The Effect of Serum Magnesium Levels and Serum Endothelin-1 Levels on Bone Mineral Density in Protein Energy Malnutrition

CF Ozturk[1], C Karakelleoglu[1], Z Orbak[1,2], L Yildiz[3]

ABSTRACT

An inadequate and imbalanced intake of protein and energy results in protein-energy malnutrition (PEM). It is known that bone mineral density and serum magnesium levels are low in malnourished children. However, the roles of serum magnesium and endothelin-1 (ET-1) levels in the pathophysiology of bone mineralization are obscure. Thus, the relationships between serum magnesium and ET-1 levels and the changes in bone mineral density were investigated in this study.

There was a total of 32 subjects, 25 of them had PEM and seven were controls. While mean serum ET-1 levels of the children with kwashiorkor and marasmus showed no statistically significant difference, mean serum ET-1 levels of both groups were significantly higher than that of the control group. Serum magnesium levels were lower than normal value in 9 (36%) of 25 malnourished children. Malnourished children included in this study were divided into two subgroups according to their serum magnesium levels. While mean serum ET-1 levels in the group with low magnesium levels were significantly higher than that of the group with normal magnesium levels ($p < 0.05$*), mean bone mineral density and bone mineral content levels were significantly lower (*$p < 0.05$*).*

In conclusion, many factors play a role in the pathophysiology of changes in bone mineral density in malnutrition. Our study suggested that lower magnesium levels and higher ET-1 levels might be important factors in changes of bone mineral density in malnutrition. We recommend that the malnourished patients, especially with hypomagnesaemia, should be treated with magnesium early.

Keywords: Bone mineral density, endothelin-1, magnesium, protein-energy malnutrition

Efecto de los Niveles Séricos de Magnesio y los Niveles Séricos de Endotelina-1 sobre la Densidad Mineral Ósea en la Malnutrición Calórico-proteica

CF Ozturk[1], C Karakelleoglu[1], Z Orbak[1,2], L Yildiz[3]

RESUMEN

El consumo inadecuado y desbalanceado de proteínas y calorías energía conduce a la malnutrición calórico-proteica (MCP). Se sabe que la densidad mineral ósea y los niveles séricos de magnesio son bajos en los niños malnutridos. Sin embargo, no está claro el papel que desempeñan los niveles séricos de magnesio y los niveles séricos de endotelina-1 (ET-1) en la patofisiología de la mineralización del hueso. Por consiguiente, las relaciones entre los niveles séricos de magnesio y los niveles séricos de ET-1, y los cambios en la densidad mineral ósea, constituyen el objeto de investigación de este estudio. Hubo un total de 32 sujetos; 25 de ellos tenían DCP y 7 eran considerados. Si bien los niveles séricos promedios de ET-1 de los niños con kwashiorkor y marasmo no mostraron diferencia estadística significativa, los niveles séricos promedio de ET-1 de ambos grupos fueron significativamente más

From: [1]Department of Paediatrics, [2]Department of Paediatric Endocrinology and Metabolism and [3]Department of Biochemistry, Atatürk University, Faculty of Medicine, Erzurum, Turkey.

Correspondence: Dr Z Orbak, Atatürk University, Faculty of Medicine, Paediatric Endocrinology, 25240 Erzurum, Turkey. E-mail: zerrinorbak @yahoo.com

altos que los del grupo de control. Los niveles séricos de magnesio estuvieron por debajo del valor normal en 9 (36%) de 25 niños malnutridos. Los niños malnutridos incluidos en este estudio fueron divididos en dos sub-grupos según sus niveles de magnesio en suero.
Mientras que los niveles séricos promedio de ET-1 en el grupo con niveles bajos de magnesio fueron significativamente más altos que los del grupo con niveles normales de magnesio ($p < 0.05$*), la densidad mineral ósea promedio y los niveles promedio del contenido mineral óseo fueron significativamente más bajos (*$p < 0.05$*).*
En conclusión, muchos factores juegan un papel en la patofisiología de los cambios en la densidad mineral ósea por la malnutrición. Nuestro estudio sugirió niveles más bajos de magnesio y niveles más altos de ET-1 podrían ser factores importantes en los cambios de densidad mineral ósea en la malnutrición. Se recomienda que los pacientes malnutridos, especialmente a causa de hipomagnesemia, sean tratados con magnesio lo más pronto posible.

