

Special Edition

"Polyphenols for improving food
quality and nutraceuticals"

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Blueberry Polyphenols and Their Effects on Bone and Joint

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ABSTRACT

In addition to their pleasing taste, blueberries are a significant source of flavonoid polyphenols, especially anthocyanins. This review summarizes findings regarding anthocyanin content and bioavailability from blueberries. Blueberries possess a remarkable variety of anthocyanins. Of this array the major ones are reviewed in terms of their chemical structures. *In vitro* and *in vivo* studies are presented that indicate blueberry anthocyanins have the potential to improve the balance between bone-forming osteoblasts and bone-resorbing osteoclasts. These are promising results that suggest a potential role for blueberry anthocyanins in preventing osteoporosis. Reports that directly measure blueberry anthocyanin effects on joint are not widespread; however, multiple studies that measure effects from isolated anthocyanins common to the blueberry but from other foods are reviewed. Preliminary *in vitro* and *in vivo* studies on anthocyanin effects in joint tissue show positive antioxidant and anti-inflammatory activity. Future research areas involving anthocyanin metabolite studies, genetic manipulation to improve anthocyanin profile, and processing technologies to preserve beneficial anthocyanins are briefly explored in closing.

KEYWORDS: Blueberry; Polyphenol; Flavonoid; Anthocyanin; Osteoporosis; Osteoarthritis.

ABBREVIATIONS: IL-6: interleukin 6; TNF- α : tumor necrosis factor- α ; RANKL: receptor activator of nuclear factor κ B-ligand; Runx2: runt related transcription factor-2; OSX: osterix; BMP: bone morphogenetic protein; Wnt: wingless/integration-1; ALP: alkaline phosphatase; IGF-1: insulin growth factor-1; OVX: ovariectomized; MMP: matrix metalloproteinase; TIMP-1: MMP inhibitor; PBMC: peripheral blood mononuclear cells; Th17: type-17 helper T cells; CBP: cyclic AMP response element binding protein; mRNA: messenger RNA.

INTRODUCTION

Ten-thousand different phytochemicals have been identified in the plant-based foods we eat, and many scientists believe thousands more are awaiting identification. Studies indicate that eating an abundance of these from whole foods reduces the risk of each of the following: cardiovascular disease, Alzheimer's disease, cancer, diabetes, cataracts, and age-related decline.¹ Much of the recent research of phytochemical influence on skeletal health has focused on polyphenols, especially the flavonoid sub-group.² The effects of these polyphenols are worthy of study considering the need for new therapies to prevent and treat osteoporosis as well as arthritic diseases. The World Health Organization (WHO) estimates that worldwide, 9.6% of men and 18% of women over sixty have symptomatic osteoarthritis. Worldwide osteoporosis estimates by WHO are 15% for those 50-59 and 70% for those over 80.³ The scope of these problems is also reflected in the health care costs associated with osteoporotic fractures as well as arthritis in the U.S. In 2005, there were approximately two million osteoporosis-related fractures with costs of more than \$19 billion dollars. By 2025, annual fractures and costs are projected to rise by almost 50%.⁴ The prevalence of arthritic disease (including osteoarthritis and rheumatoid arthritis) in the U.S. in 2005 was approximately 45 million with an estimated \$383 million in medical expenditures.⁵ Currently osteoporosis treatments include anti-resorptives, bone anabolic drugs, and combinations of the two.⁶ Treatments for arthritic diseases vary but in

general include any number of the following: weight loss if the patient is overweight, use of heat, exercise, acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), intra-joint corticosteroid injections, and potentially joint replacement surgery. Most agree that there is not a cure. For both osteoporosis and arthritis, effective treatments do exist, but most have associated limitations and side effects, making the development of alternatives a necessity.⁷ Interest in natural products is high, especially the polyphenol flavonoids: perhaps they will serve a role as these much needed alternatives.

