Occam's Razor

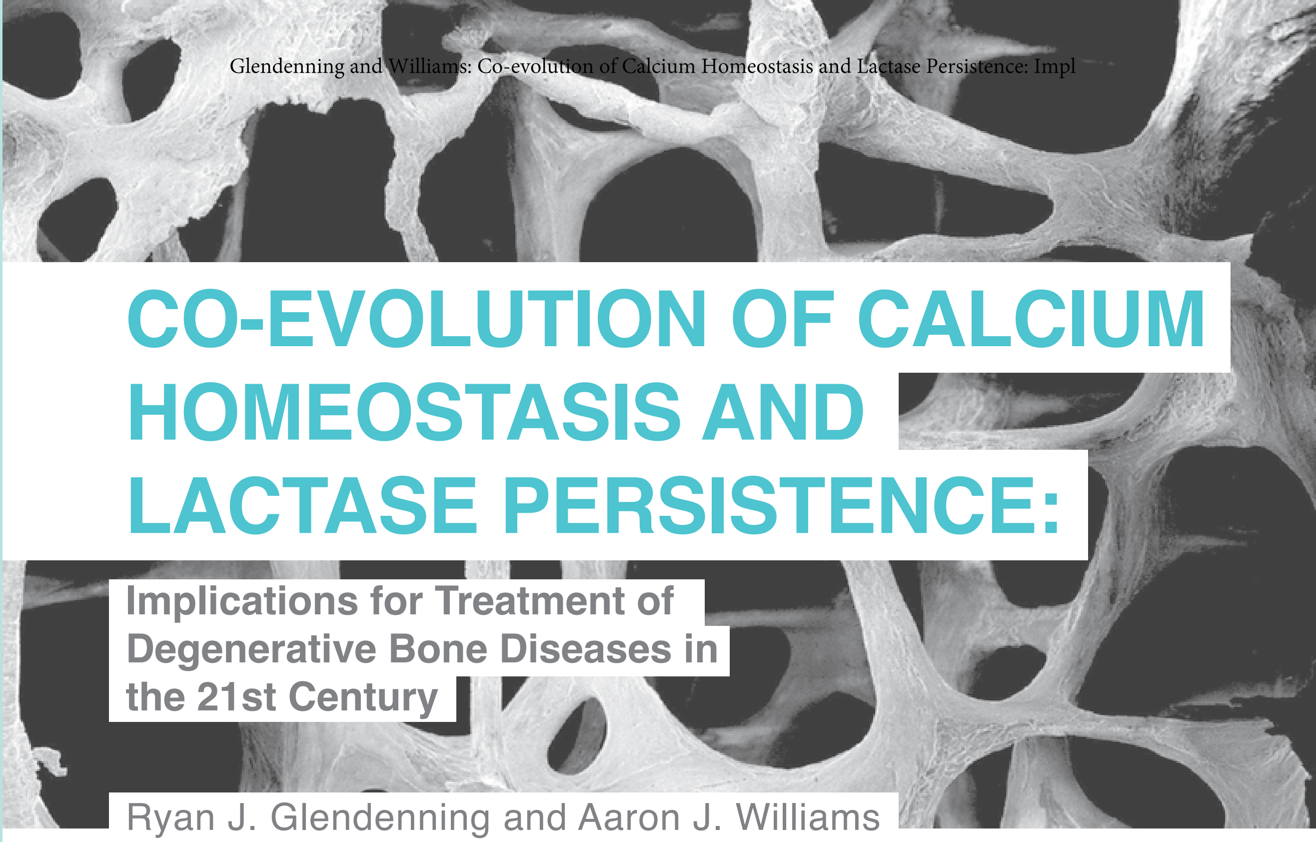
Volume 7 (2017) Article 6

2017

Co-evolution of Calcium Homeostasis and Lactase Persistence: Implications for Treatment of Degenerative Bone Diseases in the 21st Century