Palabras claves: densidad mineral ósea, endotelina-1, malnutrición calórico-proteica

West Indian Med J 2011; 60 (3): 214

INTRODUCTION

Inadequate and imbalanced intake of protein and energy results in protein-energy malnutrition (PEM). Although PEM occurs more frequently in low-income countries, numerous children from higher-income countries are also affected, including children from large urban areas and of low socio-economic status, children with chronic diseases, and children who are institutionalized.

Malnutrition is "the syndrome that results from the interaction between poor diets and disease and leads to most of the anthropometric deficits observed among children in the world's less developed countries" (1). Kwashiorkor, marasmus and marasmic-kwashiorkor represent three clinical forms of serious protein-energy malnutrition. Marasmus results mainly from an inadequate intake of calories. Kwashiorkor is very limited protein intake (2).

Protein-energy malnutrition results in not only high mortality but also morbidity and suboptimal neurological development. It is known that hypomagnesaemia is a frequent complication in PEM. In malnutrition, insufficient magnesium (Mg) is related to less intake in diet, diarrhoea, vomiting, long-lasting liquid therapy and insufficient protein.

On the other hand, osteopaenia and osteoporosis are the other complications of PEM. In our previous study, it was found that bone mineral density (BMD) was lower in children with malnutrition than in controls and mineralization was significantly affected by the severity of malnutrition (3). But the pathophysiology of insufficient mineralization in malnutrition is still not fully understood. The role of serum magnesium and endothelin-1 (ET-1) levels in the patho-physiology of bone mineralization are obscure. Thus, the relationships between serum magnesium and ET-1 levels and the changes in bone mineral density were investigated in malnutrition.

SUBJECTS AND METHODS

There was a total of 32 subjects: 25 children with malnutrition and seven healthy controls. Twenty of the children with malnutrition had marasmus (80%), the others had kwashiorkor (20%). In study groups, malnutrition was not related to malabsorption. By medical history, there were data with regards to the insufficient diet and nutrition in children with marasmus and kwashiorkor. All children were white. The healthy subjects were normally growing children free of chronic disease and who had not received any prior therapy with a known effect on bone metabolism. The mean age in children with malnutrition (4.6 ± 2.7 months for marasmus, 6.1 ± 3.6 months for kwashiorkor) and healthy controls (6.2 ± 3.3 months) was similar. In groups, the female:male ratio was similar.

Using the Wellcome Classification (4), marasmus was diagnosed in 20 children and kwashiorkor in five. Weights were measured using the same equipment, by the same observer. Weight was measured to the nearest 10 g using a digital electronic instrument (Seca 727 Digital Baby Scale, serial interface RS 232, Seca Corporation, Vogel&Holke, Germany). Subjects were naked when weighed. Data were evaluated using standard weight and length measurements for Turkish children developed by Neyzi *et al* (5). Intra-observer differences were of the order of $\pm 5\%$ and were not statistically significant.

Venous blood samples of children were collected from the antecubital vein for serum Mg and ET-1 measurements. Samples were immediately centrifuged, separated and stored at -80°C until assay. Bone mineral densitometry for each case was measured by dual-energy X-ray absorptiometry (DXA) method.

Serum magnesium levels of patients and controls were studied by spectrophotometrical xylidyl blue method (Olympus AU2700 autoanalyzer, commercially available Boehrin-

ger Mannheim System kit). The reference range was accepted as 1.71–2.5 mg/dL. Endothelin-1 levels were studied by the ELISA method (BioTEK Power-WXS, commercially avail-able kit; catalog No. 900–022).

Bone mineral density can be evaluated by DXA which is a noninvasive method and has lower radiation dose (under 3 mRem), higher accuracy and precision, and shorter scanning time. This method is the most appropriate method for evaluating the BMD in children (6, 7). Bone mineral density was measured by a Hologic QDR 4500 Elite densitometer (Hologic, Inc, Waltham, MA, USA) which uses an X-ray tube as the radiation source. During the measurements, the child was supine, and the physiological lumbar scoliosis was flattened by elevation of the knees. The system scans the lumbar spine in a rectilinear way. The scanning time for the region of interest (L1-L4) ranges from 3–7 minutes. Results for vertebrae L-1 to L-4 were averaged to obtain the patient's total vertebral bone mass. All images were processed by the same investigator. The results were expressed as BMD in grams per cm^2 and bone mineral content (BMC) in grams.

