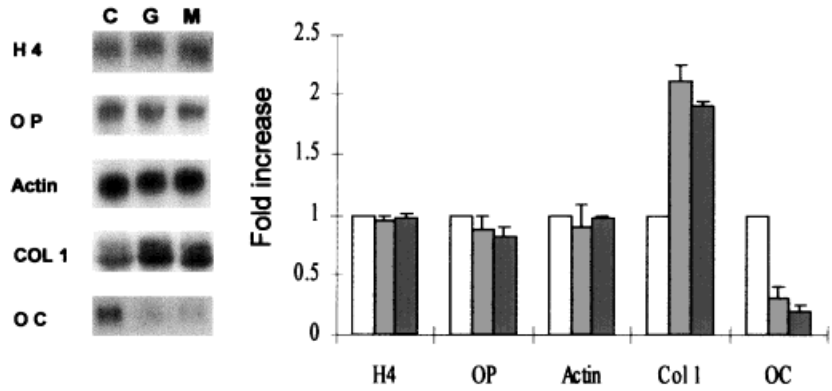

Fig. 1. Elevated levels of extracellular glucose or mannitol influence osteoblast gene expression 24 h after treatment. Osteoblasts were fed every 2 days with media containing 5.5 mM glucose. On day 11 (24 h after the last feeding) glucose (G, 16.5 mM) or mannitol (M, 16.5 mM) were added directly to the tissue culture media of confluent cultures of osteoblasts to yield a final concentration of 22 mM monosaccharide. Control plates (C) were treated with PBS. Twenty-four h after treatment the cells were harvested for RNA. Autoradiographs of Northern blot membranes hybridized with cDNAs to histone H4 (H4), osteopontin (OP), actin, collagen I (COL I), and osteocalcin (OC) are shown. Levels of mRNAs (as determined by phosphoimager analysis) for control (white), 22 mM glucose (gray), or 22 mM mannitol (dark gray) treated cells were expressed relative to ribosomal 18S subunit RNA levels and are graphed as a fold increase relative to control levels set at 1. Values were obtained from three to seven separate experiments and are expressed ± SE.

tracted and analyzed by Northern blot, as previously described [McCabe et al., 1995; Chomczynski and Sacchi, 1987]. Northern blots were hybridized with random primed (Random primed DNA labeling kit, Gibco) 32P-labeled complementary DNA probes specific for each AP-1 family member (generously provided by Dr. Rodrigo Bravo) and to cDNA probes for markers of osteoblast differentiation [McCabe et al., 1995, 1996; Lian and Stein, 1992]. Hybridization signals were quantitated by phosphoimaging analysis and expressed relative to 18S ribosomal subunit levels.

Statistical Analysis

All statistical analyses were performed using Microsoft excel data analysis program for *t*-test analysis. Experiments were repeated at least three times unless otherwise stated. The autoradiographs shown are of one representative experiment. Values are expressed as a mean ± SEM except where indicated.

RESULTS

The use of an osteoblast cell line, MC3T3-E1 cells, which exhibits gene expression in developmental manner similar to primary rat osteoblasts and bone in vivo [Quarles et al., 1992; Rodan et al., 1988], allowed us to examine modulation of one factor associated with diabetes, glucose, while leaving insulin levels constant. Previously we found that chronic treatment of osteoblasts with 22 mM glucose, a level seen in untreated diabetics, results in decreased mineralization [Zayzafoon et al., 1998]. To determine if osteoblasts respond to acute glucose treatment, MC3T3-E1 cells were treated (24 h after feeding/media change) with 16.5 mM glucose, which was directly added to the tissue culture medium. Cells were harvested at 1 and 24 h after treatment. Within 24 h of treatment gene expression was clearly modulated when compared to control cultures that have 5.5 mM glucose (normal in vivo and in vitro levels). Specifically, the level of collagen I mRNA was significantly increased by greater than two-fold (Fig. 1). In contrast, the level of osteocalcin mRNA was decreased to 30% of control levels. Osteopontin, actin, and histone H4 expression was unaffected (Fig. 1). A two-fold increase in collagen I expression was seen within 1 h of treatment (Fig. 2) demonstrating that collagen I expression is highly and rapidly responsive to elevation in extracellular glucose levels. In addition, the influence of extracellular glucose on collagen I expression was detectable with as little as 4.5 mM glucose addition (data not shown), a level that would be commonly seen in a diabetic patient.

