Review Article

Osteoporosis in Children: Possible Risk Factors and Role of Antioxidants

Salah A. Sheweita, Awad S. Al Samghan[1], Owais K. Khoshhal[2]

Department of Clinical Biochemistry, Faculty of Medicine, King Khalid University, [1]Department of Family and Community Medicine, Faculty of Medicine, King Khalid University, Abha, Saudi Arabia, [2]College of Medicine, Taibah University, Almadinah Almunawwarah, Saudi Arabia

Abstract

Osteoporosis is well recognized in children as a consequence of several factors. Therefore, the present review sheds light on the role of diabetes mellitus (DM), malabsorption, glucocorticoids, nutrition, free radicals, and oxidative stress in the induction of osteoporosis. It may also provide valuable information regarding the early detection of osteoporosis to improve not only the bone health of schoolchildren but also their general quality of life. Measurement of bone mineral density (BMD) does not capture all the risk factors of bone fractures and/or osteoporosis. Therefore, bone resorption and formation markers such as osteoprotegerin; prolidase; osteocalcin; bone alkaline phosphatase and Vitamin D; parathyroid hormones; and macroelements such as calcium, phosphorus, and magnesium should be measured beside BMD in the plasma of school-aged children. Moreover, endocrine abnormalities, high levels of free radicals, and induction of oxidative stress showed an adverse effect on the skeleton and cause osteoporosis. It has been found that there is a strong correlation between osteoporosis and DM, malnutrition, and glucocorticoids in both pediatric and adult patients. Inhibition of antioxidant enzyme activities, such as superoxide dismutase, catalase, and glutathione peroxidase, was found to increase the production of reactive oxygen species by osteoclasts. Therefore, oxidative stress and other factors are important mediators of bone loss and also osteoporosis. Furthermore, antioxidants should be provided to maintain bone integrity because a deficiency of antioxidant vitamins has been found in the osteoporotic children.

Keywords: Diabetes mellitus, free radicals, glucocorticoids, malabsorption, nutrition, osteoporosis, oxidative stress

Introduction

Osteoporosis

Approximately 200 million people in the world are threatened with osteoporosis, and therefore, it has become a health problem globally.[1,2] Osteoporosis is a silent disease because no asymptomatic signs are observed until a fracture occurs.[3] The main characteristics of osteoporosis include systemic skeletal disorders, which are associated with low bone mass and micro-architectural deterioration of bone tissues, leading to an increase in bone fragility and susceptibility to fracture.[4] However, assessment of bone mineral density (BMD) alone in the absence of any fractures in adults is used to detect osteoporosis. Several epidemiological studies have demonstrated a link between the measurements of bone density at the spine, hips, and wrists, with a subsequent risk of fractures using dual-energy X-ray absorptiometry (DEXA). However, in children, on the basis of the measurements of bone density alone, it is not possible to define osteoporosis. Several previous studies have shown the relationship and the differences between bone density in both healthy children and those with bone fractures.[4,5] Currently, the reasons of association between the fracture risk and bone density are unknown in children with chronic diseases, and therefore, it is not possible to define the level of bone density below which the fracture risk increases. An additional reason is that body size was found to affect the bone density measurements in children using DEXA scans because there is a strong correlation between areal bone density and bone size. This was illustrated in the study by Gafni and Baron where up to 50% of children had received an inappropriate diagnosis of osteoporosis.[6]

Access this article online

Quick Response Code:

Website: www.journalmsr.com

DOI: 10.4103/jmsr.jmsr_40_19

Address for correspondence: Prof. Salah A. Sheweita, Department of Clinical Biochemistry, Faculty of Medicine, King Khalid University, Abha, Saudi Arabia.
E-mail: ssheweita@yahoo.com

Received : 08-05-2019 **Revised :** 15-06-2019
Accepted : 20-07-2019 **Published Online :** 20-08-2019

How to cite this article: Sheweita SA, Al Samghan AS, Khoshhal OK. Osteoporosis in children: Possible risk factors and role of antioxidants. J Musculoskelet Surg Res 2019;3:319-25.

Bone resorption markers

The extracellular matrix of bones is degraded by osteoclasts and released into the circulation. Therefore, measurable concentrations of collagen degradation products in both serum and urine are used as indicators of bone resorption.[7] These indicators include cross-linked carboxyterminal-telopeptide and cross-linked aminoterminal-telopeptide, as well as free pyridinolines and deoxypyridinolines. In addition, acid phosphatase isoenzyme called tartrate-resistant acid phosphatase (TRAP) is produced by osteoclasts.[7] However, the measurement of total TRAP activity can be influenced by various circulating inhibitors because it is originating from both platelets and erythrocytes.[8] In addition, assay of the kinetic activity of the desialylated isoenzyme (type-5b TRAP) which is present only in osteoclasts has been described[9,10] because the increased activity has been associated with bone resorption as observed in conditions of hemodialysis, end-stage renal failure, bone disease, and metastatic bone disease.[11,12] In addition, there are also markers of osteoblastic activity such as osteocalcin levels and bone-specific alkaline phosphatase (ALP) activity which increase after bone fractures.[13] It has been found that the assay of markers of collagen production could indicate the status of bone health at the fracture sites.

The evaluation of bone turnover markers in various metabolic bone diseases as observed in diabetes mellitus (DM) has been improved through the development of biochemical markers which are specific and sensitive in reflecting the overall rate of bone metabolism.[14,15]

Predisposing factors of osteoporosis

There are several etiological factors that adversely affect the bone development of a child with a chronic condition. These factors can act either singly or in combination and increase the development of osteoporosis.