Informed consent was obtained from parents. This study was approved by the Ethics Committee of the Faculty of Medicine, Atatürk University.

Statistical analyses were perfomed with the help of SPSS/PC (version 11) programme. Mann-Whitney U and Kruskal-Wallis tests for comparison of means of each group; Spearman's correlation analysis for evaluation of the relations among parameters were used. A p-value of < 0.05 was considered significant.

RESULTS

Serum mean ET-1, Mg levels of groups and statistical differences are shown in Table 1. While mean serum ET-1 levels of the children with kwashiorkor and marasmus showed no statistically significant difference, mean serum ET-1 levels of both groups were significantly higher than that of the control group. When serum Mg levels were evaluated, they were similar for the three groups.

The mean BMD, BMC measurements of lumbar spine and statistical differences for the groups are presented in Table 2.

Table 1: Mean ± SD and significance values for serum endothelin-1 and magnesium levels for each group

		Marasmus n = 20	Kwashiorkor n = 5	Malnutrition n = 25	Control n = 7
Endothelin-1 (pg/mL)	Mean ± SD	44.9 ± 14.9	38.6 ± 8.91	43.7 ± 14.0	24.4 ± 9.9
	p	Marasmus – Kwashiorkor		$p > 0.05$	
		Marasmus – Control		$p < 0.001$	
		Kwashiorkor – Control		$p < 0.05$	
		Malnutrition – Control		$p < 0.01$	
Magnesium (mg/dL)	Mean ± SD	2.05 ± 0.32	1.86 ± 0.46	2.02 ± 0.35	2.2 ± 0.41
	p	Marasmus – Kwashiorkor		$p > 0.05$	
		Marasmus – Control		$p > 0.05$	
		Kwashiorkor – Control		$p > 0.05$	
		Malnutrition – Control		$p > 0.05$	

Table 2: Mean ± SD and significance values for BMD and BMC measurements for each group

		Marasmus n = 20	Kwashiorkor n = 5	Malnutrition n = 25	Control n = 7
BMD (g/cm^2)	Mean ± SD	0.18 ± 0.71	0.22 ± 0.72	0.19 ± 0.07	0.25 ± 0.57
	p	Marasmus – Kwashiorkor		$p > 0.05$	
		Marasmus – Control		$p < 0.01$	
		Kwashiorkor – Control		$p > 0.05$	
		Malnutrition – Control		$p < 0.01$	
BMC (g)	Mean ± SD	1.76 ± 0.42	2.31 ± 1.05	1.87 ± 0.61	3.09 ± 0.48
	p	Marasmus – Kwashiorkor		$p > 0.05$	
		Marasmus – Control		$p < 0.001$	
		Kwashiorkor – Control		$p > 0.05$	
		Malnutrition – Control		$p < 0.001$	

BMD – bone mineral density, BMC – bone mineral content

Serum magnesium levels were lower than normal value in 9 (36%) of 25 malnourished children. Malnourished children included in this study were divided into two subgroups according to their serum magnesium levels. While mean serum ET-1 levels in the group with low magnesium levels were significantly higher than that of the group with normal magnesium levels ($p < 0.05$), mean BMD and BMC levels were significantly lower [$p < 0.05$] (Table 3).

Table 3: Mean ± SD and significance values for serum ET-1, Mg, BMD and BMC measurements according to serum magnesium levels

	Mg level: low n = 9	**Mg level: normal n = 16**	***p***
Mg (mg/dL)	1.66 ± 0.31	2.21 ± 0.18	< 0.001
ET-1 (pg/mL)	53.81 ± 15.54	38.04 ± 9.43	< 0.05
BMD (g/cm^2)	0.16 ± 0.03	0.21 ± 0.08	< 0.05
BMC (g)	1.54 ± 0.29	2.06 ± 0.68	< 0.05

DISCUSSION

In a previous study, we found elevated plasma ET-1 levels in eclamptic – pre-eclamptic mothers and their newborns (8). In the present study, high ET-1 levels were detected in malnutrition. No data are available on the possible changes in ET-1 levels during severe malnutrition – chronic starvation. Messaoudi *et al* (9) investigated ET-1 concentrations in obese patients during an eight-day period of very low calorie diet and they found that there was no significant variation in ET-1 concentrations, which remained within the normal range (9). However, this study was different from ours because the starvation period was shorter.