Ryan J. Glendenning
Western Washington University, glender@wwu.edu

Aaron J. Williams
Western Washington University, willi336@wwu.edu



CO-EVOLUTION OF CALCIUM HOMEOSTASIS AND LACTASE PERSISTENCE:

Implications for Treatment of Degenerative Bone Diseases in the 21st Century

Ryan J. Glendenning and Aaron J. Williams

ABSTRACT

Recent research on the relationship between osteoporosis and lactase persistence has revealed that these two factors are positively correlated. There is existing evidence that shows the domestication of cattle was a selective force for the lactase persistence allele. We hypothesize that this genetic change caused a shift in the ancestral physiological mechanism for calcium homeostasis, resulting in a derived calcium homeostasis. Consequently, individuals with this derived calcium homeostasis are more susceptible to degenerative bone diseases, such as osteoporosis. Osteoporosis is a topic of major health concern in the United States, considering that it is responsible for more hospital stays for women aged 45 or older than any other disease in America. Geriatric populations are the demographic most heavily affected by osteoporosis—particularly postmenopausal women. Research has also indicated that roughly 20 percent of elderly patients die within the first year of a hip fracture and less than 50 percent return to their previous lifestyle. The health concerns associated with osteoporosis along with the expensive costs of treatment place a priority on alternative ways to treat and prevent this disease. We review the development of lactase persistence along with important biological molecules involved in calcium homeostasis. We also discuss the physiology behind the ancestral calcium homeostasis and the derived calcium homeostasis, as well as potential paths for further research.

INTRODUCTION

Osteoporosis is a degenerative bone disease recognizable by a loss of bone mass density (BMD). Dual-energy X-ray absorptiometry (DXA) is a diagnostic technique used to evaluate the BMD of a patient.[1] While a DXA scan can assess BMD of the whole skeleton, it is usually focused on the hips and spine of a patient when searching for osteoporosis.[1] The results of a DXA scan are then measured against a reference

ALLELE:

an alternative form of a gene

population of young, healthy individuals. This comparison against the reference population is widely referred to as a *T*-score. The *T*-score measures how many standard deviations away from the reference population the patient's BMD is. A diagnosis of osteoporosis occurs when a patient's *T*-score is -2.5 or lower, i.e. 2.5 standard deviations below the reference population.[2] Elderly women are the most likely demographic group to develop the disease, although it can develop in anyone at any point in life.[3, 4] Osteoporosis accounts for more hospital stays for women aged 45 or older than any other disease in America and treatments cost Americans over $22 billion per year.[5, 6] For this reason, a drug that can effectively treat or even prevent this disease is a priority in the pharmaceutical community. Historically, osteoporosis was thought to be rather simple. However, new research has increased our understanding of the complex immunological and physiological systems behind the disease.[7, 8]

Recently, a team of researchers led by Dr. Constance Hilliard from the University of North Texas ran a comparative study between West and East Africans. West Africans have some of the lowest rates of osteoporosis in the world, with around 3 hip fractures per 100,000 postmenopausal women, while East Africans have high rates of osteoporosis, with around 243 hip fractures per 100,000 postmenopausal women.[9] The fracture rates were positively correlated ($r = 0.841$) with milk consumption and positively correlated with lactase persistence ($r = 0.735$).[9] East Africans adopted cattle domestication between 2,700–6,800 years ago, and have been consuming milk from cattle ever since.[10] Milk contains lactose, a sugar only digestible by people who produce the lactase enzyme. The domestication of cattle created a selective pressure for people who have the lactase persistence (LP) allele.[11–13]

Current guidelines on osteoporosis encourage maximum calcium consumption.[14] However, Dr. Hilliard's study contradicts this suggestion. According to Dr. Hilliard, the incidence of osteoporosis increases with calcium and milk consumption. The implications of these findings are critical to understanding the etiological processes of osteoporosis. Identifying risk factors in patients earlier, and more accurately, could prevent injuries and hospital stays. Based on Dr. Hilliard's conclusions, we hypothesize the domestication of cattle was a selective force for the LP allele. This genetic change caused a shift in the ancestral physiological mechanism for calcium homeostasis, resulting in susceptibility to developing degenerative bone diseases like osteoporosis.