Diabetes mellitus

In fact, 374 million people all over the world are suffering from diabetic complications.[16,17] Like osteoporosis, DM is a pandemic and a chronic metabolic disorder that adversely affects bones, nerves, muscles, eyes, and kidneys.[18] Several studies have shown that type 1 DM (T1DM) had increased rates of bone fractures and osteoporosis[19,20] in both children[21,22] and adults.[23] Several researchers have described different mechanisms that show how DM induces osteoporosis and bone fractures through multiple pathways.[24,25]

Supporting these findings, in our previous study, it has been found that levels of both osteocalcin and N-terminal propeptide of type I procollagen (P1NP) were much lower in the serum of diabetic children than that of nondiabetic controls. In agreement with our finding, it has been found that the serum levels of P1NP were lower in diabetic osteopenic patients, which indicated poor bone formation.[26] P1NP has several advantages due to its stability in serum at room temperature and has also been recommended to be used as a stable marker due to its low interindividual variability.[27,28] Moreover, it has been used as a preliminary biomarker on the effectiveness of a given drug on bone formation.

Bone marrow-derived endothelial progenitor cells (EPCs) play a significant role in bone healing.[29,30] Bone formation at the fracture sites in DM patients decreases due to the downregulation of the expression of EPCs.[31-33] DM is also responsible for the decrease in the rate of blood flow to the bone due to the deposition of lipid in the bone marrow, thereby leading to the expansion of the marrow cavity.[31] The reduction of osteoblasts available for bone formation might be due to the transformation of osteoblasts to adipocytes.[31,34] It has been found that advanced glycation end product expression was induced in DM, and this protein has a significant role in bone rigidity.[35,36] Other osteoporotic factors such as amylin and preptin were also secreted by pancreatic β-cells which could induce bone formation and reduce both bone resorption and apoptosis of osteoblasts.[37] Osteogenesis has been regulated by osteocalcin which is diminished in DM, which consequently leads to the decrease of insulin, amylin, and preptin synthesis.

Malabsorption

Celiac disease (CD) and inflammatory bowel disease (IBD) symptoms are varied, and metabolic bone disease is not well recognized among all extradigestive manifestations.[38] Both diseases are associated with osteoporosis, osteopenia, and osteomalacia. The most common causes of malabsorption among gastrointestinal diseases are mainly due to CD and IBD.[39] In contrast to osteoporosis, which occurs in postmenopausal women, patients with CD and IBDs are much younger, and are prone to develop vertebral fractures. Impaired absorption of nutrients such as calcium and Vitamin D and the use of glucocorticoids in the treatment of IBD are the main pathophysiologic factors specific to gastrointestinal diseases.[40] Hyperparathyroidism is associated with increased bone remodeling and results from CD. Therefore, children with CD may be at risk of bone fractures.[41] A gluten-free diet is known to improve BMD, but it cannot normalize bone mass in all patients.[42] Therefore, CD patients should be screened for low BMD, and appropriate follow-up and management of bone disease should be made based on BMD and fracture risk.[43,44] IBD has been recognized in both the United States (1.4 million) and Europe (2.2 million people).[45] It has been found that osteomalacia and Vitamin D deficiency are not the main causes of diminished BMD in IBD.

Nutraceuticals

The normal growth and development of bones are mainly dependent on adequate nutrition.[46] Recently, it has been found that antioxidant vitamins play a significant role in the healing of bone fractures.[47] It is not surprising; therefore, nutritional and low-body-weight disorders could lead to osteoporosis.[48,49] Various etiological factors including Vitamin D and protein intake, low body weight, low calcium, gonadal deficiency, growth hormone resistance, and malabsorption are found to play a significant role in the induction of osteoporosis[50] because calcium and Vitamin D are essential for skeletal

mineralization.[51] In healthy adults, it has been found that calcium supplementation causes short-term gains in BMD.[52] It is not well known whether such gains are sustainable and could improve peak bone mass or most importantly increase bone strength. Both healthy and ill children should receive calcium as recommended daily doses. Similar to the case of chronically ill children, children living in sunny climates can become Vitamin D deficient without adequate sun exposure.[53] Therefore, the level of Vitamin D in chronically ill children should be evaluated and supplemented at 400 IU/day.

Growing evidences show that nutritional imbalance and endocrine abnormalities could be involved in the pathogenesis of osteoarthrosis (OA) and osteochondritis dissecans (OCD).[54,55] Therefore, it has been found that dietary programs play an important role in the management of these disorders.[56] Vitamins (particularly Vitamin C) and essential trace elements (zinc, magnesium, and copper) are critically important for articular cartilage.[54] Therefore, nutraceuticals used with nonsteroidal anti-inflammatory drugs may be beneficial for patients with joint disorders including OA and OCD.[57]

The metabolites of essential fatty acids (EFAs) such as γ-linolenic acid (GLA), eicosapentaenoic acid (EPA), and docosahexaenoic acid show a beneficial effect in the prevention of osteoporosis.[58,59] Nephrocalcinosis in animals can be prevented by fish oil (FO) by reducing the excretion of calcium in urine. In addition, it has been found that diet rich in saturated fat interferes with calcium absorption.[60] Maintaining higher BMD was found after feeding of mouse for a long term with FO. These improvements in BMD might be due to the induction of antioxidant enzyme activities, decreased expression of receptor activator of nuclear factor kappa-B ligand (RANKL), and increased expression of osteoprotegerin (OPG) in FO-fed mouse. These effects on BMD suggested that FO is important to prevent BMD loss in patients with rheumatoid arthritis.[61]

Severe osteoporosis and increased renal and arterial calcification were found in EFA-deficient animals,[58] which is similar to those occurred in elderly people. Enhanced calcium absorption and bone calcium content were found after the administration of a combination of GLA and EPA.[58] The mechanism of the prevention of osteoporosis by GLA and EPA might be due to the inhibition of pro-inflammatory cytokines such as interleukin (IL) IL-1, IL-2, and tumor necrosis factor (TNF) TNF-α,[58] which have a significant role in osteoporosis.