Nutrition, and specifically adequacy of protein and energy intake, is important in several ways. Firstly, malnutrition predisposes to falls. Secondly, a soft-tissue mass over bony prominences distributes the energy sustained in falls and thereby reduces point loads on bone (10). We evaluated malnourished patients together, both BMD and BMC measurements were lower than those of controls. This result was expected (3). When the BMD measurements of both malnourished groups were compared with that of controls, the marasmic cases were lower than the control group, but not the kwashiorkor group. The causes of low BMD and BMC measurements in kwashiorkor may be related to the low number of cases, hypoproteinaemia induced generalized oedema, and the changes of body composition during kwashiorkor.

In the present study, serum Mg levels were lower in malnutrition than in controls, but was not significant. Serum magnesium levels were also lower than normal value in 9 (36%) of 25 malnourished children. Singla *et al* (11) reported that serum magnesium levels were significantly low in children with moderate and severe malnutrition in 46 malnourished and 12 healthy children aged three months to five years and nearly half of the marasmic children had serum magnesium levels in the hypomagnesaemic range.

Dietary Mg deficiency has also been implicated as a risk factor for osteoporosis (12). In premenopausal women, one study found a significant correlation between the BMD of the lumbar spine and Mg intake (13). Another study of premenopausal women found a positive correlation between the BMD of the forearm, but not of the femur or spine and Mg intake (14). In pre-adolescent girls, Mg intake was positively related to quantitative ultrasound properties of the bone; this finding suggested that Mg was important in skeletal growth and development (15). On the other hand, in a small uncontrolled trial, a significant increase in the bone density of the proximal femur and lumbar spine was found in a patient with gluten-sensitive enteropathy who received Mg for two years (16). Several potential mechanisms may contribute to Mg-deficiency-induced bone loss (17, 18): i. low Mg enhances the release of substance P which then stimulates tumour necrosis factor-*alpha* (TNFα) and interleukin-1 *beta* (IL-1β) production by monocytes that in turn activate osteoclast; ii. the development of oxidative stress during low Mg levels may increase oxyradicals that may also induce osteoclastic bone resorption; iii. decreased PTH secretion and/or decreased 1,25(OH)2-vitamin D production secondary to hypomagnesaemia may decrease osteoblast bone formation (17). Magnesium also affects crystal formation. It is not directly incorporated into the hydroxyapatite crystal but binds to the crystal surface, leading to smaller crystals and denser bone. A lack of Mg results in a larger hydroxyapatite crystal which may affect bone stiffness and/or hardness (19). We suggested that hypomagnesaemia is an important complication of PEM, resulting in osteoporosis and increased skeletal fragility.

In one study, Weglicki *et al* (20) developed a rodent model of diet-induced magnesium-deficiency. During the development of histologically defined cardiac lesions, the magnesium-deficient rodents exhibited dramatic elevations of the macrophage-derived inflammatory cytokines together with significantly elevated circulating levels of the endothelial cell-derived cytokine, endothelin. Endothelin-1 may play a role in the promotion of cardiovascular pathology associated with Mg deficiency.

Magnesium-deficiency results in two predominant vascular complications: development of cardiomyopathic lesions and altered vascular tone with accompanying vascular spasm (20). In another study by the same authors, it was reported that free radicals participated in cardiomyopathic lesion formation (21). However, significantly elevated levels of inflammatory cytokines in the circulation suggest that the inflammatory state is not restricted to localized tissue reactions (22). On the other hand, patients with hypomagnesaemia are at high risk for potentially life-threatening vascular spasm (20). A release of TNFα and IL-1 was seen at approximately one week after initiation of a low Mg diet in mice (23). It was speculated that IL-1 can stimulate endothelial cells to produce and release endothelin (20). While the mechanism is poorly understood, endothelin is

known to have effects on vascular tone. What is the clinical importance of high serum endothelin-1 level? Laurant *et al* (24) reported that magnesium deficiency increases vasoconstrictor activity without affecting blood pressure of aged spontaneously hypertensive rats. Epidemiological studies of populations living in areas of low Mg intake have consistently shown a higher cardiovascular morbidity. An experimental study showed that magnesium deficiency was associated with increased ET-1 levels and with significant proaggregatory and coagulation alterations (25). Magnesium deficiency contributes to not only increased cardiovascular disease but also impaired haemostatic profile. Hypomagnesaemia and elevated serum ET-1 levels in malnutrition may be related to cardiovascular disease in older ages. While the present data do not confirm a primary or secondary role of ET-1 in the pathophysiology of Mg deficiency, the detection of elevated ET-1 levels in the circulation of Mg deficient patients indicates a generalized pro-inflammatory state. Magnesium-deficiency associated ET-1 may be a common initiator of pathological events associated with both formation of cardiomyopathic lesions and altered vascular tone. Thus, we think that additional studies are needed to define the pathophysiology and clinical importance of hypomagnesaemia and elevated endothelin-1 levels.

