Chelation of Extracellular Zinc Inhibits Proliferation in 3T3 Cells Independent of Insulin-Like Growth Factor-I Receptor Expression[1] (44317)

WILLIAM H. THORNTON, JR.,[2] RUTH S. MACDONALD, LAVONNA C. WOLLARD-BIDDLE, JIMMY D. BROWNING, AND BOYD L. O'DELL

Department of Food Science and Human Nutrition, and Department of Biochemistry, University of Missouri, Columbia, Missouri 65211

Abstract. **Depletion of zinc inhibits growth in animals and proliferation of cultured cells. Additionally, zinc can serve as an antioxidant protecting many compounds, including proteins, from oxidation. Regulation of cell division also involves insulin-like growth factor type I (IGF-I) and its receptor, especially during late G_1 phase, allowing progression of the cell to S phase with subsequent DNA synthesis. We examined the effects of zinc depletion from the culture media of Swiss 3T3 cells on the cell cycle and IGF-I receptor expression. Cells were exposed to reduced fetal bovine serum concentrations to induce growth arrest, then returned to normal fetal bovine serum concentrations with the divalent cation chelator diethylenetriamine pentaacetic acid. Reducing the fetal bovine serum concentration did not induce quiescence in the cells as previously suggested. Zinc depletion reduced the proliferative fraction (S and G_2/M phases) of the cell cycle. The addition of glutathione to the zinc-depleted media partially returned the proliferative fraction to the control level. Fetal bovine serum deprivation reduced IGF-I receptor expression whereas the absence of zinc had little effect on receptor expression. We conclude that depletion of zinc from culture media inhibits 3T3 cell proliferation independent of insulin-like growth factor-I receptor expression, and part of this inhibition is due to the antioxidant capacity of this divalent cation.** [P.S.E.B.M. 1998, Vol 219]

Zinc is an essential micronutrient required for cell division. In the 3T3 cell line, zinc is required for maintenance of a protein necessary for progression to S phase (1). Thymidine kinase activity is reduced in the absence of zinc (2). Additionally, zinc is essential for the proper function of a long tRNA synthetase required for cell growth (3). Zinc is also required for maintenance of cell membrane integrity, protecting the plasma membrane of mammalian cells (4). Endothelial cells cultured in media containing 1% fetal bovine serum (FBS) with low zinc concentrations showed a reduced membrane barrier function that was completely restored when zinc concentrations were increased by supplementation (5).

Insulin-like growth factor type I (IGF-I) is also essential for progression of cell division from late G_1 through S phase. The requirement of this growth factor has been demonstrated in competent cells (i.e., cells in G_0 that have been primed by the addition of serum rich in other growth factors such as platelet-derived growth factor (6) and epidermal growth factor (7)). In exponentially growing 3T3 cells, IGF-I was required to initiate DNA synthesis, acting post-transcriptionally late in G_1 (8). Insulin-like growth factor acts *via* its membrane receptor, which phosphorylates tyrosine residues of several intracellular proteins including insulin receptor substrate-1 and c-Crk (9). Expression of the IGF-I receptor (IGF-IR) is key to eliciting a mitogenic re-

[1] Presented in part at Experimental Biology 96, (Thornton, WH Jr, Wollard, LC and MacDonald, RS. Deprivation of zinc in 3T3 cells alters proliferation and IGF-I binding leading to G_1 arrest. FASEB J **10:**A530, 1996.)

[2] To whom requests for reprints should be addressed at Dept. of Food Science and Human Nutrition, 122 Eckles Hall, University of Missouri, Columbia, MO 65212.

Received January 12, 1998. [P.S.E.B.M. 1998, Vol 219]
Accepted May 28, 1998.

0037-9727/98/2191-0064$10.50/0

sponse. A cell line derived from BALB/c 3T3 cells has been developed that overexpresses IGF-IR (10). Compared to the parent cell line, IGF-IR overexpression in the daughter cell line required a significantly shorter time of exposure to serum-containing medium. In a mouse skeletal myoblast line transfected with the human IGF-IR gene and expressing high levels of the protein, supplementation of medium with IGF-I prevented differentiation but stimulated proliferation (11). Disruption of the IGF-IR gene in a fibroblast cell line led to growth arrest in media supplemented with growth factors whereas wild type cells grew normally (12).