Osteoporosis accounts for more hospital stays than any other disease in America, and treatments cost Americans over $22 billion per year.

DEVELOPMENT OF LACTASE PERSISTENCE

For ninety-nine percent of human history, humans would become lactose intolerant around early adulthood.[15–17] Milk was primarily consumed during infancy and over time there became no need to digest milk.[15] Around 10,000 years ago, mutations developed in the genes regulating lactase, the enzyme that breaks down the lactose sugar.[10, 11] Without the ancestral need to digest the milk into adulthood the lactase non-persistent (LNP) trait naturally downregulates lactase production.[16] The lactose sugar is a disaccharide, which provides no caloric benefit because it can not be absorbed through the lumen of the

DISACCHARIDE
a molecule comprised of two individual sugar molecules

LUMEN:
the inner space of a tube-shaped structure

LACTASE PERSISTENCE
the activity of lactase (an enzyme that breaks down the sugar lactose) is continued into adulthood

HOMEOSTASIS
the regulation of a system within an organism to remain at a nearly constant level

small intestine in its natural form. However, lactase cleaves lactose into glucose and galactose, two monosaccharides capable of providing calories. Mutations in the lactase (LCT) gene, located on chromosome 2, allowed humans to successfully digest milk through adulthood.[10, 11, 15]

Upon the domestication of cattle, milk became an easy source of calories for those who could digest the lactose sugar.[18] Consuming lactose in the absence of lactase can create painful abdominal cramps and gas, discouraging those who are lactose intolerant from consuming milk.[18] Those who had the LP mutations in milk-consuming populations possessed a selective advantage over those who did not, since only LP individuals could benefit from the high nutritional content of milk.[19] This selective advantage allowed those who expressed the LP trait to pass on their genes.[20] This selecting force, along with other cultural and environmental factors, allowed LP to replace lactose intolerance in certain parts of the world.[10, 18] In populations with European ancestry, the T-13910 allele on the LCT gene is responsible for the expression of lactase. The T-13910 variant is only present at significant levels in populations with European ancestry.[10, 11] In Africa, several other variants of the LCT gene have been identified as causes of LP trait expression.[10]

Individuals selected for the LP trait shifted away from the ancestral state of LNP. This shift resulted in LP individuals consuming high levels of milk and thus a high intake of dietary calcium. It was this continuous high intake of dietary calcium, made possible by a naturally selected genetic change, that led to a physiological change in the calcium homeostasis for LP individuals. That change will be referred to as the derived calcium homeostasis. LNP individuals did not have access to excess calcium from milk and retained their ancestral calcium homeostasis.

IMPORTANT BIOLOGICAL MOLECULES INVOLVED IN CALCIUM HOMEOSTASIS

Calcium is an element found in plants and animals. It is classified as a micronutrient and a mineral, meaning it is needed in small amounts to sustain life. In humans, calcium serves three important functions of normal physiology: muscle contraction, cellular signaling, and blood clotting.[21] Humans have more hormones that raise blood calcium levels as opposed to hormones that lower blood calcium levels, indicating that adequate calcium is necessary for maintaining many cellular processes.[21] One such hormone is parathyroid hormone (PTH).

PTH is synthesized in the parathyroid glands located posterior to the thyroid gland in the lower neck.[22] This hormone has direct effects on bones and kidneys. It binds to cell surface receptors on certain bone cells to increase the ratio of bone resorption to bone building.[22] PTH also binds to cell surface receptors on the kidney tubules to cause reabsorption of calcium into the plasma.[22] Reabsorption of calcium from the kidneys helps to minimize the loss of calcium through excretion. The last function of PTH is indirect. PTH causes cellular modifications of Vitamin D_3 in the kidney to turn Vitamin D_3 into its biologically active form, 1α,25-dihydroxyvitamin D_3, also known as calcitriol. PTH accomplishes this by increasing production of the enzyme 1-α-hydroxylase, which converts 25-hydroxyvitamin D_3 into calcitriol. Calcitriol increases plasma calcium by absorbing it from the small intestinal lumen. It also acts to cause bone resorption by increasing osteoclast production.[23] In a correctively functioning calcium homeostasis, average bone building rate roughly equals average bone resorption rate. This maintains BMD.

