Metabolic bone disease in the preterm infant: Current state and future directions

Moghis Ur Rehman, Hassib Narchi

Moghis Ur Rehman, Department of Pediatrics, Tawam Hospital, Al Ain, PO Box 15258, United Arab Emirates

Hassib Narchi, Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, PO Box 17666, United Arab Emirates

Author contributions: Both Ur Rehman M and Narchi H have made substantial contributions to the conception and design of the editorial, drafting the article or making critical revisions related to important intellectual content of the manuscript and final approval of the version of the article to be published.

Conflict-of-interest statement: The authors have no commercial, personal, political, intellectual, or religious conflict-of-interest to report in relation to this work.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Correspondence to: Hassib Narchi, Professor, Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, PO Box 17666, United Arab Emirates. hassib.narchi@uaeu.ac.ae

Telephone: +971-3-7137414
Fax: +971-3-7672022

Received: January 19, 2015
Peer-review started: January 20, 2015
First decision: March 6, 2015
Revised: July 1, 2015
Accepted: August 4, 2015
Article in press: August 7, 2015
Published online: September 26, 2015

Abstract

Neonatal osteopenia is an important area of interest for neonatologists due to continuing increased survival of preterm infants. It can occur in high-risk infants such as preterm infants, infants on long-term diuretics or corticosteroids, and those with neuromuscular disorders. Complications such as rickets, pathological fractures, impaired respiratory function and poor growth in childhood can develop and may be the first clinical evidence of the condition. It is important for neonatologists managing such high-risk patients to regularly monitor biochemical markers for evidence of abnormal bone turnover and inadequate mineral intake in order to detect the early phases of impaired bone mineralization. Dual-energy X-ray absorptiometry has become an increasingly used research tool for assessing bone mineral density in children and neonates, but more studies are still needed before it can be used as a useful clinical tool. Prevention and early detection of osteopenia are key to the successful management of this condition and oral phosphate supplements should be started as soon as is feasible.

Key words: Premature; Osteopenia; Bone metabolism; Calcium; Alkaline phosphatase; Phosphorus; Nutrition

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Osteopenia of prematurity remains an important challenge in neonatal medicine due to continuing increased survival of preterm infants. The risk is higher with long-term diuretics or corticosteroids. It is important when managing such infants to regularly monitor biochemical markers for evidence of abnormal bone turnover and inadequate mineral intake. Dual-energy X-ray absorptiometry is increasingly used in research for assessing bone mass density in neonates. Prevention and early detection of osteopenia are key to the successful management of this condition and oral phosphate supplements should be started as soon as it is feasible.
INTRODUCTION
Neonatal metabolic bone disease (MBD), osteopenia of prematurity (OOP), neonatal rickets or rickets of prematurity, are terms used to describe a reduction in bone mineral content (BMC) of the preterm infant. Although its exact prevalence is difficult to quantify because of the different methods used to screen infants at risk and also because of the difficulty in the interpretation of these results, it has been steadily increasing with the survival of more immature neonates as a result of advances in neonatal care. Born before term pregnancy and thus deprived of a period of intrauterine supply of minerals, these infants already suffer at birth from suboptimal bone mineralization. The prevalence of MBD is inversely associated with birth weight and gestational age, with up to a third of infants weighing less than one kilogram at birth being osteopenic, more so if they are breastfed. Other factors impeding normal bone mineralization include inadequate postnatal intake of vitamin D, calcium (Ca) and phosphorus (P), extended periods of total parenteral nutrition, lengthy duration of immobilization and as also a side effects of diuretics and corticosteroids prescribed to these infants. Depending on the severity of the demineralization, osteopenia can remain clinically silent or develop as rickets, and, if severe, can even result in fractures.

As it is an important determinant of skeletal strength structure and density of the skeletal system throughout life, bone mineral density (BMD) in infants is an important topic for neonatologists, pediatricians and also endocrinologists. Guidelines for preventing, screening and treating MBD are not always consistent nor are they universally agreed upon, as still illustrated in a recently published review of this topic.