Some of the findings summarized in this review require an understanding of the cells of the bone and joint—this paragraph outlines key concepts for that comprehension. In the normal bone microenvironment, both the bone-resorbing osteoclast and the bone-forming osteoblast work in concert to renew and remodel bone structure. The osteoclast initiates the process by resorbing old bone followed by the closely coupled action of osteoblasts following behind to secrete and mineralize bone matrix. Imbalances that favor greater osteoclast activity over osteoblast activity lead to osteoporosis. Closely associated with the bone microenvironment, the normal synovial joint is intricate, consisting of articular cartilage covering the ends of the bones. This articular cartilage is synthesized by chondrocytes. The joint cavity is enclosed by a fibrous articular capsule, lined by an inner synovial membrane comprised of synoviocytes that make and secrete synovial fluid into the joint cavity. Reinforcing ligaments as well as nerves and blood vessels are also present.⁸ During joint disease the pathologic fibroblast-like synoviocyte produces inflammatory cytokines, adhesion molecules, and matrix-degrading enzymes that inflict damage and destruction to bone and cartilage.⁹

This review examines our current understanding of blueberry polyphenols, especially the highly abundant anthocyanin flavonoids, and their actions on preventing age-related decline in the bone and joint. Perspectives include the anthocyanin content and bioavailability from this tasty fruit as well as the actions of blueberry anthocyanins on the cells of the bone and joint as revealed by *in vitro* and *in vivo* studies.

ANTHOCYANIN POLYPHENOL CONTENT AND BIOAVAILABILITY IN BLUEBERRIES

Blueberries are part of the *Angiosperm* group of flowering plants, the *Ericaceae* family, and the *Vaccinium* genus. There are currently over two hundred accepted species of *Vaccinium*.¹⁰ Commercial species of note include the northern highbush (*Vaccinium corymbosum* L.), the lowbush (*Vaccinium angustifolium* L.), the southern highbush (*Vaccinium darrowii* Camp.), the rabbit eye (*Vaccinium virgatum* Aiton.), and Elliott's (*Vaccinium elliotii* Chapm.). Hybrids of the *Vaccinium* species are also grown commercially. In the U.S. and other parts of the world, the Northern highbush is the most frequently cultivated because of its ability to withstand lower temperatures and retain high fruit quality. Each variety has different growth requirements in

regards to soil and temperature as well as different drought-resistance. The U.S. is the largest producer at over 200 thousand tons annually, typically accounting for over half of the world's production.¹¹

The structure of the blueberry fruit resembles other soft fruits in that a cuticle and epicuticular wax cover a single epidermal layer.¹² This outer layer protects the flesh and seeds located underneath as well as contains nearly all of the beneficial anthocyanin polyphenols.¹³ The total polyphenol content measured in blueberries is influenced by several factors, including the variety of plant, growing conditions, maturity of the berry, and the measurement technique used. As a result the total polyphenol value range reported by one review author was 48 to 304 mg/100 g of fresh fruit weight.¹¹ A potential expansion of this range becomes apparent in a 2007 study by Hosseinian and Beta in which six Manitoba berries (Saskatoon blueberry, wild blueberry, raspberry, chokecherry, strawberry, and seabuckthorn) were each analyzed for their anthocyanin polyphenol subclass content. Saskatoon and wild blueberries had the highest anthocyanin content at 562.4 mg/100 g dry weight and 558.3 mg/100 g dry weight, respectively. The next closest value was 365.2 mg/100 g, measured in raspberry.¹⁴

Blueberries possess a large array of polyphenols. Flavonoid polyphenol subclasses that are found in blueberry include monomeric and oligomeric procyanidins, anthocyanidins, and flavonols (kaempferol, quercetin, and myricetin). In addition other polyphenols found in blueberries include phenolic acids (primarily hydroxycinnamic acids) and stilbene derivatives.^{11,15-19} During blueberry ripening all of these subclasses diminish with the exception of the anthocyanins, which appear to increase in synthesis.^{11,20} The word anthocyanin is derived from the Greek *anthos* for flowers and *kyanos* for blue.²¹ Unique among berries, the blueberry contains an astounding variety of anthocyanins.¹¹ In 2015, Lee et al. compared the anthocyanin content of three different berries in a study on the bone health of a diet-induced obesity mouse model. Blueberry anthocyanin content differed from that of blackberry and black currant in that reversed phase HPLC analysis revealed nineteen different anthocyanins in blueberry, only two in blackberry, and just four in black currant. The nineteen anthocyanins in blueberry were of relatively equal concentration whereas cyanidin-3-glucoside was the major anthocyanin in blackberry and the major anthocyanins of black currant were delphinidin-3-rutinoside and cyanidin-3-rutinoside.²² Of the nineteen blueberry anthocyanins, the eight major ones were cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, delphinidin-3-glucoside, delphinidin-3-galactoside, delphinidin-3-arabinoside, petunidin-3-glucoside, and malvidin-3-glucoside.²³ The red, blue, and purple pigments of blueberries are glycosides of cyanidin, delphinidin, and pelargonidin, respectively.^{11,24} Growing condition variables, storage conditions, and processing methods will impact flavonoid content, including the anthocyanins.²⁵