Based on the role of AP-1 in immediate response of cells to extracellular stimuli [Morgan and Curran, 1991; Angel and Karin, 1991] and in

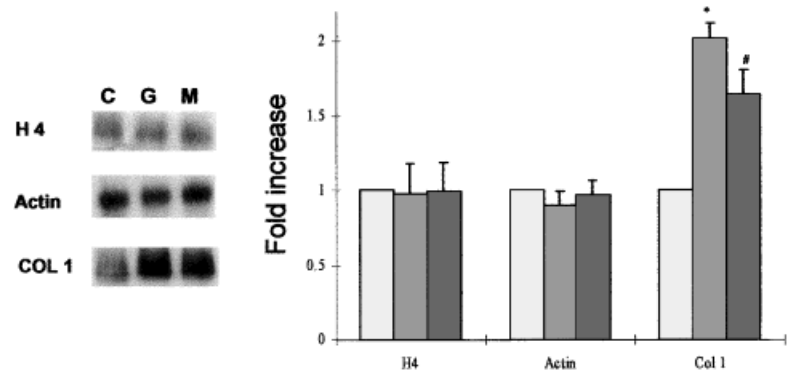

Fig. 2. Elevated levels of extracellular glucose or mannitol influence osteoblast gene expression 1 h after treatment. Osteoblasts were fed every 2 days with media containing 5.5 mM glucose. On day 11 (24 h after feeding) glucose (G, 16.5 mM) or mannitol (M, 16.5 mM) were added directly to the tissue culture media of confluent cultures of osteoblasts to yield a final concentration of 22 mM monosaccharide. Control plates (C) were treated with PBS. One h after treatment the cells were harvested for RNA. Autoradiographs of Northern blot membranes hybridized with cDNAs to histone H4 (H4), actin, and collagen I (COL I) are shown. Levels of mRNAs (as determined by phosphoimager analysis) for control (white), 22 mM glucose (gray), or 22 mM mannitol (dark gray) treated cells were expressed relative to ribosomal 18S subunit RNA levels and are graphed as a fold increase relative to control levels set at 1. Values were obtained from three to seven separate experiments and are expressed ± SE; $*P < 0.01$, $\#P < 0.05$.