The interaction between zinc and IGF-I in cell proliferation has been demonstrated in the osteoblastic cell line MC3T3-E1 (13). Addition of IGF-I to the media increased DNA synthesis, and this increase was significantly enhanced by the addition of zinc sulfate (13). Supplementing the media with zinc sulfate or β-analyl-L-histidinato zinc also induced an increase in IGF-I secretion by this same osteoblast cell line (14). Activation of protein kinase C with 12-O-tetradecanoylphorbol-13-acetate inhibited secretion of IGF-I into the media while increasing IGF-IR expression (15). When dibutyryl cAMP was used, IGF-I secretion was elevated with no effect on IGF-IR. These observations suggest that when IGF-I levels are reduced, receptor levels on the cell increase to compensate, and when IGF-I levels are normal or even increased, no change in membrane receptor level occurs.

In the current study we examined the role of zinc in cell cycle progression and IGF-I receptor expression using the Swiss 3T3 cell line. The cells were grown in medium containing low concentrations of FBS to induce growth arrest (16) then returned to FBS-adequate media containing the membrane-impermeable divalent cation chelator diethylenetriaminepentaacetic acid (DTPA; 4, 16, 17) or DTPA and zinc sulfate. Cell proliferation and IGF-I receptor expression were measured using flow cytometric techniques.

Materials and Methods

Materials. Antibody to IGF-I receptor was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). Biotinylated anti-IgG came from Pierce (Rockford, IL). Extravidin phycoerythrin, FBS, DTPA, and dimethyl sulfoxide (DMSO) was from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of the highest quality obtained from Sigma or Fisher Scientific (St. Louis, MO).

Cell Culture. 3T3-Swiss albino murine fibroblast cells (ATCC, Rockville, MD) were maintained in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% FBS and 2 m*M* L-glutamine. Cells were kept at 37°C in a humidified atmosphere with 5% CO_2 and passaged weekly.

Experimental Protocol. *Experiment 1.* This experiment was designed to examine the effect of zinc deprivation on the proliferative fraction of 3T3 cells, and to determine the interaction, if any, between zinc deprivation and pretreatment. Cells were plated at 1×10^5 cells/ml onto 35-mm plastic dishes in 2 ml DMEM supplemented with 10% FBS. Cells were allowed to grow for 3 days; then medium was replaced with DMEM supplemented with 0%, 0.5%, or 2% FBS and grown for an additional 2 days. The 3 days in 10% FBS medium followed by 2 days in 2% FBS medium was used to induce quiescence following the work of Chesters *et al.* (16). Following 2 days in the low FBS media, cells were washed with PBS and returned to 10% FBS medium (no DTPA), 10% FBS medium with 600 μ*M* DTPA (DTPA), or 10% FBS medium with 600 μ*M* DTPA and 400 μ*M* zinc sulfate (DTPA + Zn) for 24 hr. Cells were then examined for proliferative fraction. Concentrations for DTPA and DTPA + Zn were established by Chesters *et al.* (16). Cell monolayers were subconfluent following the 3 + 2 day protocol, and cell number per dish increased following return to 10% FBS medium or medium containing DTPA + Zn (data not shown). Zinc content of a sample of FBS was 3.50 ppm, the content of 10% FBS medium was 0.33 ppm, of 2% FBS medium was 0.07 ppm, and of medium alone was 0.003 ppm (18).

Experiment 2. This experiment was designed to determine the effect of pretreatment and zinc deprivation on IGF-I receptor expression. Cells were cultured as in Experiment 1, and the fraction of cells that stained positively for receptor was determined immediately after pretreatment and 24 hr after the experimental treatments.

Experiment 3. The effect of adding glutathione (GSH) during the 24-hr period of stimulation with 10% FBS in the presence of DTPA was measured in cells pretreated with 2% FBS as in Experiment 1. During the 24-hr treatment period, medium was supplemented with the experimental treatments with or without 400 μ*M* GSH. The proliferative fraction was then examined.