Figure 1 presents the basic core structure of the antho-

cyanidin. After consumption the glycoside form is digested to the aglycone form of the anthocyanin and readily absorbed into the bloodstream. Evidence also exists that anthocyanins can be absorbed in the glycoside form; however, the aglycone forms are thought to absorb more rapidly and in greater quantity.²⁷⁻²⁹ Human and/or the microflora enzymes of the intestine can further metabolize these aglycones into glucuronate or sulfate on absorption with eventual elimination.²⁸ After absorption the maximal post-meal plasma concentration range of polyphenols appears to reach 0.1 to 10 μM .²

BLUEBERRY ANTHOCYANINS AND BONE EFFECTS

During aging the increased activity of two key processes is linked with increased bone loss: low-grade inflammation in the form of inflammatory cytokine release and reactive oxygen species production.²³ It is not surprising that flavonoids in general as well as vitamins A, C, E, and carotenoids appear to decrease bone turnover through their antioxidant abilities.¹ Flavonoid supplementation reduces the circulating inflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α).²⁵ Both of these cytokines induce receptor activator of NF- κB ligand (RANKL), a ligand needed to trigger osteoclast differentiation and hence bone resorption.²⁵ Additional evidence suggests that plant flavonoids decrease the matrix metalloproteinases MMP-2 and MMP-9, resulting in less bone collagen turnover.²⁵ Anabolic pathways in osteoblasts get a boost from the flavonoids in that runt related transcription factor-2 (Runx2,

a critical transcription factor that signals osteoblast differentiation), osterix (OSX, a transcription factor essential for osteoblast differentiation), osteocalcin (a noncollagenous protein made by osteoblasts and deposited in bone), bone morphogenetic protein (BMP, a group of growth factors that stimulate bone and cartilage growth as well as orchestrate tissue architecture), smad (intracellular protein group that signal increased gene transcription downstream of transforming growth factor- β), wingless/integration-1 (Wnt, a family of signaling proteins, some of which facilitate osteoblastogenesis), alkaline phosphatase (ALP, synthesized by osteoblasts and involved in calcification of bone matrix), and insulin growth factor-1 (IGF-1) levels are all enhanced.^{2,25,30} Several preliminary studies add to this list of osteoblast function enhancements *via* plant polyphenol nutrition and include activation of the following pathways: activator protein-1, mitogen-activated protein kinase, estrogen receptor, and osteoprotegerin (OPG, also known as osteoclastogenesis inhibitory factor or the decoy receptor for RANKL).³⁰

Studies more specifically examining blueberry or blueberry extract effects on bone included a 2006 *in vitro* study by Bickford et al. in which 500 ng/ml blueberry extract for 72 hours increased human bone marrow cell proliferation and decreased RANKL-dependent osteoclast numbers.³¹ In 2008 Devareddy et al. utilized female, 6 month old ovariectomized (OVX) rats to demonstrate that a 5% blueberry diet for 100 days increased whole body bone mineral density and serum alkaline phosphatase levels.³² Two years later Chen et al. tested Sprague-Dawley

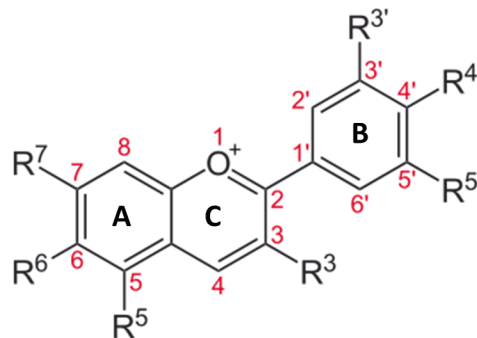


Figure 1: Basic Structure of the Flavylium Cation, the Structural Core of the Anthocyanidin.