Inflammatory cytokines

Several chronic inflammatory conditions have been found to be associated with osteoporotic children with juvenile idiopathic arthritis, systemic lupus erythematosus, and Crohn's disease. It has been found that suppression of osteoblast recruitment and stimulation of osteoclastogenesis occur by increased circulating levels of cytokines such as IL-1A, IL-6, IL-7, TNF-α, and TNF-β. This increment in IL was found to cause an imbalance in bone turnover, leading to osteoporosis. It has been shown that activated T-cells produce higher levels of TNF-α in children with Crohn's disease than those from controls.[62] Glucocorticoids have been used for the treatment of these conditions. Such treatments with glucocorticoids make it difficult to distinguish the impact of the inflammatory condition on bone integrity because osteoporotic fractures can occur in the absence of the use of glucocorticoids.[63] In addition, inflammatory cytokines show an adverse effect on the skeletal muscles, which could compromise the mechanical loading on the skeleton and consequently impact on bone metabolism. Identified significant defects in lean body mass was found in adults and also in children with Crohn's disease.[64] Increases in bone density and levels of bone formation markers have been observed after treatment of patients with Crohn's disease and also treated with infliximab.[65]

Glucocorticoids

Bone fractures are the most serious common adverse events related to the long-term use of glucocorticoids which are used for many medical treatments.[66] It has been found that osteoporosis develops in a time- and dose-dependent manner of glucocorticoid treatment which is slightly induced at low doses, but at higher doses markedly increased risk of bone fractures.[67] Because of potent anti-inflammatory actions of glucocorticoids, they are used in many chronic childhood conditions. However, glucocorticoids have various effects on calcium and bone metabolism. In addition, glucocorticoids show a direct effect on osteoblasts and cause a reduction in bone formation, an inhibition of OPG leading to increased bone resorption by stimulating osteoclastogenesis. It has been found that glucocorticoids reduce calcium absorption by intestines and also increase renal tubular calcium excretion. The vertebral fractures were associated with children with juvenile idiopathic arthritis received 0.62 mg/kg per day of prednisolone.[68] In addition, it has been found that children receiving four or more courses of systemic steroids have an increased incidence of bone fracture.[69] However, Leonard *et al*. demonstrated that bone mineral content of the lumbar spine and whole body was not different from that of controls.[70]

The toxic effect of glucocorticoids on osteoblasts might be due to their bond to the promoter region of response elements, ultimately leading to altered protein synthesis and regulation. Hormonally active glucocorticoids can be converted into inactive hormone forms by 11 β-hydroxysteroid dehydrogenase; therefore, polymorphism of the gene of this enzyme may explain the susceptibility to glucocorticoid toxicity.[71] The deleterious effect of glucocorticoids on bone formation might be due to change in the expression of gamma receptor 2 (PPARγ2)[72] and also might be due to the inhibition of signaling pathway of the canonical Wnt/β catenin.[73] Decreased production of osteoblasts and ultimately less bone formation after exposure to glucocorticoids could be due to the enhanced expression of Wnt antagonists, sclerostin, and Dickkopf-related protein 1. Glucocorticoids increase the production of RANKL and decrease the production of OPG, resulting in enhanced bone resorption.[73]

Reactive oxygen species and bone integrity

Reactive oxygen species (ROS) have an important role in bone metabolism because they have a dual effect including physiological and pathological conditions.[74,75] Under physiological conditions, ROS assists in accelerating the destruction of calcified tissue and hence assists in bone remodeling.[76,77] Increased bone resorption through the activation of nuclear factor-κB (NF-κB) has been found due to high levels of free radicals.[78] It has been found that oxidative stress has adverse effects on bones; therefore, insufficient dietary intake of antioxidant Vitamins (E and C) may substantially increase the risk of hip fracture.[64] Moreover, NADPH oxidase capable of cytokine-regulated generation of ROS is also present in osteoclasts.[69,79,80]

A remarkably high yield of free radicals is possible when bone fractures occur.[81] However, many skeletal pathologies are linked to enhanced osteoclastic activity and increased production of ROS. It is suggested that increased ROS production overwhelms the antioxidant defenses, subjecting the individual to hyperoxidant stress. There are many complex steps of calcified tissue destruction by osteoclasts. It has been found that osteoclasts could accelerate the destruction of calcified tissue and assist in bone remodeling under controlled production of free radicals.[77] Increased production of ROS, which is evident by increased levels of serum malondialdehyde (MDA) levels, was found to be mainly due to enhanced osteoclastic activity that is observed in bone disorders. Lipid peroxidation is one of the most damaging effects of ROS, and MDA is its end product.[82] In addition, MDA serves as an index of lipid peroxidation and also serves as a measure of osteoclastic activity. Superoxide dismutase and glutathione peroxidase (GSH-P_X) activities are one of the defense mechanisms against free radicals that could reduce the antioxidants capacity in the body, and consequently increased superoxide production by the osteoclasts.[82]