Proliferative Fraction. Following treatment protocol, cells were harvested from 35-mm plates following two PBS washes by adding 500 μl 0.05% trypsin/1 m*M* EDTA in PBS for 10 min at 37°C, humidified. Once cells were detached, 1 ml of DMEM with 10% FBS and 10% DMSO was added; then cells were transferred to microfuge tubes and frozen at −20°C. Prior to flow cytometry, cells were rapidly thawed, mixed, transferred to 12 × 75-mm polystyrene tubes, and centrifuged at 2500 rpm for 5 min. Supernatant was discarded, and cell pellet was resuspended in 250 μl 0.04 *M* phosphate buffer containing 0.1% Triton X-100, 0.2 *M* sucrose and 0.1 *M* EDTA followed by 250 μl 0.07 *M* phosphate buffer containing 0.1 *M* sodium chloride and 20 μg/ml acridine orange. Cells were evaluated using a Coulter EPICS 753 flow cytometer (Coulter Corp., Hialeah, FL) gating on forward angle light scatter and measuring DNA fluorescence at 520 nm and RNA fluorescence at 650 nm. Dual parameter contour plots were evaluated using the Quadstat program-Cytologic software (Coulter Corp.). The proliferative fraction represented those cells falling in the S and G_2/M fractions and was established by setting one quadrant to encompass the G_1 fraction of the cells that did

not receive DTPA treatment. Cells falling outside of this quadrant were considered in the S and G_2/M phases.

IGF-I Receptor Expression. Following treatment protocol, cells were harvested from 35-mm plates by washing two times with PBS. Cells were then scraped into 0.5 ml PBS and transferred to 12 × 75-mm polystyrene tubes. Cells were fixed with 0.5 ml paraformaldehyde/acetic acid (2%/1%, v/v) in PBS for 15 min at 4°C followed by two PBS washes with centrifugation at 2500 rpm. Cells were then resuspended in 200 μl anti-human IGF-I receptor antibody at a concentration of 2 μg/ml in PBS with 0.5% BSA overnight at 4°C. Cells were again washed twice with PBS and resuspended in 200 μl biotinylated rabbit anti-chicken IgG diluted 1:500 (2.4 μg/ml) in PBS for 1 hr at room temperature, then washed twice with PBS and resuspended in 200 μl extravidin phycoerythrin diluted 1:100 in PBS for 1 hr at room temperature. Cells were washed and resuspended in 200 μl PBS for flow cytometry. Cells were evaluated using a Coulter EPICS 753 gating on forward angle light scatter and measuring log green fluorescence at 520 nm. The percentage of cells staining positive was determined by analysis of single parameter fluorescent histograms compared to a negative control using Cytologic software.

Statistical Analysis. Statistical analysis was performed on each experiment using commercially available software (SAS v6.12, SAS Institute, Inc., Cary, NC) and two-way ANOVA with mean differences ascertained by calculating a least significant difference. Treatment and pretreatment were the variables for Experiments 1 and 2. Treatment and GSH were the variables for Experiment 3. When significant differences were present, mean significant differences were ascertained using Fishers Least Significant Difference. Each experiment was examined for effect with a significance level of $P < .05$.

Results

The results of Experiment 1 are shown in Figure 1. Within each pretreatment, chelation of zinc by DTPA markedly reduced the proliferative fraction (i.e., those cells considered to be in the S + G_2/M phases) when compared to the control, which received no DTPA. Adding zinc with the DTPA restored the proliferative fraction to the control (no DTPA) level in all cases. Cells treated with DTPA after pretreatment with 0% FBS demonstrated a smaller proliferative fraction compared to those pretreated with 0.5% and 2% FBS (ANOVA, $P < .01$). The proliferative fraction was significantly lower in all cells pretreated with 0% FBS compared to those pretreated with 0.5% or 2% FBS (ANOVA, $P < .05$). Additionally, there was a significant pretreatment and treatment interaction (ANOVA, $P < .04$).