Bones release and sequester calcium based on their anatomical composition. The hard part of bone is made of a calcium phosphate crystal known as calcium hydroxyapatite.[24] These crystals are built by bone building cells, osteoblasts, and are broken down by bone dissolving cells, osteoclasts. Osteoblasts form these hydroxyapatite crystals by combining water with secreted calcium and phosphates.[24] The

These guidelines are insufficient for prevention and treatment of osteoporosis under our model of the derived calcium homeostasis.

bone matrix is maintained by osteoblasts trapped within the bone matrix known as osteocytes. When these crystals are broken down by osteoclasts, calcium leaves the bone and enters the bloodstream.[25] Once in the bloodstream, the body utilizes this calcium for life sustaining functions.[21] Bones serve as storage banks for calcium in this homeostatic mechanism. Proper regulation of osteoblasts and osteoclasts is necessary for bones to effectively serve this purpose.

Receptor activator of nuclear factor kappa-B ligand (RANK-L), receptor activator of NF-κB (RANK), and osteoprotegerin (OPG) are key signaling-proteins involved in osteoblast and osteoclast regulation.[26] When calcitriol binds to osteoblasts, RANK-L is released into the extracellular environment. RANK-L then binds RANK on osteoclast precursors, causing production of osteoclasts by a process called osteoclastogenesis.[26] OPG is a signaling-protein that functions as a RANK receptor. It binds to RANK-L with a high affinity and prevents RANK-L from binding to RANK, therefore inhibiting osteoclastogenesis and conserving BMD.[26] OPG is vitally important in maintaining a healthy BMD.

THE EVOLUTION OF THE DERIVED CALCIUM HOMEOSTASIS

Preagricultural diets had extremely low dietary calcium levels, which can be partially attributed to the absence of milk.[27–29] Therefore, this ancestral calcium homeostasis would have been extremely advantageous for our early ancestors before dairy agriculture was present. We theorize that the ancestral calcium homeostasis is defined as having chronically high PTH and OPG levels that are necessary to efficiently utilize the low levels of dietary calcium.

High PTH levels in the presence of low dietary calcium is beneficial for the ancestral calcium homeostasis because PTH allows for maximum absorption of calcium through the small intestine via calcitriol and the maximum reabsorption of calcium from the renal tubules.[22] The calcium conserving purposes of PTH in the ancestral calcium homeostasis allows individuals to maintain a healthy BMD. The high presence of OPG mediates the negative bone resorption associated with PTH and conserves BMD by inhibiting osteoclastogenesis.[22] This ancestral calcium homeostasis allowed our ancestors to maintain bone health despite having a low dietary calcium intake. Research shows that having high levels of OPG and PTH present at the same time significantly increases bone mineral density.[22, 30, 31] Further studies have shown that the body can adapt its calcium homeostasis physiology in the presence of low calcium levels without compromising bone health in the process.[32]

The introduction of dairy agriculture along with the selection for LP genotypes allowed individuals to consume high amounts of dietary calcium in the form of milk.[18] This large influx of dietary calcium led to high levels of calcium in the bloodstream, which would decrease the relative amount of PTH.[22, 33, 34] With PTH levels decreased, osteoblastic OPG production would also decrease in an effort to conserve energy. This would create a new calcium homeostasis setpoint for LP individuals where both PTH and OPG levels are low. Although this high calcium intake leads