Role of antioxidants

It has been found that generation of high levels of free radicals could cause several diseases. It has been found that free radical scavengers have a great role in the amelioration of diseases. For example, polyphenols, which are one of the free radical scavengers, play a significant role in the prevention of cardiovascular diseases, cancers, neurodegenerative diseases, diabetes, or osteoporosis in both animals and human cell lines.[83] 2,6-diisopropylphenol, similar to alpha-tocopherol, has been used for the induction and maintenance of anesthesia and has shown antioxidant effects.[83] Therefore, it has been found that 2,6-diisopropylphenol protects osteoblasts from sodium nitroprusside- and hydrogen peroxide-induced cell damage.[83] Moreover, ascorbic acid (AA) plays a key role in the regulation of differentiation and activation of osteoclasts[84] through the inhibition of RANKL-induced differentiation of osteoclasts (OCL) precursor cells into mature OCL and reduces the formation of bone resorption pits *in vitro*.[84] In addition, differentiation of embryonic stem cells into osteoblasts has been induced by AA. The mechanisms by which AA induced the differentiation of embryonic stem cells included synthesis of collagen type I, interaction with alpha2- and beta1-integrins, activation of the mitogen-activated protein kinase pathway, and phosphorylation of osteoblast-specific transcription factors.[85] By using DNA microarrays, it has been found that several genes cover a broad range of functional activities, including cell growth, metabolism, morphogenesis, cell death, and cell communication at early-stage stimulation of preosteoblasts by AA in MC3T3-E1 cultured with AA for 24 h.[85] Elucidation of the molecular mechanism of AA may facilitate the clinical implications of AA to accelerate bone regeneration.[85]

Oxidative stress is an important mediator of bone loss because TNF-α, which increases as a result of oxidative stress, was found to play a critical role in bone loss after menopause.[85] In HS-5 hBMSC, TNF-α and H_2O_2 increase intracellular ROS levels and induce cell apoptosis through the activation of caspases, JNK, and NF-κB. Alpha-lipoic acid, an antioxidant, prevents the induction of ROS_2, suggesting its potential therapeutic action in preventing bone loss.[85,86]

Reduction in oxidative stress, inhibition of inflammatory cytokine activation, and NF-κB DNA-binding activity were found after the treatment of mice with alpha-lipoic acid.[87] Moreover, inhibition of bone destruction *in vivo* and osteoclastogenesis *in vitro* have been seen after treatment with alpha-lipoic acid.[87] The risk of osteoporosis is associated with oxidative stress induced by ROS, and can be reduced by certain dietary antioxidants. Lycopene is an antioxidant known to decrease the risk of osteoporosis[88] through the reduction of oxidative stress and also the levels of bone turnover markers, and may be beneficial in osteoporosis treatment.[88] Markers of oxidative stress including reduced glutathione (GSH), oxidized glutathione (GSSG), and MDA were found in loose and stable hips revised for high rate of wear and osteolysis.[89] Collagen in periprosthetic tissues measured as hydroxyproline content[89] was correlated with MDA, GSH, and GSSG levels.[89] This study provided a new evidence regarding the role of MDA in the destruction of collagen through releasing of hydroxyproline.[89]

Elderly osteoporotic patients have common subclinical vitamin deficiency.[90] Administration of vitamins has been found to treat and prevent osteoporosis in these patients.[91,92] Bone health and muscle strength in the elderly require higher Vitamin D intake and more sun exposure to keep them in a good condition.The risk of bone fractures in osteoporosis are also linked to the deficiency of Vitamins K, C, or B.[92,93] Therefore, a diet rich in fruit and vegetables along with fish could fulfill a balance among these vitamins and should be recommended for prevention and/or treatment of osteoporosis.[93]

Oxidative stress in various experimental models could be alleviated after administration of antioxidant vitamins and/or radical scavengers.[94] Furthermore, melatonin can enhance osteogenesis in the case of an individual with a head injury because osteoblastic activity raises up with increased melatonin levels. Melatonin might cause early bone healing

and hypertrophic callus,[94] and healing of a fracture of long bone can be accelerated in patients with severe traumatic brain injury after melatonin therapy. Flavones, in contrast to soybean isoflavones, are the most abundant phytoestrogens in Western diets, being present in onions, beans, fruits, red wine, and tea. Quercetin is the most widely distributed type of flavonols, which occurs mainly as glycoside and rutin, but the published data are very scarce regarding the precise mechanism of action of quercetin on bone-resorbing cells.[95] Estrogen receptor (ER)-alpha (ER-alpha), ER-beta, and RANK proteins are present in osteoclasts and osteoclast progenitors. Flavones increase nuclear ER-beta protein and decrease ER-alpha protein of osteoclast progenitors. Moreover, rutin reduces RANK protein, whereas 17 beta-estradiol and quercetin promote apoptosis by cleavage of caspase-8 and caspase-3. Flavones exert the antiresorbing properties of ER proteins through the inhibition of RANK protein or the activation of caspases.[96] In our previous studies, we had found that antioxidants could protect organs against the toxicity of toxic compounds and also improved male infertility.[97-100]

Conclusion

It is concluded from this review that increased free radical production overwhelms the natural antioxidant defense mechanisms, subjecting individuals to hyperoxidant stress and thus leading to osteoporosis. In addition, administration of antioxidants might protect bones from osteoporosis and also might help in the acceleration of healing of fractured bones.

Recommendations

Unexplained fractures in children or fractures after minor trauma call for an investigation to rule out underlying bone disease, including osteoporosis.