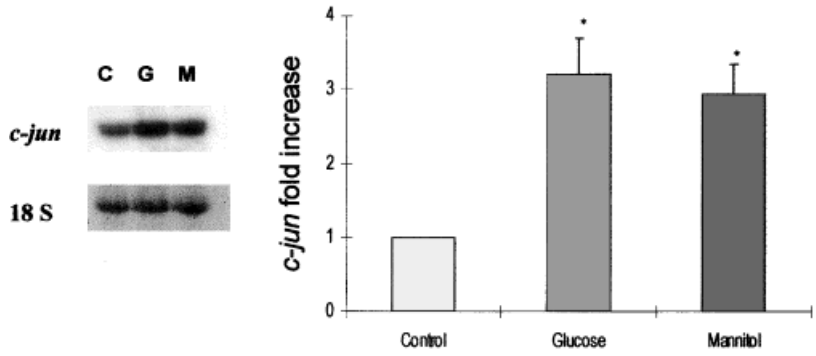

Fig. 3. Elevated extracellular glucose levels stimulate c-jun expression 1 h after treatment. On day 11 osteoblasts were treated with 16.5 mM glucose or mannitol to yield a final concentration of 22 mM. Cells were harvested 1 h after addition of sugar for RNA extraction. The autoradiograph was obtained a Northern blot hybridized to a cDNA specific for *c-jun.* The membrane contains RNA from control (C), 22 mM glucose (G), or 5.5 mM glucose + 16.5 mM mannitol (M) treated osteoblasts. Two bands were detected, 3.2 and 2.6 kb, as has been previously reported. Levels of *c-jun* mRNA (as determined by phosphoimager analysis) were expressed relative to ribosomal 18S subunit RNA levels and graphed relative to control values set at 1: control (white), 22 mM glucose (gray), or 22 mM mannitol (dark gray). Values were obtained from six separate experiments and are expressed ± SE; $*P < 0.01$.

the regulation of osteoblast growth, differentiation, and gene expression [McCabe et al., 1996] we next examined whether AP-1 family member expression in osteoblasts was modulated by glucose treatment. MC3T3-E1 cells were treated with 16.5 mM glucose for 1 h. Northern blot analysis demonstrates a greater than three-fold induction of *c-jun* expression (Fig. 3), a rapid and transient induction in *c-fos* which is not always detectable by Northern blot, and a small but not statistically significant induction of *fra-1* (data not shown). RNA levels of other AP-1 members (*jun B, jun D, fos B, fra-2*) did not change. To examine the characteristics of the *c-jun* response a complete time course was performed with osteoblasts being harvested at 0, 0.5, 1, 3, 8, and 24 h following the addition of 16.5 mM glucose. Figure 4 demonstrates that the induction of *c-jun* was maximal at 0.5–1 h and was maintained even 24 h after the addition of glucose. This response is unlike a serum response where *c-jun* levels rapidly decline after 1 h [McCabe et al., 1995]. Thus, glucose treatment leads to long-term changes in transcription factor levels.

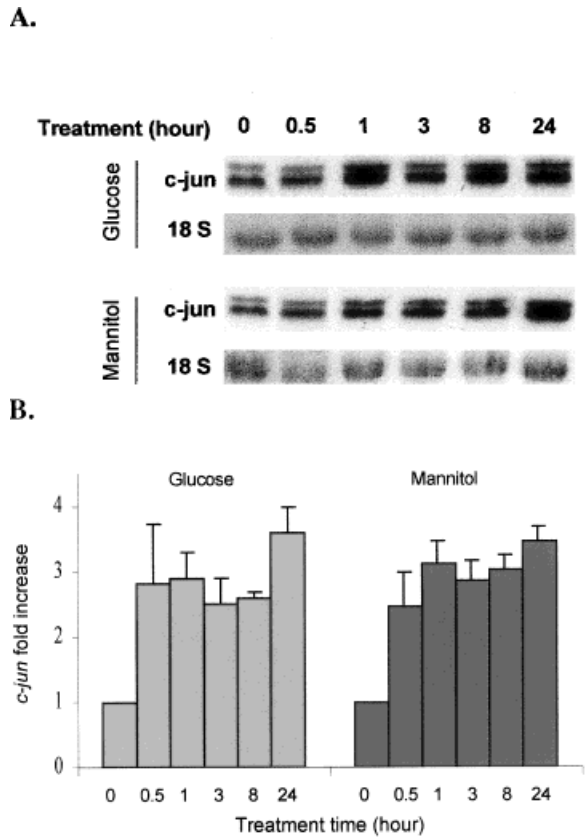

Fig. 4. Glucose and mannitol treatment results in chronic elevation of *c-jun* expression. Osteoblasts were fed every 2 days with media containing 5.5 mM glucose. At day 11 osteoblasts were treated with 16.5 mM glucose or mannitol to yield a final concentration of 22 mM. Cells were harvested at 0, 0.5, 1, 3, 8, and 24 h after the addition of sugar for RNA extraction. **A:** A representative autoradiograph containing RNA from glucose or mannitol treated osteoblasts hybridized to a cDNA specific for *c-jun* or 18 S ribosomal subunit RNA. **B:** Levels of *c-jun* mRNA (as determined by phosphoimager analysis) from glucose treated (gray) or mannitol treated (dark gray) osteoblasts were expressed relative to ribosomal 18S subunit RNA levels and graphed as a fold increase relative to basal control (0 h) levels. Values were obtained from three separate experiments and are expressed ± SE.