LIGAND:

a molecule that binds to another molecule

CALCITRIOL:

the biologically active form of vitamin D

DECOY RECEPTOR:

a receptor that can recognize and bind cell-signaling proteins as an inhibitor to prevent normal binding to energy conservation, the derived calcium homeostasis is also susceptible to dysfunction. In times of stress, such as low calcitriol production or low calcium consumption, PTH levels rise and cause bone resorption.[35] Without the protective effects of OPG, osteoclast activity is minimally inhibited and outpaces osteoblast activity.[26] More simply stated, the breakdown of bone via PTH would go unchecked and bone health would suffer. Due to the decrease in estrogen production levels associated with the post-menopausal stage of life, postmenopausal women with this calcium homeostasis are at a high risk for developing osteoporosis. Estrogen has been seen to provide a protective effect against osteoporosis since it positively regulates OPG expression.[26]

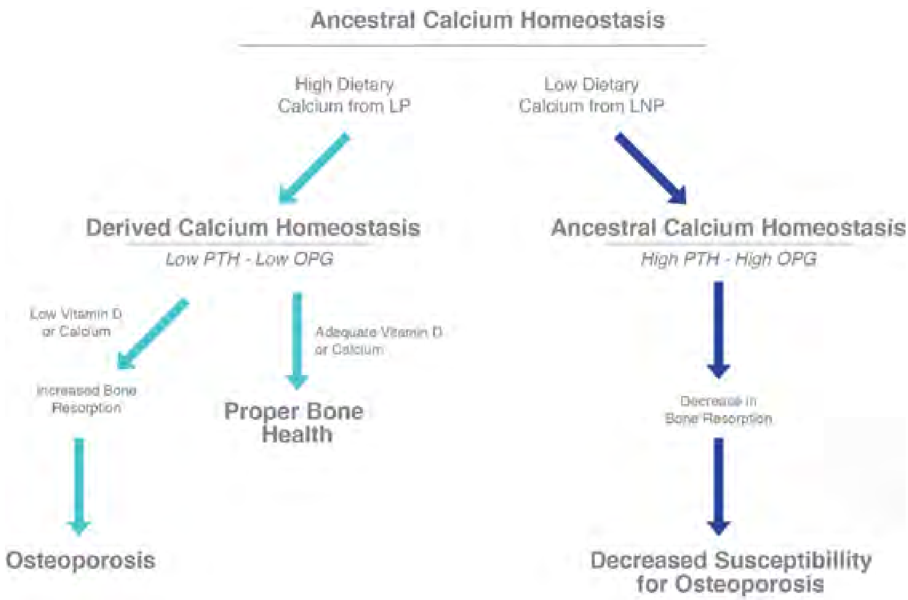


FIGURE 1

Figure 1. The diagram above shows the divergence of the derived calcium homeostasis from the ancestral calcium homeostasis along with the basic functions of each homeostatic mechanism. Parathyroid hormone (PTH) binds to surface receptors on osteoclasts to increase bone resorption activity. PTH also causes resorption of calcium into blood plasma by binding to receptors on kidney tubules. Osteoprotegerin (OPG) inhibits the bone degrading effects of PTH. In the derived calcium homeostasis, low PTH and OPG levels increase the risk for osteoporosis development since PTH levels can rise with inadequate calcitriol or calcium. With PTH levels raised, bone resorption increases. In the ancestral calcium homeostasis, high OPG levels inhibit the bone resorption aspect of PTH and therefore decreases the risk for osteoporosis development.

IMPLICATIONS FOR THE TREATMENT OF OSTEOPOROSIS

Current guidelines for osteoporosis prevention from the National Osteoporosis Foundation (NOF) suggest "get enough calcium, eat a well-balanced diet, engage in regular exercise, eat foods that are good for bone health such as fruits and vegetables, and avoid smoking and limit alcohol to 2–3 drinks per day."[14] These guidelines are insufficient for prevention and treatment of osteoporosis under our model of the derived calcium homeostasis. Studies have shown that dietary calcium and vitamin D supplements alone have mixed results in their effectiveness to reduce fractures.[36–38] Current treatments for osteoporosis do not take into account this difference between an ancestral and a derived calcium homeostasis. These treatments include supplementing with calcium, vitamin D, estrogen, calcitonin, and/or bisphosphonate derivatives. While bisphosphonate derivatives have been shown to effectively treat osteoporosis, there have been major concerns regarding long-term safety of their use.[39, 40]