Many different factors could induce and enhance osteoporosis in children. To avoid such a disease at an early stage, it is advisable to check and screen for osteoporosis using biomarkers including OPG, prolidase, bone ALP, osteocalcin, Vitamin D, and parathyroid hormone in susceptible children.

It is recommended that children whom are at risk of osteoporosis at an early stage should receive Vitamin D and antioxidant vitamins. Moreover, children with osteoporotic fractures need supplements to enhance fracture healing.

Ethical approval

This article does not contain any study with human participants or animals performed by any of the authors.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Authors' contributions

SAS suggested the idea and concept of the review article and shared in writing the text. ASA shared in writing the text. OKK shared in writing the text and the concept of the review article. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

References

1. Lampropoulos CE, Papaioannou I, D'Cruz DP. Osteoporosis – A risk factor for cardiovascular disease? Nat Rev Rheumatol 2012;8:587-98.
2. Chen HL, Deng LL, Li JF. Prevalence of osteoporosis and its associated factors among older men with type 2 diabetes. Int J Endocrinol 2013;2013:285729.
3. Khoshhal KI. Childhood osteoporosis. J Taibah Univ Med Sci 2011;6:61-76.
4. Goulding A, Cannan R, Williams SM, Gold EJ, Taylor RW, Lewis-Barned NJ. Bone mineral density in girls with forearm fractures. J Bone Miner Res 1998;13:143-8.
5. Clark EM, Ness AR, Bishop NJ, Tobias JH. Association between bone mass and fractures in children: A prospective cohort study. J Bone Miner Res 2006;21:1489-95.
6. Gafni RI, Baron J. Overdiagnosis of osteoporosis in children due to misinterpretation of dual-energy x-ray absorptiometry (DEXA). J Pediatr 2004;144:253-7.
7. Gunczler P, Lanes R, Paz-Martinez V, Martins R, Esaa S, Colmenares V, *et al.* Decreased lumbar spine bone mass and low bone turnover in children and adolescents with insulin dependent diabetes mellitus followed longitudinally. J Pediatr Endocrinol Metab 1998;11:413-9.
8. Camacho P, Kleerekoper M. Biochemical markers of bone turnover. In: Favus MJ, editor. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 6th ed. Washington, D.C.: American Society for Bone and Mineral Research; 2006. p. 127-33.
9. Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ, Väänänen HK. Tartrate-resistant acid phosphatase 5b: A novel serum marker of bone resorption. J Bone Miner Res 2000;15:1337-45.
10. Henriksen K, Tanko LB, Qvist P, Delmas PD, Christiansen C, Karsdal MA. Assessment of osteoclast number and function: Application in the development of new and improved treatment modalities for bone diseases. Osteoporos Int 2007;18:681-5.
11. Małyszko J, Małyszko JS, Pawlak K, Wołczyński S, Myśliwiec M. Tartrate-resistant acid phosphatase 5b and its correlations with other markers of bone metabolism in kidney transplant recipients and dialyzed patients. Adv Med Sci 2006;51:69-72.
12. Lyubimova NV, Pashkov MV, Tyulyandin SA, Gol'dberg VE, Kushlinskii NE. Tartrate-resistant acid phosphatase as a marker of bone metastases in patients with breast cancer and prostate cancer. Bull Exp Biol Med 2004;138:77-9.
13. Bowles SA, Kurdy N, Davis AM, France MW, Marsh DR. Serum osteocalcin, total and bone-specific alkaline phosphatase following isolated tibial shaft fracture. Ann Clin Biochem 1996;33(Pt 3):196-200.
14. Garnero P, Delmas PD. Biochemical markers of bone turnover: Clinical usefulness in osteoporosis. Ann Biol Clin (Paris) 1999;57:137-48.
15. Burch J, Rice S, Yang H, Neilson A, Stirk L, Francis R, *et al.* Systematic review of the use of bone turnover markers for monitoring the response to osteoporosis treatment: The secondary prevention of fractures, and primary prevention of fractures in high-risk groups. Health Technol Assess 2014;18:1-18.
16. Wongdee K, Charoenphandhu N. Osteoporosis in diabetes mellitus: Possible cellular and molecular mechanisms. World J Diabetes 2011;2:41-8.
17. Sealand R, Razavi C, Adler RA. Diabetes mellitus and osteoporosis. Curr Diab Rep 2013;13:411-8.
18. Hamann C, Kirschner S, Günther KP, Hofbauer LC. Bone, sweet bone – Osteoporotic fractures in diabetes mellitus. Nat Rev Endocrinol 2012;8:297-305.
19. Räkel A, Sheehy O, Rahme E, LeLorier J. Osteoporosis among patients with type 1 and type 2 diabetes. Diabetes Metab 2008;34:193-205.
20. Hui SL, Epstein S, Johnston CC Jr. A prospective study of bone mass in patients with type I diabetes. J Clin Endocrinol Metab 1985;60:74-80.
21. Santiago JV, McAlister WH, Ratzan SK, Bussman Y, Haymond MW, Shackelford G, *et al.* Decreased cortical thickness & osteopenia in