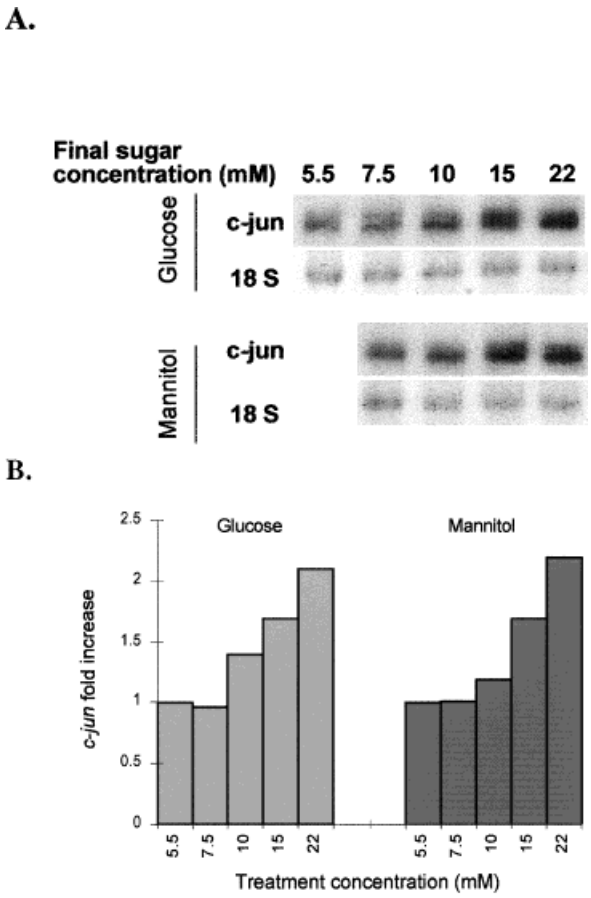

Fig. 5. Induction of c-jun by glucose or mannitol is concentration dependent. Osteoblasts were fed every 2 days with media containing 5.5 mM glucose. At day 11 (24 h after feeding) osteoblasts were treated with additional glucose or mannitol to yield a final sugar concentration of 7.5, 10, 15, or 22 mM. Cells were harvested 1 h after the addition of sugar and RNA extracted. **A:** A representative autoradiograph of a Northern blot containing RNA from glucose or mannitol treated osteoblasts hybridized to a cDNA specific for *c-jun* or 18 S ribosomal subunit RNA. **B:** Levels of *c-jun* mRNA (as determined by phosphoimager analysis) from glucose treated (gray) or mannitol treated (dark gray) osteoblasts were expressed relative to ribosomal 18S subunit RNA levels and graphed as a fold increase relative to basal control (5.5 mM) levels. Values were obtained from two separate experiments.

To determine if the effect on *c-jun* expression was concentration dependent, osteoblasts were treated with 2, 4.5, 9.5, and 16.5 mM glucose (final concentration equal to 7.5, 10, 15, and 22 mM glucose, respectively) and *c-jun* mRNA levels were examined 1 h after treatment. Figure 5 demonstrates that as little as 4.5 mM of glucose added to osteoblasts is enough to stimulate *c-jun* mRNA levels. This level of glucose (10 mM final concentration; 180 mg/dL) as well as the addition of 9.5 mM glucose (15 mM final concentration; 270 mg/dL) is often seen in patients who are diagnosed with diabetes.