The drug known as denosumab targets the RANK-L-RANK-OPG pathway, a key aspect of the derived calcium homeostasis, and provides ample evidence to support an effective treatment of osteoporosis.[39] The derived calcium homeostatic mechanism needs to be considered in order to develop more effective treatments and preventions for osteoporosis. Sampling serum biomarkers from a large population of diverse individuals would allow for further evidence to support our hypothesis. Specifically, research might include looking at the serum levels of PTH and OPG in lactase persistent and lactase non-persistent individuals.

Genetic screening for the presence of the LP allele, as well as sampling PTH and OPG serum levels, could be an important step in the prevention and treatment of osteoporosis. This method would identify risk factors for young patients and could lead to the early implementation of preventative treatment for osteoporosis. Currently, US health insurance companies will not cover the use denosumab derivatives for patients.[41, 42] In order for insurance companies to cover this preventative treatment, studies need to be conducted to determine if this type of preventative treatment would save them money.

Recent research suggests that mortality rates for hip fractures in North America alone are between 14 percent–36 percent within 1 year of surgery and less than 50 percent of patients return to their previous lifestyle.[43, 44] Hip fracture surgery has also been shown to be associated with an increase in dependency on long-term institutional care, an increased incidence of entering a low-income status, an increased risk of coronary heart disease and other postoperative complications like perioperative anemia, gastrointestinal anemia, cognitive alterations, and embolisms.[45–47] These potential health and lifestyle consequences combined with the immense cost of treating osteoporosis indicate the dire need for future osteoporosis-related research.

ACKNOWLEDGEMENTS

Department of Anthropology
Western Washington University
Professor: Dr. MJ Mosher
Peer Reviewers: Candice Orfao and Sam Legg