BURDEN OF OOP
Although the burden is not easy to quantify and available data remains conflicting, the known short-term complications are dominated by fractures of the long bones and ribs in the neonatal period. These respond well to therapy and there have no known residual long-term complications. The duration of hospital stay is unaffected by the diagnosis of OOP and preterm infants are routinely given mineral to prevent or treat neonatal rickets. Growth alteration of the skull (dolichocephalic flattening) has been reported in association with poor BMC.

The weight, height, body mass index, lumbar BMC and BMD in 7-year-old children born prematurely and weighing less than 1500 g at birth are lower than those of the reference population. Dual-energy X-ray absorptiometry (DEXA) assessment of areal BMD (aBMD; measured as grams per square centimeter) shows lower values at the level of the radial metaphysis, femoral neck and total hip in ex-preterm girls, but similar values at the radial and femoral diaphysis, with femoral neck aBMD remaining lower 12 mo later. After adjusting for age, weight, height and jump power, prepubertal boys born at term have greater bone size and mass on DEXA scan at the age of at 5.7-8.3 years than those born before 34 wk of gestation. It is still unknown if these changes in BMD in infancy and childhood increase the risk of developing early osteoporosis in adulthood.

PATHOPHYSIOLOGY AND RISK FACTORS
Antenatally
To develop normally, the skeleton of the growing fetus requires considerable active materno-fetal transfer of energy, protein, Ca and P. Serum Ca and P levels in the fetus are 20% more elevated than in the mother in the second trimester. Bone mineralization which occurs predominantly during the third trimester, will be inadequate if the fetal increased demands in Ca and P are not met. During pregnancy, augmented maternal intestinal absorption and increased skeletal mobilization increase maternal Ca supply to the fetus. The reduction in the Ca supply by the placenta results in a postnatal increase of parathyroid hormone (PTH) level that continues 48 h after birth when the peak serum Ca and the stabilization of serum Ca and P levels are attained.

Vitamin D also affects BMC and maternal hypovitaminosis D negatively affects the development of the fetal skeleton. It is transferred across the placenta predominantly as 25-hydroxyvitamin D before conversion in the fetal kidney to the active form 1,25-dihydroxy-vitamin D.

Chronic damage to the placenta, with the resulting altered phosphate transport, also contributes to poor bone mineralization and explains the high postnatal incidence of rickets in neonates born with intrauterine growth retardation. Such placental pathologies include pre-eclampsia and also chorioamnionitis and placental infections.

Mechanical force patterns, such as fetal movements, including kicking against the wall of the uterus, also stimulate the growth of cortical bone. As a result, preterm infants have a decrease in cortical bone growth leading to a reduction in bone strength. This, added to the reduction in transplacental accretion of Ca and P in the fetus, increases the risk of osteopenia in premature infants.

Postnatally
In infants who are exclusively breast fed, OOP is not
If the biomarkers include higher birth weight, short duration of parenteral nutrition, absence of intraventricular hemorrhage, exclusive feeding of fortified breast milk, and older age at discharge. \[^{[18]}\]

Candidate genes associated with adult osteoporosis have recently been evaluated in VLBW infants where MBD was found to be associated with a lower number of thymidine-adenine (TA) repeats polymorphism of the estrogen receptor gene, compared to a higher number in those without MBD. \[^{[19]}\]

### SCREENING AND MONITORING

As MBD is usually asymptomatic in most infants, its diagnosis depends essentially on screening. This is based on a set of criteria defined by the presence of clinical manifestations, radiologic findings, biochemical markers and BMC measurements. The recognized clinical-radiological associations include, bone demineralization, periosteal reactions and, in severe cases of osteopenia, rickets and pathological fractures may be present. \[^{[20]}\]

Infants at high risk of osteopenia, including VLBW infants or neonates on long term diuretic therapy should be regularly monitored for that condition as serious complications can be avoided by early diagnosis with appropriate management. Measuring BMC and BMD relies on a few surrogate markers (Table 1).