Several factors produced by endothelial cells may affect osteoblast function or differentiation and that osteoblasts not only express receptors for endothelial-derived factors but also produce paracrine factors that influence endothelial cell function (26, 27). One of the important factors involved in this communication is ET-1. It is a 21-amino-acid peptide. Endothelin, which is synthesized by vascular endothelium, is one of the most powerful vasoconstrictor substances known (28). Osteoblasts also produce ET-1 (29). Several studies showed that ET-1 stimulates osteoprogenitor proliferation and differentiation and recommended that ET-1 affects vascular endothelial growth factor-A production, an important mediator of the angiogenic process required for bone formation and repair (30–31). However, little information is available about the effects of ET on the differentiation and mineralization of osteoblastic cells. Hiruma *et al* (33) proposed that ET-1 might act as a local factor to inhibit the maturation and mineralization of osteoblastic cells *via* an interaction with the ET_A receptor (A-type receptor for ET), with generation of IP_3 (inositol 1, 4, 5-triphosphate) as the intracellular signal (33).

In conclusion, many factors play a role in the pathophysiology of changes in bone mineral density in malnutrition. Our study suggested that lower magnesium levels and higher ET-1 levels might be important factors contributing to the changes of bone mineral density in malnutrition. So, we suggest that hypomagnesaemia in malnutrition causes the elevated ET-1 levels and then elevated ET-1 level also causes impaired bone mineralization. We recommend that malnourished patients, especially with hypomagnesaemia, should be treated with Mg early.

REFERENCES

1. World Health Organization. Physical status: The use and interpretation of anthropometry. Report of a WHO Expert Committee. WHO Technical Report Series 854. Geneva: World Health Organization; 1995.
2. Nelson-Barness LA, Curan JS. Nutritional disorders. In: Behrman RE, Kliegman RM, Arvin AM, eds. Nelson Textbook of Pediatrics. Philadelphia: WB Saunders; 1996: 166–9.
3. Alp H, Orbak Z, Kemren T, Uslu H. Bone mineral density in malnourished children without rachitic manifestations. Pediatr Int 2006; **48:** 128–31.
4. Tietz NW. Textbook of Clinical Chemistry. Philadelphia: WB Saunders; 1987: 674–7.
5. Neyzi O, Binyıldız P, Alp H. Türk çocuklarında büyüme ve gelişme normlar l. Tartı ve boy değerleri. İst Tıp Fak Mecm 1978; **41:** 74–86.
6. Salle BL, Glorieux FH. Assessment of bone mineral content in infants: the new age. Acta Paediatr 1993; **82:** 709–10.
7. Wahner HW, Dunn WL, Brown ML, Morin RL, Riggs BL. Comparison of dual-energy X-ray absorptiometry of lumbar spine. J Clin Endocrinol Metab 1988; **67:** 839–44.
8. Orbak Z, Zor N, Energin M, Selimoglu MA, Alp H, Akcay F. Endothelin-1 levels in mothers and eclampsia-pre-eclampsia and their newborns. J Trop Pediatr 1998; **44:** 47–9.
9. Messaoudi L, Donckier J, Stoffel M, Ketelslegers J, Kolanowski J. Changes in blood presure and vasoactive and volume regulatory hormones during semistarvation in obese subjects. Metabolism 1998; **47:** 592–7.
10. Heaney RP. Nutrition and osteoporosis. In: Favus MJ, ed. Primer on the metabolic bone diseases and disorders of mineral metabolism. 4th ed. Philadelphia: Lippincot Williams and Wilkins; 1999: 270–3.
11. Singla DN, Chand P, Kumar A, Kachhawoba JS. Serum magnesium levels in protein-energy malnütrition. J Trop Pediatr 1998; **44:** 117–9.
12. Rude RK. Magnesium deficiency: a heterogeneous cause of disease in humans. J Bone Miner Res 1998; **13:** 749–58.
13. New SA, Bolton-Smith C, Grubb DA, Reid DM. Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. Am J Clin Nutr 1997; **65:** 1831–9.
14. Angus RM, Sambrook PN, Pocock NA, Eisman JA. Dietary intake and bone mineral density. Bone Miner 1988; **4:** 265–77.
15. Wang MC, Moore EC, Crawford PB, Hudes M, Sabry ZI, Marcus R et al. Influence of pre-adolescent diet on quantitative ultrasound measurements of the calcaneus in young adult women. Osteoporos Int 1999; **9:** 532–5.
16. Rude RK, Olerich M. Magnesium deficiency: possible role in osteoporosis associated with gluten-sensitive enteropathy. Osteoporos Int 1996; **6:** 453–61.
17. Rude RK, Gruber EH. Magnesium deficiency and osteoporosis: animal and human observations. J Nutr Biochem 2004; **15:** 710–6.
18. Launius BK, Brown PA, Cush EM, Mancini MC. Osteoporosis: The dynamic relationship between magnesium and bone mineral density in the heart transplant patient. Osteoporosis 2004; **27:** 96–100.
19. Lian JB, Stein GS, Canalis E, Robey PG, Boskey AL. Bone formation: Osteoblast lineage cells, growth factors, matrix proeins, and the mineralization process. In: Favus MJ, ed. Primer on the metabolic bone diseases and disorders of mineral metabolism. 4th ed. Philadelphia: Lippincott Williams and Wilkins; 1999: 14–29.
20. Weglicki WB, Phillips TM, Freedman AM, Cassidy MM, Dickens BF. Magnesium-deficiency elevates circulating levels of inflammatory cytokines and endothelin. Mol Cell Biochem 1992; **110:** 169–73.
21. Freedman AM, Atrakchi AH, Cassidy MM, Weglicki WB. Magnesium deficiency-induced cardiomyopathy: protection by vitamin E. Biochem Biophys Res Comm 1990; **170:** 1102–6.
22. Billingham ME. Cytokines as inflammatory mediators. Br Med Bull 1987; **43:** 350–70.
23. Weglicki WB, Dickens BF, Wagner TL, Chemielinska JJ, Phillips TM. Immunoregulation by neuropeptides in magnesium deficiency: ex vivo effect of enhanced substance P production on circulation T lymphocytes from magnesium-deficient mice. Manges Res 1996; **9:** 3–11.