BIBLIOGRAPHY

1. Kanis, John A. "Assessment of Fracture Risk and Its Application to Screening for Postmenopausal Osteoporosis: Synopsis of a WHO Report." *Osteoporosis International* 4, no. 6 (1994): 368-81.
2. Kanis, John A. "Diagnosis of Osteoporosis and Assessment of Fracture Risk." *The Lancet* 359, no. 9321 (June 1, 2002): 1929-936.
3. "What Women Need to Know - National Osteoporosis Foundation." *National Osteoporosis Foundation*. Accessed December 5, 2016. https://www.nof.org/prevention/general-facts/what-women-need-to-know/.
4. Gronholz, M. Jill, DO. "Prevention, Diagnosis, and Management of Osteoporosis-Related Fracture: A Multifactoral Osteopathic Approach." *The Journal of the American Osteopathic Association* 108, no. 10 (October 2008): 575-85.
5. Kanis, J. A., P. Delmas, P. Burckhardt, C. Cooper, and D. Torgerson. "Guidelines for Diagnosis and Management of Osteoporosis." *Osteoporosis International* 7, no. 4 (1997): 390-406.
6. Blume, S. W., and J. R. Curtis. "Medical Costs of Osteoporosis in the Elderly Medicare Population." *Osteoporosis International* 22, no. 6 (2010): 1835-844.
7. Baccaro, Luiz Francisco, Délio Conde, Lúcia Costa-Paiva, and Aarão Mendes Pinto-Neto. "The Epidemiology and Management of Postmenopausal Osteoporosis: A Viewpoint from Brazil." *Clinical Interventions in Aging* (March 2015): 583-91.
8. Mcclung, Michael. "Role of RANKL Inhibition in Osteoporosis." *Arthritis Research & Therapy* 9, no. 1 (June 29, 2007).
9. Hilliard, Constance B. "High Osteoporosis Risk among East Africans Linked to Lactase Persistence Genotype." *BoneKEy Reports* 5 (June 29, 2016): 803.
10. Ranciaro, Alessia, Michael C. Campbell, Jibril B. Hirbo, Wen-Ya Ko, Alain Froment, Paolo Anagnostou, Maritha J. Kotze, Muntaser Ibrahim, Thomas Nyambo, Sabah A. Omar, and Sarah A. Tishkoff. "Genetic Origins of Lactase Persistence and the Spread of Pastoralism in Africa." *The American Journal of Human Genetics* 94, no. 4 (April 3, 2014): 496-510.
11. Bersaglieri, Todd, Pardis C. Sabeti, Nick Patterson, Trisha Vanderploeg, Steve F. Schaffner, Jared A. Drake, Matthew Rhodes, David E. Reich, and Joel N. Hirschhorn. "Genetic Signatures of Strong Recent Positive Selection at the Lactase Gene." *The American Journal of Human Genetics* 74, no. 6 (2004): 1111-120.
12. Enattah, Nabil Sabri, Aimee Trudeau, Ville Pimenoff, Luigi Maiuri, Salvatore Auricchio, Luigi Greco, Mauro Rossi, Michael Lentze, J.k. Seo, Soheila Rahgozar, Insaf Khalil, Michael Alifrangis, Sirajedin Natah, Leif Groop, Nael Shaat, Andrew Kozlov, Galina Verschubskaya, David Comas, Kazima Bulayeva, S. Qasim Mehdi, Joseph D. Terwilliger, Timo Sahi, Erkki Savilahti, Markus Perola, Antti Sajantila, Irma Järvelä, and Leena Peltonen. "Evidence of Still-Ongoing Convergence Evolution of the Lactase Persistence T-13910 Alleles in Humans." *The American Journal of Human Genetics* 81, no. 3 (2007): 615-25.
13. Plantinga, Theo S., Santos Alonso, Neskuts Izagirre, Montserrat Hervella, Rosa Fregel, Jos W M Van Der Meer, Mihai G. Netea, and Concepcion De La Rúa. "Low Prevalence of Lactase Persistence in Neolithic South-West Europe." *European Journal of Human Genetics* 20, no. 7 (January 11, 2012): 778-82.
14. "General Facts - National Osteoporosis Foundation." *National Osteoporosis Foundation*. Accessed November 22, 2016. https://www.nof.org/prevention/general-facts/.
15. Jones, Bryony Leigh, Tamiru Oljira, Anke Liebert, Pawel Zmarz, Nicolas Montalva, Ayele Tarekeyn, Rosemary Ekong, Mark G. Thomas, Endashaw Bekele, Neil Bradman, and Dallas M. Swallow. "Diversity of Lactase Persistence in African Milk Drinkers." *Human Genetics* 134, no. 8 (June 09, 2015): 917-25.
16. Ingram, Catherine J. E., Charlotte A. Mulcare, Yuval Itan, Mark G. Thomas, and Dallas M. Swallow. "Lactose Digestion and the Evolutionary Genetics of Lactase Persistence." *Human Genetics* 124, no. 6 (November 26, 2008): 579-91.
17. Sahi, T. "Hypolactasia and Lactase Persistence Historical Review and the Terminology." *Scandinavian Journal of Gastroenterology* 29, no. Sup202 (1994): 1-6.
18. Gerbault, P., A. Liebert, Y. Itan, A. Powell, M. Currat, J. Burger, D. M. Swallow, and M. G. Thomas. "Evolution of Lactase Persistence: An Example of Human Niche Construction." *Philosophical Transactions of the Royal Society B: Biological Sciences* 366, no. 1566 (2011): 863-77.
19. Hollox, Edward. "Evolutionary Genetics: Genetics of Lactase Persistence—Fresh Lessons in the History of Milk Drinking." *European Journal of Human Genetics* 13, no. 3 (December 15, 2004): 267-69.
20. Enattah, Nabil Sabri, Timo Sahi, Erkki Savilahti, Joseph D. Terwilliger, Leena Peltonen, and Irma Järvelä. "Identification of a Variant Associated with Adult-type Hypolactasia." *Nature Genetics* 30, no. 2 (2002): 233-37.
21. Beto, Judith A. "The Role of Calcium in Human Aging." *Clinical Nutrition Research* 4, no. 1 (2015): 1.
22. Coetzee, Magdalena, and Marlena C. Kruger. "Osteoprotegerin-Receptor Activator of Nuclear Factor-κB Ligand Ratio: A New Approach to Osteoporosis Treatment?" *Southern Medical Journal* 97, no. 5 (2004): 506-11.
23. Hewison, M., D. Zehnder, R. Bland, and P. M. Stewart. "1alpha-Hydroxylase and the Action of Vitamin D." *Journal of Molecular Endocrinology* 25, no. 2 (2000): 141-48.
24. Ducy, Patricia, Thorsten Schinke, and Gerard Karsenty. "The Osteoblast: A Sophisticated Fibroblast under Central Surveillance." *Science* 289, no. 5484 (2000): 1501-504.
25. Teitelbaum, S. L. "Bone Resorption by Osteoclasts." *Science* 289, no. 5484 (September 01, 2000): 1504-508.