Although the levels of glucose used in this study are not normally used to study osmotic effects on cells, we still wanted to determine if any component of the osteoblast response results from osmotic stress. Therefore osteoblasts were also treated with mannitol, a nonabsorbable sugar, to control for osmotic stress. Surprisingly, Figures 1, 2, 3, and 4 demonstrate that mannitol can completely mimic the effects seen with glucose. The influence of mannitol on *c-jun* was seen with addition of as little

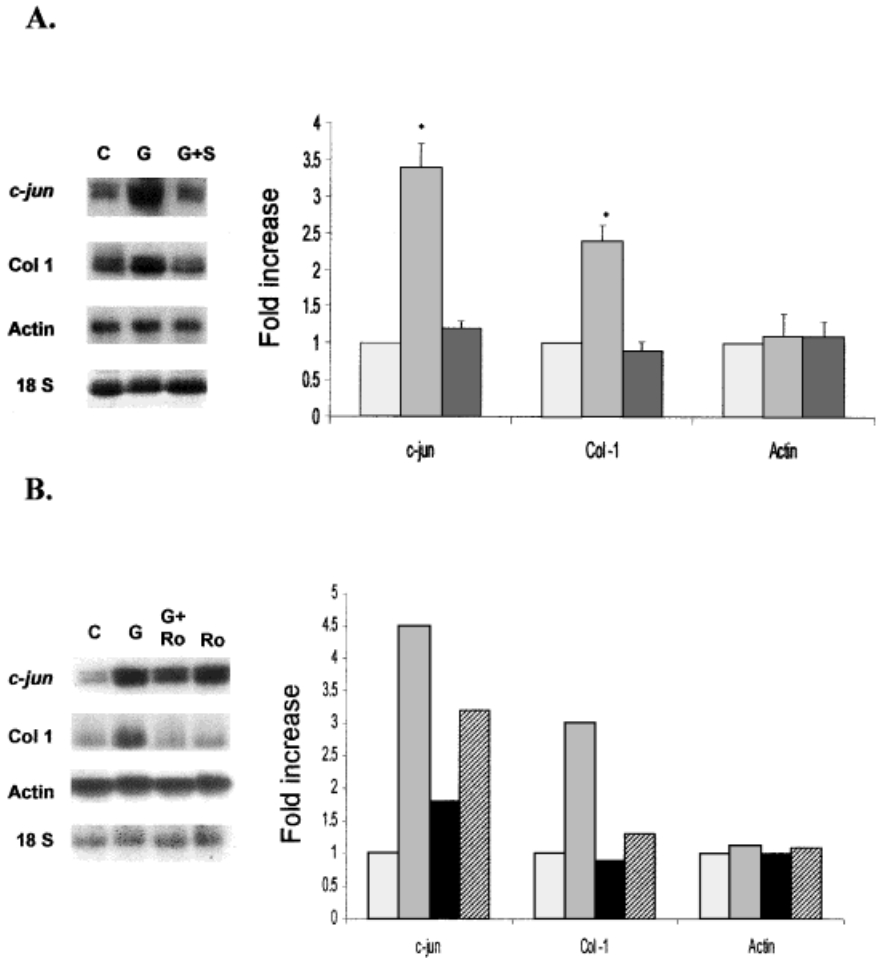

Fig. 6. Inhibition of protein kinase C blocks glucose induction of c-jun and collagen I expression. Osteoblasts were fed every 2 days with media containing 5.5 mM glucose. At day 11 (24 h after feeding) osteoblasts were pretreated for 30 min with 50 nM staurosporine (**A**) or with 200 nM of Ro-31-8220 (**B**). Cells were then treated with 16.5 M glucose. After 1 h cells were harvested and RNA extracted. A: Representative autoradiograhs of a Northern blot containing RNA from control (5.5 mM glucose), glucose (22 mM final sugar concentration), and glucose + staurosporine treated cells. Levels of *c-jun,* collagen I (COLI), and actin mRNA were quantitated (by phosphoimager analysis) from control (white), glucose treated (gray), or glucose + staurosporine treated (dark gray) cells. Values (± SE) were expressed relative to 18S rRNA levels and expressed relative to control levels set at 1; n = 3, $*P < 0.02$. Values for staurosporine alone treated cells did not differ from controls (data not shown). B: Representative autoradiographs of a Northern blot containing RNA from control (5.5 mM sugar), glucose (22 mM final sugar concentration), glucose + Ro-31-8220, and Ro-31-8220 alone treated cells. Levels of *c-jun,* collagen I (COLI), and actin mRNA were quantitated from control (white), glucose treated (gray), glucose + Ro-31-8220 treated (dark gray), or Ro-31-8220 alone treated (diagonal lines) cells and expressed relative to control cells; n = 2.

as 4.5 mM glucose. These results demonstrate that osmotic stress is the primary conveyor of the glucose-induced changes in gene expression shown in Figures 1, 2, 3, 4 and 5.

To begin to address the signaling pathways involved in the osteoblast response to glucose we treated MC3T3-E1 cells with glucose in the presence of a protein kinase C inhibitor, staurosporine. Northern blot analysis demonstrated that inhibition of protein kinase C blocked the induction of collagen I and *c-jun* expression after 1 h of glucose treatment (Fig. 6A) while actin expression was not affected. Ro-31-8220, another inhibitor of protein kinase C, also suppressed the influence of glucose on *c-jun* and collagen I expression (Fig. 6B). Treatment with Ro-31-8220 alone had no effect on basal collagen I expression but did enhance *c-jun* expression as has previously been reported [Beltman et al., 1996]. These findings further support an important role for protein kinase C in the osteoblast response to osmotic stress.