### Serum biomarkers

As a normal serum Ca level can still be maintained to the detriment of Ca loss from the bone, it should not be used to screen infants at risk. Furthermore, serum Ca may also be affected by unrelated conditions such as MBD do not normalize, consider either vitamin D supplementation with up to 600 IU/d (although not well supported by evidence) or initiate instead ergocalciferol or alphacalcidiol therapy in which case regular monitoring of urinary calcium/creatinine ratio is necessary to detect hypercalcinia.

| Infants at risk | Prevention | Monitoring | Management |
|-----------------|------------|------------|------------|
| Born with birth weight below 1500 g | Early enteral nutritional intervention | Biochemical | If the biomarkers of MBD do not normalize, consider either vitamin D supplementation with up to 600 IU/d (although not well supported by evidence) or initiate instead ergocalciferol or alphacalcidiol therapy in which case regular monitoring of urinary calcium/creatinine ratio is necessary to detect hypercalcinia. |
| Born before 28 wk of gestation | Maintain a sufficient supply of Ca and P. Start oral P supplements as soon as its feasible. The P absorption rate is very good in the presence of Ca, with absorption rates exceeding 90% with both human and formula milk. The Ca absorption rate increases from 35 to 60 mg/kg per day when both Ca and P are supplemented and to 90 mg/kg per day when the appropriate dietary Ca/P ratio is attained. High Ca and P retention rates are attained with high-mineral preterm milk formulæ or with fortified human milk | Monitor weekly serum “bone profile” (Ca, P and ALP); maintain serum Ca concentration between 2.05-2.75 mmol/L and serum P between 1.87-2.91 mmol/L. If serum P < 1.8 mmol/L and ALP > 500 IU/L, renal TRP should be measured and, if it exceeds 95%, P supplementation should be started. | |
| Having received total parenteral nutrition for more than four weeks | Vitamin D supplementation | Being increasingly used for assessing BMD in neonates, but not recommended as yet as a clinical tool. | |
| On long-term diuretics or corticosteroid therapy | Ensure a minimum daily supplement of 400 IU vitamin D. Doses above 400 IU/d do not improve Ca and P absorption | Monitor for metabolic acidosis and hypercalcuria which may result from an increase in parenteral mineral delivery during parenteral nutrition. | |
| Suffering from neuromuscular disorders | Parenteral nutrition | | |
| | Parenteral nutrition | | |
| | Preparations providing 1.45 to 1.9 mmol/kg per day of Ca and 1.23 to 1.74 mmol/kg per day of P result in Ca and P retention rates of 88%-94% and 83%-97% respectively. The optimal Ca/P ratio in the intravenous solution fluid is between 1.3:1 and 1.7:1.5. | | |
| | If needed, parenteral P delivery can also be enhanced by using special preparations of organic P. Exercises | | |
| | Daily exercises such as gentle compression and movements of the limbs | | |
| | Regular review of medications in use | | |
| | Discontinuation of diuretics and steroids when appropriate | | |

ALP: Alkaline phosphatase; BMD: Bone mass density; Ca: Calcium; IU: International units; P: Phosphorus; TRP: Tubular reabsorption of phosphate. DEXA: Dual-energy X-ray absorptiometry.
as hypophosphataemia\textsuperscript{[16,21]}. Serum P concentration is correlated with BMD, is highly specific but is not sensitive enough to identify infants with osteopenia. While serum P concentration adequately reflects P levels in the bone, serum Ca concentration remains maintained at the cost of Ca content in the skeleton.