The sugar moieties are most often attached to the A ring at the 5 position or to the C ring at the 3 position. Seven side groups are designated by the letter "R." The "R" can be a hydrogen atom, a methoxy group, or a hydroxide. The "R" group identities in the most common edible plant anthocyanidins are R⁶=H, R³, R⁴, R⁵, and R⁷ each=OH while the R³ and R⁵ identities are as follows:

R ³	R ⁵	Anthocyanidin
OH	OH	delphinidin
OH	H	cyanidin
H	H	pelargonidin
OCH ₃	H	peonidin
OCH ₃	OH	petunidin
OCH ₃	OCH ₃	malvidin

(Adapted from Miguel as well as Watson and Schönlaue^{21,26})

rats (both male and female) on a 10% blueberry diet for 40 days to observe bone mass and bone mineral content increases as well as increased osteoblast numbers but decreased osteoclast numbers.³¹ In 2011 Zhang et al. used female Sprague-Dawley rats fed a 10% blueberry diet, but only between 20-34 days postnatal. Their findings from this early blueberry supplementation were that osteoblast senescence and adult bone loss were prevented with increases of trabecular bone volume, osteoblast number, bone formation rate, and osteocalcin levels.³¹ Work by Welch et al. published in 2012 demonstrated that regular flavonoid intake, especially anthocyanins (on average of 13.7 mg/day), had bone-protective effects in 3,160 women from the Twins UK Adult Twin Registry.³³

So in both rodent and human, anthocyanins show benefit to the skeleton. In the 2015 Lee et al. study mentioned in the first section of the review above, the high-fat/blueberry diet group exhibited greater bone mineral density at the distal femoral epiphysis compared to the high-fat/blackberry diet group. This study strengthened findings by others that different berries with different anthocyanin profiles and concentrations have different effects on bone health.²² To understand at the molecular level the specific anthocyanin in blueberries working to increase bone density, Moriwaki et al. in 2014 tested three major anthocyanidins from blueberry, delphinidin, cyanidin, and peonidin. The authors used a widely accepted *in vitro* model of osteoclast formation, the RANKL-treated RAW264.7 mouse macrophage line. In the presence of RANKL, these macrophages differentiate into mature, functional osteoclasts. Moriwaki found that delphinidin dose-dependently inhibited osteoclast differentiation over the range tested, 0.25-20 µg/ml. Cyanidin only showed a mild inhibitory effect at the top dosage and peonidin did not demonstrate any significant effect on osteoclastogenesis.²⁷ Moriwaki's group tested the three anthocyanidins on osteoblasts from C57BL/6J mouse calvariae and found no effect on the cells' ability to mineralize matrix. Then they further evaluated the action of delphinidin *in vivo*, measuring bone morphology and microarchitecture by microcomputed tomography. In both RANKL-induced osteoporotic mice and in OVX female mice, orally administered delphinidin (10 mg/kg/day) significantly reduced bone loss. At the molecular level, delphinidin significantly decreased gene expression of key transcription factors that activate osteoclastogenesis genes as measured by quantitative real-time polymerase chain reaction.²⁷

BLUEBERRY ANTHOCYANINS AND JOINT EFFECTS

Although reports directly addressing blueberry anthocyanin effects on the joint are lacking, some *in vitro* as well as pre-clinical studies suggest a protective role for polyphenols in the joint, primarily measured as the ability to limit osteoarthritis progression.³⁴ The specific parameters tested included reduction of chondrocyte inflammation, thus lowering damage to and pain within the joint. Preliminary data suggests that polyphenol mechanisms may include a decrease in inflammatory activity, decreases in apoptosis of joint cells, a decrease of oxidative damage, and decreased matrix degradation. Decreased

inflammatory activity was indicated by decreases in inducible nitric oxide synthase, cyclooxygenase-2, and prostaglandin E₂, a mediator of inflammation. Cell apoptosis decreases were indicated by a measured decrease in caspase activity and increased proliferation in chondrocytes. Oxidative damage control was indicated by increased superoxide dismutase and glutathione peroxidase antioxidant enzyme activities. Lessened matrix degradation was determined by increased matrix synthesis (collagen type II, glycosaminoglycan, and aggrecan levels), increased anabolic cytokines, decreased matrix degrading enzyme activity (MMPs), and increased MMP inhibitor (TIMP-1) levels.³⁴ Further investigations of the polyphenols and their joint effects are needed because as of this date, there are no nutritional aids, including glucosamine, that have proven effective at decreasing osteoarthritis. The ultimate goal would be to successfully use a nutritional aid either alone or in combination with the current treatment of NSAIDs to limit NSAID toxicity associated with their long-term use.³⁴