DISCUSSION

Diabetes (IDDM) is marked by high glucose levels and is associated with bone loss, decreased osteoblast number, and decreased osteoid surface suggesting that osteoblast phenotype is altered. Few studies have examined the influence of increasing extracellular glucose on osteoblast growth, differentiation, and gene expression. Our results demonstrate that modulation of extracellular glucose concentration has an immediate and significant effect on osteoblast gene expression 24 h after treatment: c-jun and collagen I expression is elevated while osteocalcin expression is decreased. Treatment with mannitol, a nonabsorbable sugar, completely mimics the effects of glucose hence the role of glucose metabolites can also be excluded and the role of osmotic stress can be concluded as a primary inducer of the effects seen on gene expression. This concept may be specific for osteoblasts, which may be more sensitive to this form of stress than other types of cells. Osteoblast sensitivity to osmotic stress is demonstrated by the small addition of (4.5–16.5 mM) of either glucose or mannitol to osteoblasts, which results in increase in *c-jun* expression and in collagen I expression. Elevation of extracellular glucose levels to 10–30 mm has been shown to influence gene expression in other cells including mesangial cells [Ingram et al., 1999], monocytes [Manduteanu et al., 1999], and fibroblasts [Benazzoug et al., 1998], potential mechanisms causing these changes are thought to stem from metabolic effects although effects on monocytes involve an osmotic component [Manduteanu et al., 1999].

Examination of glucose transporter expression by osteoblasts further supports a role for increasing extracellular glucose concentration in causing osmotic stress. It is known that osteoblasts do not express Glut 2 (the only high Km, 20 mM, glucose transporter) [Waeber et al., 1994] but do express Glut 1 and Glut 3 glucose transporters [Thomas et al., 1996a, b], which are known to have low Kms (1–2 mM). Thus, entry of glucose into the osteoblast is not significantly affected by increases in extracellular glucose [Zayzafoon et al., unpublished results]. Therefore, an elevation in extracellular glucose should cause an osmotic stress that could be continuous or the osteoblast may adapt by generating osmolytes or modulating water and ion channel transport.