Serum alkaline phosphatase (ALP) is a marker of bony turnover. Elevated levels indicate increased bone cellular activity and when exceeding 700 to 750 IU/L, they are associated with osteopenia, which is still asymptomatic at that stage\textsuperscript{[22,23]}. The diagnosis of MBD in the preterm infant is usually suggested by the presence of low serum P levels in association with elevated serum ALP levels\textsuperscript{[1]}. The association of serum ALP levels exceeding 900 IU/L with a serum P level less than 1.8 mmol/L is 100\% sensitive and 70\% specific to diagnose OOP\textsuperscript{[24]}. A serum ALP level exceeding five times the upper limit of the normal range in adults is also associated with an increased risk of rickets\textsuperscript{[25]}. The diagnosis of OOP cannot be made however with certainty by elevated serum ALP concentrations, because DEXA scan measurements of BMC did not find an association between ALP levels and OOP in some studies\textsuperscript{[26]} and also because healthy preterm and osteopenic infants have higher serum ALP concentrations than full term infants. Associating multiple measurements of serum ALP with a wrist radiograph, with or without that of the knee, has been suggested for the identification of rickets in VLBW infants if the levels exceed 800 IU/L\textsuperscript{[27]}. Because it is located on osteoblast surfaces, bone-specific ALP is a more specific biomarker of bone turnover, useful to confirm OOP, when high levels of total serum ALP are found\textsuperscript{[28,29]}. Despite its limitations and, despite the absence of a clear cut-off diagnostic level, serum ALP measurement is frequently used to screen high risk infants for MBD. It is a readily available measurement in most laboratories and serial serum levels provide a trend very useful for follow up. Using it in conjunction with serum P levels as a screening tool significantly increases the sensitivity of identifying infants at risk of MBD.

Serum osteocalcin (OC), a non-collagenous protein of the bony matrix, is also a biomarker of osteoblastic activity. It is synthesized by osteoblasts and is partly regulated by 1,25-dihydroxyvitamin D levels. Its serum concentrations are elevated whenever bone turnover is increased, making it a possible useful tool to diagnose OOP\textsuperscript{[30]}. However, despite its specificity, there is no correlation between serum OC levels and BMC in the first four months of age\textsuperscript{[30]}. 

**Urinary biomarkers**

Urinary Ca and P excretion have also been used as biomarkers of postnatal skeletal mineralization. Urinary excretion of Ca exceeding 1.2 mmol/L and P exceeding 0.4 mmol/L are strongly associated with high bone mineralization. Infants born between 23 and 25 wk of gestation have a significantly lower renal P excretion threshold than other preterm neonates, resulting in elevated urinary P excretion even when serum P levels are low\textsuperscript{[21]}. As, unlike Ca, P is not bound in the plasma, it has been suggested that a better biomarker for OOP is the percentage of renal tubular reabsorption of phosphate (TRP), with TRP > 95\% indicating inadequate supplementation, bearing in mind that renal tubular leak of P can also be associated with inadequate Ca intake and increased serum PTH concentration\textsuperscript{[32]}. Similarly urinary Ca or P to creatinine ratios may also be useful as biomarkers for OOP; normal reference ranges in preterm infants have already been established for these ratios\textsuperscript{[33,34]}. However these urinary ratio results need to be carefully interpreted as they are highly dependent on the dietary intake (resulting in uncertainty if the standard reference range) and are also affected by the administration of drug such as furosemide or theophylline\textsuperscript{[35]}. 

**Radiological markers**

Old fractures and cortical thinning may be seen on plain radiographs, reflecting poor bone mineralization, but are usually very late signs because they are not usually apparent unless the BMC decreases to 40\%\textsuperscript{[36]}. 

DEXA is currently the most widely used modality to assess BMD. It correlates well with fracture risk and, in both term and preterm infants, it can be used to estimate BMC\textsuperscript{[37]}. Measuring BMD prior to adulthood however is hindered by the "areal" nature of the derived measurement. In addition, the establishment of robust, reliable neonatal, ethnic and gender specific normograms is urgently needed. Barriers to the routine use of DEXA as a screening tool for OOP include its high cost, its limited availability, the dimensions of the equipment used, the lengthy time required for imaging, as well as its sensitivity to movement artifact.

Quantitative Ultra sound (QUS), with already established reference values for both preterm and term infants, is a new inexpensive and portable modality of investigating OOP\textsuperscript{[38-40]}. This simple, non-invasive and inexpensive bedside method measures the broadband ultrasound speed attenuation, and is usually performed on the tibia. Although the measurements it provides correlate well with bone density and structure, the association is a poor with the thickness of the bony cortex\textsuperscript{[41]}. QUS is significantly lower in preterm infants than term infants and a significant correlation of QUS exists with serum ALP, supplementation with Ca, P and vitamin D as well as risk factors for reduced BMD\textsuperscript{[42]}. The combination of longitudinal QUS measurements with serum ALP and P levels are helpful to identify infants at increased risk of OOP\textsuperscript{[43]}. 