children with diabetes mellitus. J Clin Endocrinol Metab 1977;45:845-8.
22. Hamed EA, Faddan NH, Elhafeez HA, Sayed D. Parathormone–25(OH)-Vitamin D axis and bone status in children and adolescents with type 1 diabetes mellitus. Pediatr Diabetes 2011;12:536-46.
23. Won HY, Lee JA, Park ZS, Song JS, Kim HY, Jang SM, *et al.* Prominent bone loss mediated by RANKL and IL-17 produced by CD4+ T cells in TallyHo/JngJ mice. PLoS One 2011;6:e18168.
24. Leidig-Bruckner G, Ziegler R. Diabetes mellitus a risk for osteoporosis? Exp Clin Endocrinol Diabetes 2001;109 Suppl 2:S493-514.
25. Gurav AN. Advanced glycation end products: A link between periodontitis and diabetes mellitus? Curr Diabetes Rev 2013;9:355-61.
26. Elhabashy SA, Said OM, Agaiby MH, Abdelrazek AA, Abdelhamid S. Effect of physical exercise on bone density and remodeling in Egyptian type 1 diabetic osteopenic adolescents. Diabetol Metab Syndr 2011;3:25.
27. Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, Griesmacher A, *et al.* Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: A need for international reference standards. Osteoporos Int 2011;22:391-420.
28. Stokes FJ, Ivanov P, Bailey LM, Fraser WD. The effects of sampling procedures and storage conditions on short-term stability of blood-based biochemical markers of bone metabolism. Clin Chem 2011;57:138-40.
29. Schmidt-Bleek K, Schell H, Lienau J, Schulz N, Hoff P, Pfaff M, *et al.* Initial immune reaction and angiogenesis in bone healing. J Tissue Eng Regen Med 2014;8:120-30.
30. Menegazzo L, Albiero M, Avogaro A, Fadini GP. Endothelial progenitor cells in diabetes mellitus. Biofactors 2012;38:194-202.
31. Balestrieri ML, Servillo L, Esposito A, D'Onofrio N, Giovane A, Casale R, *et al.* Poor glycaemic control in type 2 diabetes patients reduces endothelial progenitor cell number by influencing SIRT1 signalling via platelet-activating factor receptor activation. Diabetologia 2013;56:162-72.
32. Gunczler P, Lanes R, Paz-Martinez V, Martins R, Esaa S, Colmen-ares V, *et al*. Decreased lumbar spine bone mass and low bone turnover in children and adolescents with insulin dependent diabetes mellitus followed longitudinally. J Pediatr Endocrinol Metab 1998;11: 413-9.
33. Schwartz AV, Sellmeyer DE, Ensrud KE, Cauley JA, Tabor HK, Schreiner PJ, *et al.* Older women with diabetes have an increased risk of fracture: A prospective study. J Clin Endocrinol Metab 2001;86:32-8.
34. Sheng HH, Zhang GG, Cheung WH, Chan CW, Wang YX, Lee KM, *et al.* Elevated adipogenesis of marrow mesenchymal stem cells during early steroid-associated osteonecrosis development. J Orthop Surg Res 2007;2:15.
35. Vashishth D. Advanced glycation end-products and bone fractures. IBMS Bonekey 2009;6:268-78.
36. Kranstuber AL, Del Rio C, Biesiadecki BJ, Hamlin RL, Ottobre J, Gyorke S, *et al.* Advanced glycation end product cross-link breaker attenuates diabetes-induced cardiac dysfunction by improving sarcoplasmic reticulum calcium handling. Front Physiol 2012;3:292.
37. Reid IR. Relationships between fat and bone. Osteoporos Int 2008;19:595-606.
38. Phan CM, Guglielmi G. Metabolic bone disease in patients with malabsorption. Semin Musculoskelet Radiol 2016;20:369-75.
39. Stobaugh DJ, Deepak P, Ehrenpreis ED. Increased risk of osteoporosis-related fractures in patients with irritable bowel syndrome. Osteoporos Int 2013;24:1169-75.
40. Southerland JC, Valentine JF. Osteopenia and osteoporosis in gastrointestinal diseases: Diagnosis and treatment. Curr Gastroenterol Rep 2001;3:399-407.
41. Ludvigsson JF, Michaelsson K, Ekbom A, Montgomery SM. Coeliac disease and the risk of fractures – A general population-based cohort study. Aliment Pharmacol Ther 2007;25:273-85.
42. Di Stefano M, Mengoli C, Bergonzi M, Corazza GR. Bone mass and mineral metabolism alterations in adult celiac disease: Pathophysiology and clinical approach. Nutrients 2013;5:4786-99.
43. Fouda MA, Khan AA, Sultan MS, Rios LP, McAssey K, Armstrong D. Evaluation and management of skeletal health in celiac disease: Position statement. Can J Gastroenterol 2012;26:819-29.
44. Ludvigsson JF, Bai JC, Biagi F, Card TR, Ciacci C, Ciclitira PJ, *et al.* Diagnosis and management of adult coeliac disease: Guidelines from the British Society of Gastroenterology. Gut 2014;63:1210-28.
45. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO study group. World Health Organ Tech Rep Ser 1994;843:1-29.
46. Bunout D, Barrera G, Leiva L, Gattas V, de la Maza MP, Haschke F, *et al.* Effect of a nutritional supplementation on bone health in Chilean elderly subjects with femoral osteoporosis. J Am Coll Nutr 2006;25:170-7.
47. Kruger MC, Booth CL, Coad J, Schollum LM, Kuhn-Sherlock B, Shearer MJ. Effect of calcium fortified milk supplementation with or without Vitamin K on biochemical markers of bone turnover in premenopausal women. Nutrition 2006;22:1120-8.
48. Ward L, Glorieux FH. The spectrum of pediatric osteoporosis. In: Glorieux FH, Pettifor J, Jueppner H, editors. Pediatric Bone: Biology and Disease. San Diego: Academic Press; 2003. p. 401-42.
49. Aris RM, Merkel PA, Bachrach LK, Borowitz DS, Boyle MP, Elkin SL, *et al.* Guide to bone health and disease in cystic fibrosis. J Clin Endocrinol Metab 2005;90:1888-96.
50. Daci E, van Cromphaut S, Bouillon R. Mechanisms influencing bone metabolism in chronic illness. Horm Res 2002;58 Suppl 1:44-51.
51. Bailey DA, Martin AD, McKay HA, Whiting S, Mirwald R. Calcium accretion in girls and boys during puberty: A longitudinal analysis. J Bone Miner Res 2000;15:2245-50.
52. Ward K, Alsop C, Caulton J, Rubin C, Adams J, Mughal Z. Low magnitude mechanical loading is osteogenic in children with disabling conditions. J Bone Miner Res 2004;19:360-9.
53. Greenway A, Zacharin M. Vitamin D status of chronically ill or disabled children in victoria. J Paediatr Child Health 2003;39:543-7.
54. Sanghi D, Mishra A, Sharma AC, Raj S, Mishra R, Kumari R, *et al.* Elucidation of dietary risk factors in osteoarthritis knee – A case-control study. J Am Coll Nutr 2015;34:15-20.
55. Díaz-López B, Cannata-Andía JB. Supplementation of Vitamin D and calcium: Advantages and risks. Nephrol Dial Transplant 2006;21:2375-7.
56. Turgut M, Yenisey C, Bozkurt M, Ergin FA, Biçakçi T. Analysis of zinc and magnesium levels in pinealectomized chicks: Roles on development of spinal deformity? Biol Trace Elem Res 2006;113:67-75.
57. Goggs R, Vaughan-Thomas A, Clegg PD, Carter SD, Innes JF, Mobasheri A, *et al.* Nutraceutical therapies for degenerative joint diseases: A critical review. Crit Rev Food Sci Nutr 2005;45:145-64.
58. Das UN. Essential fatty acids and osteoporosis. Nutrition 2000;16:386-90.
59. Zajickova K, Hill M, Vankova M, Zofkova I. Low-density lipoprotein receptor-related protein 5 and Vitamin D receptor gene polymorphisms in relation to Vitamin D levels in menopause. Clin Chem Lab Med 2006;44:1066-9.
60. Wohl GR, Loehrke L, Watkins BA, Zernicke RF. Effects of high-fat diet on mature bone mineral content, structure, and mechanical properties. Calcif Tissue Int 1998;63:74-9.
61. Ostalowska A, Birkner E, Wiecha M, Kasperczyk S, Kasperczyk A, Kapolka D, *et al*. Lipid peroxidation and antioxidant enzymes in synovial fluid of patients with primary and secondary osteoarthritis of the knee joint. J Clin Invest 1990;85:632-9.
62. Sylvester FA, Davis PM, Wyzga N, Hyams JS, Lerer T. Are activated T cells regulators of bone metabolism in children with Crohn disease? J Pediatr 2006;148:461-6.
63. Thearle M, Horlick M, Bilezikian JP, Levy J, Gertner JM, Levine LS, *et al.* Osteoporosis: An unusual presentation of childhood Crohn's disease. J Clin Endocrinol Metab 2000;85:2122-6.
64. Burnham JM, Shults J, Semeao E, Foster B, Zemel BS, Stallings VA, *et al.* Whole body BMC in pediatric Crohn disease: Independent effects of altered growth, maturation, and body composition. J Bone Miner Res 2004;19:1961-8.
65. Bernstein M, Irwin S, Greenberg GR. Maintenance infliximab treatment is associated with improved bone mineral density in Crohn's disease. Am J Gastroenterol 2005;100:2031-5.
66. Adami G, Saag KG. Glucocorticoid-induced osteoporosis: 2019 concise clinical review. Osteoporosis Int 2019;30:1145-56.
67. Adami G, Saag KG. Glucocorticoid-induced osteoporosis update. Curr Opin Rheumatol. 2019;31:388-93.
68. Varonos S, Ansell BM, Reeve J. Vertebral collapse in juvenile chronic arthritis: Its relationship with glucocorticoid therapy. Calcif Tissue Int