24. Laurant P, Robin S, Berthelot A. Magnesium deficiency increases vasoconstrictor activity without affecting blood pressure of aged spontaneously hypertensive rats. Magnes Res 1997 **10:** 107–17.
25. Serebruany VL, Herzog WR, Edenbaum LR, Shustov AR, Gurbel PA. Changes in the haemostatic profile during magnesium deficiency in swine. Magnes Res 1996; **9:** 155–63.
26. Collin-Osdoby P. Role of vascular endothelial cells in bone biology. J Cell Biochem 1994; **55:** 304–9.
27. Wang DS, Miura M, Demura H, Sato K. Anabolic effects of 1,25-dihydroxyvitamin D3 on osteoblasts are enhanced by vascular endothelial growth factor produced by osteoblasts and by growth factors produced by endothelial cells. Endocrinology 1997; **138:** 2953–62.
28. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988; **332:** 411–15.
29. Kitten AM, Andrews CJ. Endothelin-1 expression in long-term cultures of fetal rat calvarial osteoblasts: regulation by BMP-7. J Cell Physiol 2001; **187:** 218–25.
30. Veillette CJH, von Schroeder HP. Endothelin-1 down-regulates the expression of vascular endothelial growth factor-A associated with osteoprogenitor proliferation and differentiation. Bone 2004; **34:** 288–96.
31. von Schroeder HP, Chen X, Heersche JNM. Endothelin-1 stimulates fetal rat osteoblastic progenitors to proliferate and form bone nodules. J Bone Miner Res 2000; **15:** S378.
32. von Schroeder HP, Veillette CJH, Payandeh J, Qureshi A, Heersche JNM. Endothelin-1 promotes osteoprogenitor proliferation and differentiation in fetal rat calvarial cell cultures. Bone 2003; **33:** 673–84.
33. Hiruma Y, Inoue A, Shiohama A, Otsuka E, Hirose S, Yamaguchi A et al. Endothelins inhibit the mineralization of osteoblastic MC3T3-E1 cells through the A-type endothelin receptor. Am J Physiol Regul Integr Comp Physiol 1998; **275:** 1099–105.