Although ultrasound reference values are available for term and preterm infants, there is limited information showing its usefulness.

**PREVENTION AND TREATMENT**

These are summarized in Table 1. The prevalence and also the severity of OOP can be reduced by early nutritional intervention. Maintaining a sufficient supply of
Ca and P for the growth of VLBW infants’ skeleton is challenging because of their relatively high physiological requirements. In addition, although preterm infants are capable of absorbing up to 70% of Ca from human milk, the P content affects the Ca retention rate. Supplementing milk with both Ca and P is more effective: while the Ca absorption rate is 35 mg/kg per day in the presence of P supplementation alone, it increases to 60 mg/kg per day when both Ca and P are supplemented. Ca absorption is also affected by the dietary Ca/P ratio with the retention rate reaching up to 90 mg/kg per day when the appropriate ratio is attained. The neonatal intestinal absorption of Ca is very good in the presence of Ca, with absorption rates exceeding 90% with both human and formula milk [44]. Ca and P retention rates similar to those observed in utero are attained with high-mineral preterm milk formulae or with fortified human milk [45].

It is imperative to monitor closely serum Ca, P and ALP in such high-risk infants. To prevent OOP, serum Ca concentration should be maintained between 2.05–2.75 mmol/L and serum P between 1.87–2.91 mmol/L. Although VLBW infants are routinely given vitamin D supplementation to increase intestinal absorption of Ca and P, doses above 400 IU/d do not improve their absorption [46].

Parenteral nutrition preparations providing 1.45 to 1.9 mmol/kg per day of Ca and 1.23 to 1.74 mmol/kg per day of P result in Ca and P retention rates of 88%–94% and 83%–97% respectively, equivalent to 60% to 70% of the expected in utero Ca and P accretion rates [47,48]. Ca and P delivery by parenteral nutrition are affected not only by their respective concentrations in the intravenous solution, but also by the ratio of their concentrations. The optimal Ca/P ratio in the intravenous solution fluid is between 1.3:1 and 1.7:1.54 [49,50]. The supply of these minerals to infants is limited by the poor solubility of both Ca and P in parenteral nutrition solution, resulting in an increase in the risk of OOP when enteral feeding is not possible for an extended period. Further research is required to improve Ca and P delivery with parenteral nutrition. Vigilance is required during parenteral nutrition as the increase in parenteral mineral delivery may result in metabolic acidosis and hypercalciuria [52]. If needed, parenteral P delivery can also be enhanced by using special preparations of organic P.

Because of the crucial role of mechanical forces on the development of the skeleton, daily exercises such as gentle compression and movements of the limbs are recommended in infants at risk of OOP if greater increase in body weight, forearm bone length, bone area and BMC are to be achieved [53–55].

CURRENT RECOMMENDATIONS

Guidelines for screening and treating infants at risk of OOP have been developed [56]. As summarized in Table 1, it is recommended to monitor all infants for MBD if their birth weight is below 1500 g, or if born before 28 wk of gestation, or if they have received total parenteral nutrition for more than four weeks or in case of diuretic or corticosteroid therapy. Monitoring consists of weekly serum “bone profile” (Ca, P and ALP). If serum P < 1.8 mmol/L and ALP > 500 IU/L, renal TRP should be measured and, if it exceeds 95%, P supplementation should be started. If serum P levels fail to increase and if serum ALP levels keep on rising, ergocalciferol or alphacalcidol therapy should be then considered. The American Academy of Pediatrics recommends that all breast-fed, partially breast-fed and non-breast-fed infants consuming less than 1000 mL of vitamin D fortified milk daily should be supplemented daily with a minimum of 400 IU vitamin D [57]. If the biomarkers of MBD do not normalize, vitamin D supplementation with up to 600 IU/d has been suggested, but without much supporting evidence. In addition, daily passive exercises should be encouraged and the medications in use should be regularly reviewed with discontinuation of diuretics and steroids when appropriate.