Expression of IGF-IR was examined in Experiment 2, and results are presented in Figure 2. There was no significant difference in the proportion of cells staining positive for IGF-IR when treated with DTPA for 24 hr compared to the cells treated with DTPA + Zn or receiving no DTPA when 0% or 2% FBS was the pretreatment. When 0.5% FBS

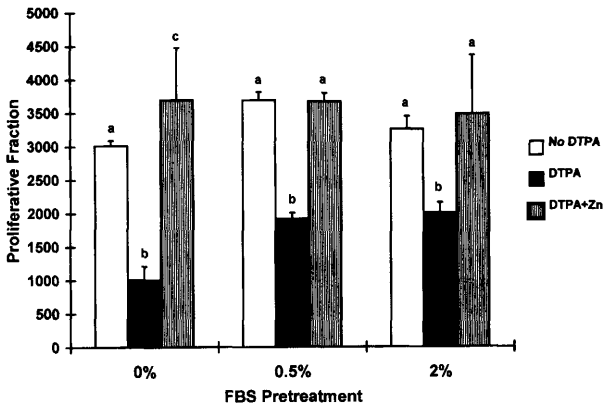

Figure 1. Proliferative fraction of 3T3 cells treated with DTPA and DTPA plus zinc after pretreatment with graded levels of FBS (Experiment 1). Following pretreatment with 0, 0.5, or 2% FBS, the cells were stimulated with 10% FBS unsupplemented (control), supplemented with 600 μ*M* DTPA (DTPA), or supplemented with 600 μ*M* DTPA and 400 μ*M* zinc sulfate (DTPA + Zn). Cells (10,000) were evaluated and the number judged to be in S and G_2M phases of the cell cycle plotted as the proliferative fraction. Bars represent means (n = 5–6), and the bar extensions represent SD. Within a pretreatment group, bars with different letters differ statistically, $P < .03$.

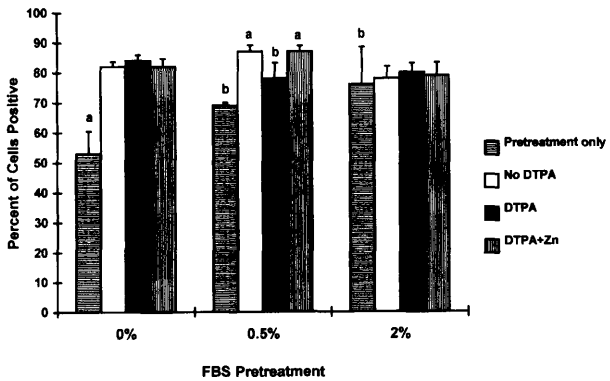

Figure 2. IGF-I receptor expression by 3T3 cells treated with DTPA and DTPA plus zinc after graded levels of FBS (Experiment 2). Following pretreatment with 0, 0.5, or 2% FBS, the cells were stimulated with 10% FBS unsupplemented (control), supplemented with 600 μ*M* DTPA (DTPA), or with 600 μ*M* DTPA and 400 μ*M* zinc sulfate (DTPA + Zn). Cells (2500–5000) were evaluated by flow cytometry, and the percentage that stained positively for IGF-IR was plotted. Bars represent means (n = 5–6), and the bar extensions represent SD. Within a pretreatment group, treatment bars with different letters differ statistically, $P < .05$. Across the pretreatment groups pretreatment only bars with different letters differ statistically, $P < .02$.

was the pretreatment, the cells receiving DTPA for 24 hr revealed a significantly smaller positive staining proportion when compared to the cells receiving DTPA + Zn and cells receiving no DTPA. Cells examined immediately following the pretreatments showed that the proportion of positive staining cells was significantly less when 0% FBS was the pretreatment compared to the 0.5% and 2% FBS pretreatments. When there was no pretreatment, there was no difference in the proportion of positive staining cells following DTPA treatment for 24 hr (61 ± 5 percent positive cells, mean ± SD) when compared to the cells receiving no DTPA (56 ± 7) or DTPA + Zn–treated cells (57 ± 4). There was a

significant interaction between pretreatment and treatment (ANOVA, $P < .001$).

The results of the addition of GSH, as described in Experiment 3, are summarized in Figure 3. Without the addition of GSH, DTPA depressed the proliferative fraction approximately 50%, as observed previously. There was not a difference between the control (no DTPA) and DTPA + Zn–treated cells. The addition of GSH significantly attenuated the effect of DTPA on the proliferative fraction without affecting the cells that did not receive DTPA or the cells treated with DTPA + Zn.