Sharing some of the anthocyanins found in blueberry, black soybean seed coat extract anthocyanins were tested for their effects on a mouse model of collagen-induced arthritis as well as human peripheral blood mononuclear cells (PBMCs). The black soybean seed coat extract anthocyanins included cyanidin-3-O-glucoside (68.3%), delphinidin-3-O-glucoside (25.2%), and petunidin-3-O-glucoside (6.5%). The authors demonstrated that the black soybean seed coat extract decreased the differentiation and hence pro-inflammatory cytokine synthesis in type-17 helper T cells (Th17), one of the cell types responsible for rheumatoid arthritis progression. They also showed that the anthocyanin extract decreased the expression of osteoclast-associated genes.³⁵ Another related report in 2013 by Decendit et al. revealed that the major anthocyanin of grapes and one of the major anthocyanins in blueberry, malvidin-3-O-beta-glucoside, decreased oxidative stress in a rat model of rheumatoid arthritis. The *in vitro* part of the study demonstrated a decrease in the transcription of inflammatory mediator genes in human macrophages. The authors validated this finding by the observation that secreted TNF- α , IL-1, IL-6, and nitric oxide levels were reduced in activated macrophages exposed to malvidin-3-O-beta-glucoside. In the rat model, arthritic paw scores were significantly reduced at therapeutic and preventive levels of the purified anthocyanin. Peritoneal macrophages isolated from these same rats displayed lowered nitric oxide levels.³⁶

Anthocyanins' effects on gene expression in cells of the joint also appear to include an epigenetic regulation mechanism. In 2011 Seong et al. tested delphinidin from pomegranate on human synovial cells, specifically a fibroblast-like synoviocyte line established from a rheumatoid arthritis patient. Delphinidin specifically inhibited the p300/CBP (cAMP response element binding protein) histone acetyltransferase. The resulting hypoacetylation included NF- κ B gene hypoacetylation which muted pro-inflammatory signaling. This is a promising finding for potential anthocyanin use in preventing inflammatory arthritis.³⁷

Perhaps the most direct study of blueberry anthocyanin

effects on joint health, Lee et al. in 2014 addressed anti-inflammatory effects of berry anthocyanins (blueberry, blackberry, and blackcurrant) *in vitro*, using macrophages as the experimental cell. Macrophage cytokine secretion was stimulated by exposure to lipopolysaccharide. Subsequent incubation with 0-20 µg/ml of each of the berry anthocyanins decreased interleukin IL-1β as well as TNF-α messenger RNA (mRNA) levels. Each berry anthocyanin extract significantly reduced cellular reactive oxygen species in bone marrow-derived mouse macrophages.²³

FUTURE RESEARCH

Further characterization of blueberry polyphenols, especially the anthocyanins that constitute the berry's major polyphenol contribution to our diets, will require a multilevel approach. One level of inquiry should ask which specific anthocyanin metabolites alter bone and joint physiology the most favorably. Many *in vitro* studies use aglycone forms or glycosides that don't represent the metabolized versions of these molecules. So using the metabolized conjugates would be more physiologically relevant.³⁰ However, one recent study on phenolic acid polyphenols did address this through the administration of hippuric acid (one of the major metabolites found in the circulation after blueberry consumption) to prepubertal female mice for two weeks. The authors noted increased gene expression related to bone-forming in parallel with decreased peroxisome proliferator-activated receptor-γ expression, leading to increased bone mass. The phenomenon was dose-dependent.³⁸ In further fine-tuning blueberry anthocyanin metabolite effect studies, physiological concentrations need to be more closely approximated, as one review author group noted that not all *in vitro* studies appear to take into account the post-meal maximal plasma circulating concentrations of polyphenols. Many studies appeared to use concentrations which just gave a response in the particular cell model.² Of course new *in vitro* findings will then require *in vivo* validation both at the animal and human clinical stages.