CONCLUSION

Preterm infants, those on long-term diuretics or corticosteroids, and those with neuromuscular disorders are at high risk of developing osteopenia. Complications such as rickets and pathological fractures may be the first manifestation of the condition. To detect the early asymptomatic phases of impaired bone mineralization and allow early intervention, all neonates at high risk of MBD appropriate biochemical markers of insufficient intake minerals and of abnormal bone turnover should be regularly monitored. DEXA is being increasingly used for assessing BMD in neonates, but more studies are still needed before it can be used as a useful clinical tool. Prevention and early diagnosis of MBD are key to the successful management of this condition and oral P supplements should be started as soon as is feasible.

Prospective studies of cohorts of preterm infants with OOP are needed with close long-term follow up for later outcomes. More research into urinary Ca and P to creatinine ratios is needed before they can reliably replace direct measurement of BMC. Similarly DEXA needs to be studied further to better define the “areal” nature of the measurement derived for BMD estimation in the newborn and also to establish reliable neonatal, ethnic and sex specific normograms. The possible role of QUS in routine screening for OOP needs also to be studied. As the poor solubility of Ca and P in parenteral nutrition solution hampers the adequacy of their supply to the growing newborn, further research in this area is required to increase their delivery.

REFERENCES

1. Koo WW, Sherman R, Succop P, Krug-Wispe S, Tsang RC, Steichen JJ, Crawford AH, Oestreich AE. Fractures and rickets in very low birth weight infants: conservative management and
outcome. J Pediatr Orthop 1989; 9: 326-330 [PMID: 2723052 DOI: 10.1097/01202412-198909030-00012]

2. Callenbach JC, Sheehan MB,Abramson SJ, Hall RT. Etiologic factors in rickets of very low-birth-weight infants. J Pediatr 1981; 98: 800-805 [PMID: 7029765 DOI: 10.1016/S0022-3476(81)80852-9]

3. Dokos C, Tsakalidis C, Tragiannidis A, Rallis D. Inside the “fragile” infant: pathophysiology, molecular background, risk factors and investigation of neonatal osteopenia. Clin Cases Miner Bone Metab 2013; 10: 86-90 [PMID: 24133523]

4. Goulding A, Jones IE, Taylor RW, Williams SM, Manning PJ. Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. J Pediatr 2001; 139: 509-515 [PMID: 1159856 DOIP: 10.1067/med.2001.116297]

5. Rustico SE. Calabria AC, Garber SJ. Metabolic bone disease of prematurity. J Pediatr 2002; 140: 509-515 [PMID: 1159856 DOI: 10.1067/med.2001.116297]

6. Dabezies EJ, Warren PD. Fractures in very low birth weight infants with rickets. Clin Orthop Relat Res 1997; (335): 233-239 [PMID: 9020223]

7. Chan GM, Armstrong C, Moyer-Mileur L, Hoff C. Growth and bone mineralization in children born prematurely. J Perinatol 2008; 28: 619-623 [PMID: 18548083 DOI: 10.1016/j.jpeds.2008.09.59]

8. Zamora SA, Beller DC, Rizzoli R, Slozman DO, Bonjour JP. Lower femoral neck bone mineral density in prepubertal former preterm girls. Bone 2001; 29: 424-427 [PMID: 11704492 DOI: 10.1016/S8756-3282(01)00596-8]

9. Abou Samra H, Stevens D, Binkley T, Specker B. Determinants of bone mass and size in 7-year-old former term, late-preterm, and preterm boys. Osteoporos Int 2009; 20: 1903-1910 [PMID: 19308302 DOI: 10.1007/s00198-009-0896-z]

10. Bozzetti V, Tagliaabe P. Metabolic Bone Disease in preterm newborn: an update on nutritional issues. Ital J Pediatr 2009; 35: 20 [PMID: 19602277 DOI: 10.1186/1828-7288-35-20]

11. Litmanovitz I, Dolfi T, Regue R, Arnon S, Friedland O, Shainkin-Kestenbaum R, Lis M, Eliakim A. Bone turnover markers and bone strength during the first weeks of life in very low birth weight premature infants. J Perin Med 2004; 32: 58-61 [PMID: 15088388 DOI: 10.1515/JPM.2004.010]