Discussion

Serum deprivation can induce cellular quiescence in certain cell lines as demonstrated by DeAngelis *et al.* (19). Following the protocol established by Chesters *et al.* (16), we pretreated 3T3 cells for 2 days in 2% FBS to induce growth arrest in an effort to produce a synchronous population of cells with which we could then examine the effects of zinc depletion on both the proliferative fraction of the cell and expression of IGF-I receptor. We observed that chelation of zinc by DTPA significantly reduced the proliferative fraction compared to untreated cells and cells treated with DTPA + Zn. This reduction was seen regardless of the pretreatment used. It is worth nothing that results from earlier experiments performed by us, in which 2% FBS supplemented media was used as the pretreatment, suggested that the cells were not quiescent. In an experiment where 3T3 cells were maintained in 2% FBS for 3 days then examined by flow cytometry, cells were present in the proliferative fraction (data not shown).

We observed a significant decrease in IGF-IR expression following the 0% FBS pretreatment compared to either the 0.5% or 2% FBS pretreatment. The reduced FBS in the media would result in reduced growth factor concentration, including IGF-I, suggesting that IGF-IR expression should increase (20). However, in the absence of certain growth factors such as platelet-derived growth factor or epidermal growth factor, cultured human fibroblasts showed reduced IGF-I binding, and this binding was increased following the addition of these growth factors to the medium (21). This would explain the reduction in IGF-IR observed with decreasing FBS in the media. There was no effect of zinc chelation by DTPA on IGF-IR expression following the 2% FBS pretreatment, suggesting the growth inhibitory effects seen in the zinc-depleted 3T3 cells involved a mechanism independent of receptor expression. However, zinc chelation with DTPA did affect IGF-IR expression when 0.5% FBS was the pretreatment. Additionally, IGF-IR expression in the DTPA-treated 3T3 cells that received no pretreatment was lower (56% positive cells) when compared to the DTPA-treated cells following 0.5% FBS pretreatment (78% positive cells). These data suggest that low FBS pretreatment in the 3T3 cells leads to a downregulation of IGF-IR, and returning the cells to 10% FBS causes an upregulation of IGF-IR that exceeds the level observed when the cells receive no pretreatment. Furthermore, zinc may have an effect at a level downstream of IGF-IR expression. Many signal proteins are involved in producing a mitogenic response once IGF-I binds to IGF-IR. One of the most downstream signal proteins is the mitogen-activated protein kinase (MAPK). MAPK activity was substantially increased in 3T3 cells following the addition of IGF-I to the culture media (22). Additionally, MAPK activity as well as that of other phosphorylated proteins increased when zinc chloride was added to cultured 3T3 cells (23).

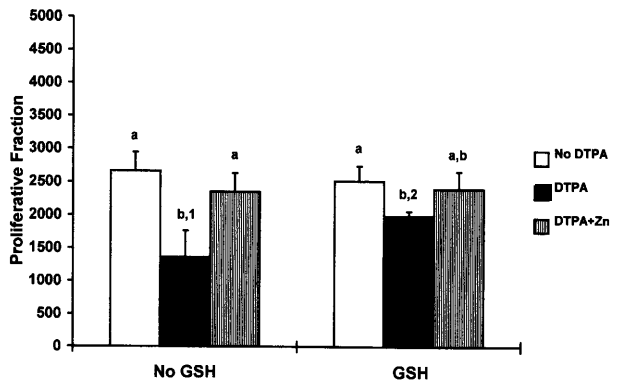

Figure 3. Effect of GSH on the proliferative fraction of 3T3 cells treated with DTPA and DTPA plus zinc after pretreatment with 2% FBS (Experiment 3). Following pretreatment, the cells were stimulated with 10% FBS unsupplemented (control), supplemented with 600 μ*M* DTPA (DTPA), or with 600 μ*M* DTPA and 400 μ*M* zinc sulfate (DTPA + Zn). Cells (10,000) were evaluated by flow cytometry, and the number in the proliferative fraction plotted. Bars represent means ($n = 3$–4), and the bar extensions represent SD. Within GSH treatment groups, different letters indicate significant differences, $P < .01$. Statistical comparison across GSH treatment groups indicated by numbers, $P < .01$.