A deeper understanding of the most beneficial metabolites of blueberry anthocyanins may lead to a second, applied level of inquiry—modern agricultural plant breeding, development, and cultivation techniques. To develop blueberry varieties that maximize the beneficial anthocyanin profile, it may be necessary to develop hybrids through conventional genetics (germplasm selection and hybridization) or through genetic modification.¹¹ Perhaps genes could be manipulated for improved anthocyanin production to elevate the nutritional content and antioxidant capabilities. There are known *Vaccinium* genes that could be manipulated to control over ripening, shelf life, better quality through machine harvest, insect resistance, and other characteristics.¹¹ Of course negative consumer reaction to genetically modified foods has inhibited these efforts. Yet another level of inquiry that is tightly associated with propagating these varieties would be the harvest, processing, and storage technologies required to preserve the optimized anthocyanin profile. Thanks to temperature-controlled and atmosphere-controlled storage as well as freezing, blueberries can be enjoyed year round. However, what storage and processing methods best preserve bio-

activity? Research to evaluate modified techniques of freezing, juicing, dewatering, and drying (especially vacuum, freeze, and radiant zone) would be required.¹¹

Since positive associations exist between good bone/joint health and the intake of vegetables and fruits, the current recommendations to consume five or more portions of a variety of fruits and vegetables each day are prudent. At this time data appears to be too incomplete to prescribe a particular type of fruit or vegetable.²⁵ Consequently the recommendation for a varied diet leads to the fourth level of inquiry. This level considers the fact that the nutritional components of the blueberry should not act by themselves. Facts uncovered about blueberry anthocyanins from a reductionist's approach should be weighed in the context of the incredibly complex mixture that is our diet. Evidence exists for synergistic interactions among different phytochemicals once they are in our bodies. Not only do phytochemicals interact with each other, but they also interact with macronutrients, vitamins, and minerals. To add further complication, these interactions change as the physiology and pathology of the body changes.¹ So opportunities for future research on the role of blueberry polyphenols in maintaining optimal bone and joint health are bountiful.

CONFLICTS OF INTEREST

The author whose name is listed below certifies that he has no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership; employment; consultancies; stock ownership; or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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REFERENCES

1. Thompson JL, Manore MM, Vaughan LA. Phytochemicals In-Depth. In: Lindelof S, ed. *The Science of Nutrition*. 3rd ed. Upper Saddle River, NJ, USA: Pearson Education, Inc.; 2014: 426-431.
2. Horcajada MN, Offord E. Naturally plant-derived compounds: role in bone anabolism. *Current Molecular Pharmacology*. 2012; 5(2): 205-218. doi: [10.2174/1874467211205020205](https://doi.org/10.2174/1874467211205020205)
3. World Health Organization: Chronic Rheumatic Conditions. Web site. www.who.int/chp/topics/rheumatic/en. Accessed February 24, 2016.
4. Burge R, Dawson-Hughes B, Solomon DH, et al. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. *J Bone Miner Res*. 2007; 22(3): 465-475. doi: [10.1359/jbmr.061113](https://doi.org/10.1359/jbmr.061113)