12. Salle BL, Glorieux FH, Delvin EE. Perinatal vitamin D metabolism. Biol Neonate 1988; 54: 181-187 [PMID: 3052601 DOI: 10.1159/000024825]

13. Holland PC, Wilkinson AR, Diez J, Lindsell DR. Prenatal deficiency of phosphate, phosphate supplementation, and rickets in very-low-birthweight infants. Lancet 1990; 335: 697-701 [PMID: 1969066 DOI: 10.1016/0140-6736(90)90114-R]

14. Bosley AR, Verrier-Jones ER, Campbell MJ. Aetiological factors in rickets of prematurity. Arch Dis Child 1980; 55: 683-686 [PMID: 7463531 DOI: 10.1136/adc.55.9.683]

15. Lucas A, Brooke OG, Baker BA, Bishop N, Morley R. High alkaline phosphatase activity and growth in preterm neonates. Arch Dis Child 1989; 64: 902-909 [PMID: 2774631 DOI: 10.1136/adc.64.7.Sec_902]

16. Yeh JK, Liu CC, Aloia JF. Effects of exercise and immobilization on bone formation and resorption in young rats. Am J Physiol 1993; 264: E182-E189 [PMID: 8447384]

17. Bandara S, Karaywasam A. Incidence of osteopenia of prematurity in preterm infants who were exclusively fed breast milk. Early Human Development 2010; 86: S18 [DOI: 10.1016/j.earlhumdev.20 10.09.053]

18. Figuera-Aloy J, Alvarez-Dominguez E, Perez-Fernandez JM, Morentinos-Suahel G, Vidal-Sicart S, Botet-Mussons F. Metabolic bone disease and bone mineral density in very preterm infants. J Pediatr 2014; 164: 499-504 [PMID: 24336169 DOI: 10.1016/j.jpeds.2013.10.089]

19. Vacharajarni AJ, Mathur AM, Rao R. Metabolic Bone Disease of Prematurity. Nooreviews 2009; 10: e402

20. Koo WW, Gupta JM, Nayanar VV, Wilkinson M, Posen S. Skeletal changes in preterm infants. Arch Dis Child Fetal Neonatal Ed 1992; 67: 373-376 [PMID: 1369567 DOI: 10.1136/adc.57.5.373]

21. Rigo J, Nyamugabo K, Picaud JC, Gerard P, Pieltain C, De Curtis M, Reference values of body composition obtained by dual energy X-ray absorptiometry in preterm and term neonates. J Pediatr Gastroenterol Nutr 1998; 27: 184-190 [PMID: 9702651 DOI: 10.1097/0158-133X(199804)30<184::AID-JPN1>3.0.CO;1-K]
Littner Y, Mandel D, Mimouni FB, Dollberg S. Bone ultrasound velocity curves of newly born term and preterm infants. *J Pediatr Endocrinol Metab* 2003; 16: 43-47 [PMID: 12585339 DOI: 10.1515/JPEM.2003.16.1.43]

Nemet D, Dolfen T, Wolach B, Elikiam A. Quantitative ultrasound measurements of bone speed of sound in premature infants. *Eur J Pediatr* 2001; 160: 736-740 [PMID: 11795683 DOI: 10.1007/s0043100100649]

Rubinacci A, Moro GE, Boehm G, De Terlizzi F, Moro GL, Cadossi R. Quantitative ultrasound for the assessment of osteopenia in preterm infants. *Eur J Endocrinol* 2003; 149: 307-315 [PMID: 14514345 DOI: 10.1530/eje.0.1490307]

Glüer CC, Wu CY, Jergas M, Goldstein SA, Genant HK. Three quantitative ultrasound parameters reflect bone structure. *Calcif Tissue Int* 1994; 55: 46-52 [PMID: 7922789 DOI: 10.1007/BF00310168]

Rack B, Lochmüller EM, Janni W, Lipowsky G, Engelsberger I, Friese K, Küster H. Ultrasound for the assessment of bone quality in preterm and term infants. *J Perinatol* 2012; 32: 218-226 [PMID: 21681177 DOI: 10.1038/jp.2011.82]