We have observed no changes in the mean total cellular zinc concentration in 3T3 cells treated with DTPA (3.32 μmol/g protein) compared to untreated cells (3.59 μmol/g protein), suggesting DTPA removes zinc from the extracellular environment (18). Additionally, DTPA is incapable of crossing the cell membrane (17). Therefore it is possible that zinc depletion and its effects on the proliferative fraction may manifest as a cell membrane aberration involving, at least in part, an oxidative process. Zinc provides a protective effect against lipid peroxidation. In an animal study, diets deficient in zinc caused significantly elevated levels of malondialdehyde, an indicator of lipid peroxidation, and significantly decreased levels of plasma and liver GSH (24). Furthermore, other membrane changes are apparent in zinc-deficient animals including impaired calcium channel function in brain synaptic membranes (25) and platelets (26, 27), and these changes may be reversed by zinc repletion. The antioxidant effect of zinc may occur *via* the prevention of oxidation of sulfhydryl groups; therefore, removal of zinc from the media may allow oxidative effects on membrane associated proteins. This was evident in zinc-deficient rats where poor platelet aggregation and reduced GSH levels were seen and which could be reversed *in vitro* with GSH treatment (28). We examined the potential oxidative effect

of zinc depletion on cell proliferation in the 3T3 cells using GSH as a reducing agent. In the presence of GSH, the DTPA-treated cells had an increased proliferative fraction compared to cells treated with DTPA alone. The proliferative fraction was still less than the DTPA + Zn–treated cells or cells receiving no DTPA, suggesting a partial antioxidant role for zinc in cell proliferation.

We conclude that zinc depletion exerts its effect at the level of cellular proliferation, and this effect is independent of IGF-IR expression but may occur downstream of receptor/ligand binding. Additionally, a portion of this inhibitory effect can be attributed to the antioxidant properties of zinc at the cell membrane since the addition of GSH to the media partially neutralizes the inhibitory effect.

Supported in part by the University of Missouri Food for 21st Century, Nutrition Cluster Program and United States Department of Agriculture Grant #95-00649. Agriculture Experiment Station journal no.: 12575.