5. Cisternas MG, Murphy LB, Yelin EH, et al. Trends in medical care expenditures of US adults with arthritis and other rheumatic conditions 1997 to 2005. *J Rheumatol.* 2009; 36(11): 2531-2538. doi: [10.3899/jrheum.081068](https://doi.org/10.3899/jrheum.081068)
6. Rachner TD, Khosla S, Hofbauer LC. New horizons in osteoporosis. *Lancet.* 2011; 377(9773): 1276-1287. doi: [10.1016/S0140-6736\(10\)62349-5](https://doi.org/10.1016/S0140-6736(10)62349-5)
7. Nelson AE, Allen KD, Golightly YM, Goode AP, Jordan JM. A systematic review of recommendations and guidelines for the management of osteoarthritis: The Chronic Osteoarthritis Management Initiative of the U.S. Bone and Joint Initiative. *Semin Arthritis Rheum.* 2014; 43(6): 701-712. doi: [10.1016/j.semarthrit.2013.11.012](https://doi.org/10.1016/j.semarthrit.2013.11.012)
8. Marieb EN, Hoehn K. Bones and Skeletal Tissues. In: Beuparlant S, ed. *Human Anatomy & Physiology*. 10th ed. Upper Saddle River, NJ, USA: Pearson Education, Inc.; 2016: 173-198.
9. Bottini N, Firestein GS. Duality of fibroblast-like synovio-cytes in RA: passive responders and imprinted aggressors. *Nat Rev Rheumatol.* 2013; 9: 24-33.
10. The Plant List: A working list of all plant species. *Vaccinium*. Web site. <http://www.theplantlist.org/1.1/browse/A/Ericaceae/Vaccinium/>. Accessed February 3, 2016.
11. Michalska A, Lysiak G. Bioactive compounds of blueberries: post-harvest factors influencing the nutritional value of products. *Int J Mol Sci.* 2015; 16(8): 18642-18663. doi: [10.3390/ijms160818642](https://doi.org/10.3390/ijms160818642)
12. Giongo L, Poncetta P, Loretto P, Costa F. Texture profiling of blueberries (*Vaccinium* spp.) during fruit development, ripening and storage. *Postharvest Biol Technol.* 2013; 76: 34-39. doi: [10.1016/j.postharvbio.2012.09.004](https://doi.org/10.1016/j.postharvbio.2012.09.004)
13. Cho MJ, Howard LR, Prior RL, Clark JR. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *J Sci Food Agric.* 2004; 84: 1771-1782.
14. Hosseinian FS, Beta T. Saskatoon and wild blueberries have higher anthocyanin contents than other Manitoba berries. *J Agric Food Chem.* 2007; 55(26): 10832-10838. doi: [10.1021/jf072529m](https://doi.org/10.1021/jf072529m)
15. Taruscio TG, Barney DL, Exon J. Content and profile of flavonoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest *Vaccinium* berries. *J Agric Food Chem.* 2004; 52(10): 3169-3176.
16. Gu L, Kelm M, Hammerstone JF, et al. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *J Agric Food Chem.* 2002; 50(17): 4852-4860. doi: [10.1021/jf020214v](https://doi.org/10.1021/jf020214v)
17. Wu X, Kang J. Blueberries: Major phytochemicals and potential health effects in cardiovascular diseases. In: Tuberoso C, ed. *Berries: Properties, Consumption and Nutrition*. New York, NY, USA: Nova Biomedical Books; 2012: 83-104.
18. Howard L, Hagar T. Berry fruit phytochemicals. In: Zhao Y, ed. *Berry Fruit: Value Added Products for Health Promotion*. Boca Raton, FL, USA: CRC Press; 2007: 73-105.
19. Sacco SM, Horcajada MN, Offord E. Phytonutrients for bone health during ageing. *Br J Clin Pharmacol.* 2012; 75(3): 697-707. doi: [10.1111/bcp.12033](https://doi.org/10.1111/bcp.12033)
20. Kalt W, Lawand C, Ryan DAJ, et al. Oxygen radical absorbing capacity, anthocyanin and phenolic content of highbush blueberries (*Vaccinium corymbosum* L.) during ripening and storage. *J Am Soc Hortic Sci.* 2003; 128: 917-923.
21. Miguel MG. Anthocyanins: Anti-oxidant and/or anti-inflammatory activities. *Journal of Applied Pharmaceutical Science.* 2011; 1(6): 7-15.
22. Lee SG, Kim B, Soung DY, et al. Relationship between oxidative stress and bone mass in obesity and effects of berry supplementation on bone remodeling in obese male mice: an exploratory study. *J Med Food.* 2015; 18(4): 476-482. doi: [10.1089/jmf.2013.0182](https://doi.org/10.1089/jmf.2013.0182)
23. Lee SG, Kim B, Yang Y, et al. Berry anthocyanins suppress the expression and secretion of proinflammatory mediators in macrophages by inhibiting nuclear translocation of NF- κ B independent of NRF2-mediated mechanism. *J Nutr Biochem.* 2014; 25(4): 404-411. doi: [10.1016/j.jnutbio.2013.12.001](https://doi.org/10.1016/j.jnutbio.2013.12.001)
24. Lee J, Durst RW, Wrolstad RE. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *J AOAC Int.* 2005; 88(5): 1269-1278.
25. Welch AA, Hardcastle AC. The effects of flavonoids on bone. *Curr Osteoporos Rep.* 2014; 12(2): 205-210. doi: [10.1007/s11914-014-0212-5](https://doi.org/10.1007/s11914-014-0212-5)
26. Watson RR, Schönlaue F. Nutraceutical and antioxidant effects of a delphinidin-rich maqui berry extract Delphinol®: a review. *Minerva Cardioangiol.* 2015; 63: 1-12.
27. Moriwaki S, Suzuki K, Muramatsu M, et al. Delphinidin, one of the major anthocyanidins, prevents bone loss through the inhibition of excessive osteoclastogenesis in osteoporosis model mice. *PLoS ONE.* 2014; 9(5): e97177. doi: [10.1371/journal.pone.0097177](https://doi.org/10.1371/journal.pone.0097177)