1987;41:75-8.
69. van Staa TP, Cooper C, Leufkens HG, Bishop N. Children and the risk of fractures caused by oral corticosteroids. J Bone Miner Res 2003;18:913-8.
70. Leonard MB, Feldman HI, Shults J, Zemel BS, Foster BJ, Stallings VA. Long-term, high-dose glucocorticoids and bone mineral content in childhood glucocorticoid-sensitive nephrotic syndrome. N Engl J Med 2004;351:868-75.
71. Diederich S, Eigendorff E, Burkhardt P, Quinkler M, Bumke-Vogt C, Rochel M, *et al.* 11beta-hydroxysteroid dehydrogenase types 1 and 2: An important pharmacokinetic determinant for the activity of synthetic mineralo- and glucocorticoids. J Clin Endocrinol Metab 2002;87:5695-701.
72. Wu Z, Bucher NL, Farmer SR. Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. Mol Cell Biol 1996;16:4128-36.
73. Ohnaka K, Tanabe M, Kawate H, Nawata H, Takayanagi R. Glucocorticoid suppresses the canonical Wnt signal in cultured human osteoblasts. Biochem Biophys Res Commun 2005;329:177-81.
74. Kasten TP, Collin-Osdoby P, Patel N, Osdoby P, Krukowski M, Misko TP, *et al.* Potentiation of osteoclast bone-resorption activity by inhibition of nitric oxide synthase. Proc Natl Acad Sci U S A 1994;91:3569-73.
75. Yalin S, Bagis S, Polat G, Dogruer N, Cenk Aksit S, Hatungil R, *et al.* Is there a role of free oxygen radicals in primary male osteoporosis? Clin Exp Rheumatol 2005;23:689-92.
76. Steinbeck MJ, Appel WH Jr., Verhoeven AJ, Karnovsky MJ. NADPH-oxidase expression and *in situ* production of superoxide by osteoclasts actively resorbing bone. J Cell Biol 1994;126:765-72.
77. Yang S, Ries WL, Key LL Jr. Nicotinamide adenine dinucleotide phosphate oxidase in the formation of superoxide in osteoclasts. Calcif Tissue Int 1998;63:346-50.
78. Kong L, Wang B, Yang X, Guo H, Zhang K, Zhu Z, *et al.* Picrasidine I from picrasma quassioides suppresses osteoclastogenesis via inhibition of RANKL induced signaling pathways and attenuation of ROS production. Cell Physiol Biochem 2017;43:1425-35.
79. Geoghegan IP, Hoey DA, McNamara LM. Estrogen deficiency impairs integrin αvβ3-mediated mechanosensation by osteocytes and alters osteoclastogenic paracrine signalling. Sci Rep 2019;9:4654.
80. Schröder K. NADPH oxidases in bone homeostasis and osteoporosis. Free Radic Biol Med 2019;132:67-72.
81. Symons MC. Radicals generated by bone cutting and fracture. Free Radic Biol Med 1996;20:831-5.
82. Yeler H, Tahtabas F, Candan F. Investigation of oxidative stress during fracture healing in the rats. Cell Biochem Funct 2005;23:137-9.
83. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr 2005;45:287-306.
84. Xiao XH, Liao EY, Zhou HD, Dai RC, Yuan LQ, Wu XP. Ascorbic acid inhibits osteoclastogenesis of RAW264.7 cells induced by receptor activated nuclear factor kappaB ligand (RANKL) *in vitro*. J Endocrinol Invest 2005;28:253-60.
85. Byun CH, Koh JM, Kim DK, Park SI, Lee KU, Kim GS. Alpha-lipoic acid inhibits TNF-alpha-induced apoptosis in human bone marrow stromal cells. J Bone Miner Res 2005;20:1125-35.
86. Kim HJ, Chang EJ, Kim HM, Lee SB, Kim HD, Su Kim G. Antioxidant alpha-lipoic acid inhibits osteoclast differentiation by reducing nuclear factor-kappaB DNA binding and prevents *in vivo* bone resorption induced by receptor activator of nuclear factor-kappaB ligand and tumor necrosis factor-alpha. Free Radic Biol Med 2006;40:1483-93.
87. Lee EY, Lee CK, Lee KU, Park JY, Cho KJ, Cho YS. Alpha-lipoic acid suppresses the development of collagen-induced arthritis and protects against bone destruction in mice. Rheumatol Int 2007;27:225-33.
88. Rao LG, Mackinnon ES, Josse RG, Murray TM, Strauss A, Rao AV. Lycopene consumption decreases oxidative stress and bone resorption markers in postmenopausal women. Osteoporos Int 2007;18:109-15.
89. Kinov P, Leithner A, Radl R, Bodo K, Khoschsorur GA, Schauenstein K, *et al.* Role of free radicals in aseptic loosening of hip arthroplasty. J Orthop Res 2006;24:55-62.
90. Hirota T, Hirota K. Osteoporosis and intake of vitamins. Clin Calcium 2005;15:854-7.
91. Kaneki M. [Protective effects of Vitamin K against osteoporosis and its pleiotropic actions]. Clin Calcium 2006;16:1526-34.
92. Södergren E, Cederberg J, Basu S, Vessby B. Vitamin E supplementation decreases basal levels of F(2)-isoprostanes and prostaglandin f(2alpha) in rats. J Nutr 2000;130:10-4.
93. Hirota K, Hirota T. Nutrition-related bone disease. Nihon Rinsho 2006;64:1707-11.
94. Cooke GM. A review of the animal models used to investigate the health benefits of soy isoflavones. J AOAC Int 2006;89:1215-27.
95. Kesemenli CC, Necmioğlu S. The role of melatonin as a link between head injury and enhanced osteogenesis. Med Hypotheses 2005;65:605-6.
96. Rassi CM, Lieberherr M, Chaumaz G, Pointillart A, Cournot G. Modulation of osteoclastogenesis in porcine bone marrow cultures by quercetin and rutin. Cell Tissue Res 2005;319:383-93.
97. Sandukji A, Al-Sawaf H, Mohamadin A, Alrashidi Y, Sheweita SA. Oxidative stress and bone markers in plasma of patients with long-bone fixative surgery: Role of antioxidants. Hum Exp Toxicol 2011;30:435-42.
98. Sheweita SA, El-Hosseiny LS, Nashashibi MA. Protective effects of essential oils as natural antioxidants against hepatotoxicity induced by cyclophosphamide in mice. PLoS One 2016;11:e0165667.
99. Sheweita SA, Al-Shora S, Hassan M. Effects of benzo[a]pyrene as an environmental pollutant and two natural antioxidants on biomarkers of reproductive dysfunction in male rats. Environ Sci Pollut Res Int 2016;23:17226-35.
100. Sheweita SA, Khoshhal KI. Calcium metabolism and oxidative stress in bone fractures: Role of antioxidants. Curr Drug Metab 2007;8:519-25.