Increased expression of collagen I and other matrix mRNAs has been previously reported for other cell types exposed to glucose including mesangial cells [Ziyadeh et al., 1995] and fibroblasts [Han et al., 1999; Benazzoug et al., 1998]. Consistent with our results, the promoter of collagen I is known to contain a functional AP-1 site [Chung et al., 1996]. Yet an elevation in collagen I expression is somewhat contradictory to histological findings of decreased osteoid surface in diabetic animals [Verhaeghe et al., 1990; Hough et al., 1981]. One possibility is that collagen protein synthesis, processing, or secretion is down regulated or altered by glucose treatment (currently under examination) thereby negating the elevation in mRNA expression. In addition our results are focused on the specific modulation of extracellular glucose only, while in vivo findings may involve the additional effects of chronic exposure and the modulation of other factors including insulin, IGF-1, and amylin. Changes in these and other factors could lead to a more complexly developed phenotype.

In contrast to the stimulation of collagen I expression, osteocalcin expression was suppressed by elevation in extracellular glucose. The osteocalcin promoter contains several AP-1 sites that have been demonstrated to be functional. c-Jun exhibits binding activity at these sites and when over expressed can suppress osteocalcin transcription [McCabe et al., 1996]. This suppression is thought to result, at least in part, by the competition with osteocalcin inducing transcription factors (such as the vitamin D receptor) for overlapping promoter binding sites [Lian et al., 1991]. Thus, suppression of osteocalcin expression in response to increased glucose is consistent with the elevation in *c-jun* expression, which may be directly suppressing osteocalcin expression.

Activation of protein kinase C in diabetic tissue is a well-known outcome. It is hypothesized that metabolism of glucose, through the pentose phosphate shunt or through glycerolipid production, leads to an increase in diacylglycerol and subsequently protein kinase C activation. Our studies demonstrate that PKC is also involved in a nonmetabolic pathway involving osmotic stress. Specifically, induction of *c-jun* and collagen I mRNA levels by either glucose (Fig. 5) or mannitol (data not shown) can be blocked by inhibitors of protein kinase

C, staurosporine and a more specific inhibitor Ro-31-8220.

Given that protein kinase C is a major activator of AP-1 expression and transactivation, it is not surprising that we found an elevation in *c-jun* expression in osteoblasts treated with increasing concentrations of extracellular glucose. In addition, the c-jun promoter contains an AP-1 site that allows for positive autoregulation. In mesangial cell cultures, Kreisberg et al. [1994] also report an increase in *c-jun* mRNA levels as well as *c-fos* mRNA levels at 0.5 and 2 h after glucose treatment. Our time course studies clearly demonstrate a long-term induction in *c-jun* mRNA expression, which is supported by an elevated *c-jun* signal at 24 h in the study by Kreisberg et al. [1994]. In two experiments we were also able to detect an induction of *c-fos* mRNA expression at 0.5 h after glucose treatment. However, this increase was not found in subsequent experiments suggesting that it is highly rapid and transient.

Given that the expression pattern of c-Jun and collagen I is highest in proliferating osteoblasts while osteocalcin is a marker of mature osteoblasts, our results suggests that glucose treated osteoblasts may be "reverting" to a less differentiated state thereby decreasing mineralization and ultimately bone mass in vivo. Since both collagen I and osteocalcin promoters contain AP-1 sites which are respectively positively and negatively responsive to c-Jun, it is highly possible that induction of *c-jun* by glucose is involved in the effects on these markers of osteoblast phenotype.

Taken together our findings demonstrate that an additional effect of glucose, osmotic stress, should be considered to contribute to the complications of diabetes with regard to osteoblasts. Osteoblasts are highly sensitive to osmotic stress and respond through a protein kinase C dependent pathway that results in an increase in *c-jun* and collagen I expression and a decrease in osteocalcin expression. Future studies will determine the precise role of c-Jun and the signaling pathways involved in the changes in osteoblast gene expression.

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