[pone.0097177](#)

29(5): 1043-1053. doi: [10.1002/jbmr.2034](https://doi.org/10.1002/jbmr.2034)

28. Matsumoto H, Inaba H, Kishi M, et al. Orally administered delphinidin 3-rutinoside and cyanidin 3-rutinoside are directly absorbed in rats and humans and appear in blood as the intact forms. *J Agric Food Chem.* 2001; 49(3): 1546-1551. doi: [10.1021/jf001246q](https://doi.org/10.1021/jf001246q)

29. McGhie TK, Walton MC. The bioavailability and absorption of anthocyanins: towards a better understanding. *Mol Nutr Food Res.* 2007; 51(6): 702-713. doi: [10.1002/mnfr.200700092](https://doi.org/10.1002/mnfr.200700092)

30. Trzeciakiewicz A, Habauzit V, Horcajada MN. When nutrition interacts with osteoblast function: molecular mechanisms of polyphenols. *Nutr Res Rev.* 2009; 22(1): 68-81. doi: [10.1017/S095442240926402X](https://doi.org/10.1017/S095442240926402X)

31. Hubert PA, Lee SG, Lee SK, Chun OK. Dietary polyphenols, berries, and age-related bone loss: a review based on human, animal, and cell studies. *Antioxidants.* 2014; 3(1): 144-158. doi: [10.3390/antiox3010144](https://doi.org/10.3390/antiox3010144)

32. Devarreddy L, Hooshmand S, Collins JK, et al. Blueberry prevents bone loss in ovariectomized rat model of postmenopausal osteoporosis. *J Nutr Biochem.* 2008; 19(10): 694-699.

33. Welch A, MacGregor A, Jennings A, et al. Habitual flavonoid intakes are positively associated with bone mineral density in women. *J Bone Miner Res.* 2012; 27(9): 1872-1878. doi: [10.1002/jbmr.1649](https://doi.org/10.1002/jbmr.1649)

34. Shen CL, Smith BJ, Lo DF, et al. Dietary polyphenols and mechanisms of osteoarthritis. *Journal of Nutritional Biochemistry.* 2012; 23(11): 1367-1377. doi: [10.1016/j.jnutbio.2012.04.001](https://doi.org/10.1016/j.jnutbio.2012.04.001)

35. Min HK, Kim SM, Baek SY, et al. Anthocyanin extracted from black soybean seed coats prevents autoimmune arthritis by suppressing the development of Th17 cells and synthesis of proinflammatory cytokines by such cells, via inhibition of NF- κ B. *PLoS ONE.* 2015; 10(11):e0138201. doi: [10.1371/journal.pone.0138201](https://doi.org/10.1371/journal.pone.0138201)

36. Decendit A, Mamani-Matsuda M, Aumont V, et al. Malvidin-3-O- β -glucoside, major grape anthocyanin, inhibits human macrophage-derived inflammatory mediators and decreases clinical scores in arthritic rats. *Biochem Pharmacol.* 2013; 86(10): 1461-1467. doi: [10.1016/j.bcp.2013.06.010](https://doi.org/10.1016/j.bcp.2013.06.010)

37. Seong AR, Yoo JY, Choi KC, et al. Delphinidin, a specific inhibitor of histone acetyltransferase, suppresses inflammatory signaling via prevention of NF- κ B acetylation in fibroblast-like synoviocyte MH7A cells. *Biochem Biophys Res Commun.* 2011; 410(3): 581-586. doi: [10.1016/j.bbrc.2011.06.029](https://doi.org/10.1016/j.bbrc.2011.06.029)

38. Chen JR, Lazarenko OP, Zhang J, et al. Diet-derived phenolic acids regulate osteoblast and adipocyte lineage commitment and differentiation in young mice. *J Bone Miner Res.* 2014;