1. Chesters JK, Boyne R. Nature of the Zn^{2+} requirement for DNA synthesis by 3T3 cells. Exp Cell Res **192:**631–634, 1991.
2. Chesters JK, Boyne R, Petrie L, Lipson KE. Role of the promoter in the sensitivity of human thymidine kinase to lack of Zn^{2+}. Biochem J **308:**659–664, 1995.
3. Landro JA, Schimmel P. Zinc-dependent cell growth conferred by mutant tRNA synthetase. J Biol Chem **269:**20217–20220, 1994.
4. Bettger WJ, O'Dell BL. Physiological roles of zinc in the plasma membrane of mammalian cells. J Nutr Biochem **4:**194–207, 1993.
5. Hennig B, Wang Y, Ramasamy S, McClain CJ. Zinc deficiency alters barrier function of cultured porcine endothelial cells. J Nutr **122:**1242–1247, 1992.
6. Pledger WJ, Stiles CD, Antoniades HN, Scher CD. An ordered sequence of events is required before BALB/c-3T3 cells become committed to DNA synthesis. Cell Biol **75:**2839–2843, 1978.
7. Duncan MD, Korman LY, Bass BL. Epidermal growth factor primes intestinal epithelial cells for proliferative effect of insulin-like growth factor-I. Dig Dis Sci **39:**2197–2201, 1994.
8. Campisi J, Pardee AB. Post-transcriptional control of the onset of DNA synthesis by an insulin-like growth factor. Mol Cell Biol **4:**1807–1814, 1984.
9. Beitner-Johnson D, LeRoith, D. Insulin-like growth factor-I stimulates tyrosine phosphorylation of endogenous c-Crk. J Biol Chem **270:**5187–5190, 1995.
10. Yoshinouchi M, Baserga R. The role of IGF-I receptor in the stimulation of cells by short pulses of growth factors. Cell Prolif **26:**139–146, 1993.
11. Quinn LS, Steinmetz B, Maas A, Ong L, Kaleko M. Type-I insulin-like growth factor receptor overexpression produces dual effects on myoblast proliferation and differentiation. J Cell Physiol **159:**387–398, 1994.
12. Sell C, Dumenil G, Deveaud C, Miura M, Coppola D, DeAngelis T, Rubin R, Efstratiadis A, Baserga R. Effect of null mutation of the insulin-like growth factor-I receptor gene on growth and transformation of mouse embryo fibroblasts. Mol Cell Biol **14:**3604–3612, 1994.
13. Matsui T, Yamaguchi M. Zinc modulation of insulin-like growth factors effect in osteoblastic MC3T3-E1 cells. Peptides **16:**1063–1068, 1995.
14. Yamaguchi M, Hashizume M. Effect of beta-alanyl-L-histinado zinc on protein components in osteoblastic MC3T3-E1 cells: Increase in osteocalcin, insulin-like growth factor-I and transforming growth factor-beta. Mol Cell Biol **136:**163–169, 1994.
15. Hakeda Y, Yoshizawa K, Hurley M, Kawaguchi H, Tezuka K, Tanaka K, Satoh T, Kumegawa M. Stimulatory effect of a phorbol ester on expression of insulin-like growth factor (IGF) binding protein-2 and level of IGF-I receptors in mouse osteoblastic MC3T3-E1 cells. J Cell Physiol **158:**444–450, 1994.
16. Chesters JK, Petrie L, Vint H. Specificity and timing of the Zn^{2+} requirement for DNA synthesis by 3T3 cells. Exp Cell Res **184:**499–508, 1989.
17. McCabe MJ, Jiang SA, Orrenius S. Chelation of intracellular zinc triggers apoptosis in mature thymocytes. Lab Invest **69:**101–110, 1993.
18. MacDonald RS, Wollard-Biddle LC, Browning JD, Thornton WH Jr., O'Dell BL. Zinc deprivation of murine 3T3 cells by use of diethylentrinitrilopentaacetate impairs DNA synthesis upon stimulation with insulin-like growth factor (IGF-I). J Nutr (in press).
19. DeAngelis T, Ferber A, Baserga R. Insulin-like growth factor-I receptor is required for the mitogenic and transforming activities of the platelet-derived growth factor receptor. J Cell Physiol **164:**214–221, 1995.
20. Yamamoto H, Prager D, Yamasaki H, Melmed S. Rat pituitary GC cell insulin-like growth factor-I receptor regulation. Endocrinology **133:**1420–1425, 1993.
21. Clemmons DR, Shaw DS. Variables controlling somatomedin production by cultured human fibroblasts. J Cell Physiol **115:**137–142, 1983.
22. Hansson A, Thoren M. Activation of MAP kinase in Swiss 3T3 fibroblasts by insulin-like growth factor-I. Growth Regul **5:**92–100, 1995.
23. Hansson A. Extracellular zinc ions induce mitogen-activated protein kinase activity and protein tyrosine phosphorylation in bombesin-sensitive Swiss 3T3 fibroblasts. Arch Biochem Biophys **328:**233–238, 1996.
24. Shaheen AA, el-Fattah AA. Effect of dietary zinc on lipid peroxidation, glutathione, protein thiols levels, and superoxide dismutase activity in rat tissues. Int J Biochem Cell Biol **27:**89–95, 1995.
25. Browning JD, O'Dell BL. Low zinc status in guinea pigs impairs calcium uptake by brain synaptosomes. J Nutr **124:**436–443, 1994.
26. Emery MP, O'Dell BL. Compromised zinc status in rats adversely affects calcium metabolism in platelets. J Nutr **121:**1763–1768, 1991.
27. Xia J, O'Dell BL. Zinc deficiency in rats decreases thrombin-stimulated platelet aggregation by lowering protein kinase C activity secondary to impaired calcium uptake. J Nutr Biochem **6:**661–666, 1995.
28. O'Dell BL, Emery M, Xia JM, Browning JD. *In vitro* addition of glutathione to blood from zinc-deficient rats corrects platelet defects-impaired aggregation and calcium uptake. J Nutr Biochem **8:**346–350, 1997.