Ashmeade T, Pereda L, Chen M, Carver JD. Longitudinal measurements of bone status in preterm infants. *J Pediatr Endocrinol Metab* 2007; 20: 415-424 [PMID: 17451080 DOI: 10.1515/JPEM.2007.20.3.415]

Salle BL, Senterre J, Putet G. Calcium, phosphorus, magnesium and vitamin D requirement in premature infants. *Nestle Nutrition Workshop Series* 1993; 32: 125-134

Ehrenkranz RA, Gettner PA, Nelli CM. Nutrient balance studies in premature infants fed premature formula or fortified preterm human milk. *J Pediatr Gastroenterol Nutr* 1989; 8: 58-67 [PMID: 2499673 DOI: 10.1097/00005176-198901000-00012]

Evans JR, Allen AC, Stinson DA, Hamilton DC, St John Brown B, Vincer MJ, Raad MA, Gundberg CM, Cole DE. Effect of high-dose vitamin D supplementation on radiographically detectable bone disease of very low birth weight infants. *J Pediatr* 1989; 115: 779-786 [PMID: 2809913 DOI: 10.1016/S0022-3476(89)80662-6]

Pelegano JF, Rowe JC, Carey DE, LaBarre DJ, Raye JR, Edgren KW, Horak E. Simultaneous infusion of calcium and phosphorus in parenteral nutrition for premature infants: use of physiologic calcium/phosphorus ratio. *J Pediatr* 1989; 114: 115-119 [PMID: 2491886 DOI: 10.1016/S0022-3476(89)80617-1]

Salle BL, David L, Chopard JP, Grafmeyer DC, Renaud H. Prevention of early neonatal hypocalcemia in low birth weight infants with continuous calcium infusion: effect on serum calcium, phosphorus, magnesium, and circulating immunoreactive parathyroid hormone and calcitonin. *Pediatr Res* 1977; 11: 1180-1185 [PMID: 593762 DOI: 10.1203/00006640-197712000-00003]

Atkinson SA. Calcium and phosphorus needs of premature infants. *Nutrition* 1994; 10: 66-68 [PMID: 8199427]

Pereira GR. Nutritional care of the extremely premature infant. *Clin Perinatol* 1995; 22: 61-75 [PMID: 7781256]

Vileisis RA. Effect of phosphorus intake in total parenteral nutrition infuses in premature neonates. *J Pediatr* 1987; 110: 586-590 [PMID: 3031260 DOI: 10.1016/S0022-3476(87)80058-9]

Pelegano JF, Rowe JC, Carey DE, LaBarre DJ, Edgren KW, Lazar AM, Horak E. Effect of calcium/phosphorus ratio on mineral retention in parenterally fed premature infants. *J Pediatr Gastroenterol Nutr* 1991; 12: 351-355 [PMID: 1649288 DOI: 10.1097/00005176-199104000-00011]

Eliakim A, Nemet D. Osteopenia of prematurity - the role of exercise in prevention and treatment. *Pediatr Endocrinol Rev* 2005; 2: 675-682 [PMID: 16208281]

Moyer-Mileur LJ, Brunstetter V, McNaught TP, Gill G, Chan GM. Daily physical activity program increases bone mineralization and growth in preterm very low birth weight infants. *Pediatrics* 2000; 106: 1088-1092 [PMID: 11061779 DOI: 10.1542/peds.106.5.1088]

Litmanovitz I, Dolfin T, Friedland O, Arnon S, Regev R, Shainkin-Kestenbaum R, Lis M, Eliakim A. Early physical activity intervention prevents decrease of bone strength in very low birth weight infants. *Pediatrics* 2003; 112: 15-19 [PMID: 12837861 DOI: 10.1542/peds.112.1.15]

Harrison CM, Gibson AT. Osteopenia in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2013; 98: F272-F275 [PMID: 22556204 DOI: 10.1136/archdischild-2011-301025]

Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* 2008; 122: 1142-1152 [PMID: 18977996 DOI: 10.1542/peds.2008-1862]