26. Walsh, Matthew C., and Yongwon Choi. "Biology of the RANKL-RANK-OPG System in Immunity, Bone, and Beyond." *Frontiers in Immunology* 5 (October 20, 2014).
27. Österdahl, M., T. Kocturk, A. Koochek, and P. E. Wändell. «Effects of a Short-term Intervention with a Paleolithic Diet in Healthy Volunteers.» *European Journal of Clinical Nutrition* 62, no. 5 (May 16, 2007): 682-85.
28. Tarantino, G., V. Citro, and C. Finelli. "Hype or Reality: Should Patients with Metabolic Syndrome-related NAFLD Be on the Hunter-Gatherer (Paleo) Diet to Decrease Morbidity?" *Journal of Gastrointestinal and Liver Diseases* 24, no. 3 (September 2015).
29. Pitt, Christopher E. "Cutting through the Paleo Hype: The Evidence for the Palaeolithic Diet." *Australian Family Physician* 45, no. 1 (January/February 2016): 35-38.
30. Samadfam, Rana, Qingwen Xia, and David Goltzman. "Co-Treatment of PTH With Osteoprotegerin or Alendronate Increases Its Anabolic Effect on the Skeleton of Oophorectomized Mice." *Journal of Bone and Mineral Research* 22, no. 1 (October 02, 2006): 55-63.
31. Leder, Benjamin Z., Joy N. Tsai, Alexander V. Uihlein, Sherri-Ann M. Burnett-Bowie, Yuli Zhu, Katelyn Foley, Hang Lee, and Robert M. Neer. "Two Years of Denosumab and Teriparatide Administration in Postmenopausal Women With Osteoporosis (The DATA Extension Study): A Randomized Controlled Trial." *The Journal of Clinical Endocrinology & Metabolism* 99, no. 5 (July 07, 2014): 1694-700.
32. Olausson, Hanna, Gail R. Goldberg, M. Ann Laskey, Inez Schoenmakers, Landing M. A. Jarjou, and Ann Prentice. "Calcium Economy in Human Pregnancy and Lactation." *Nutrition Research Reviews* 25, no. 01 (2012): 40-67.
33. Cosman, F., V. Shen, D. Morgan, S. Gordon, M. Parisien, J. Nieves, and R. Lindsay. "Biochemical Responses of Bone Metabolism to 1,25-Dihydroxyvitamin D Administration in Black and White Women." *Osteoporosis International* 11, no. 3 (2000): 271-77.
34. Jones, K. S., S. Assar, D. Vanderschueren, R. Bouillon, A. Prentice, and I. Schoenmakers. "Predictors of 25(OH) D Half-Life and Plasma 25(OH)D Concentration in The Gambia and the UK." *Osteoporosis International Osteoporos Int* 26, no. 3 (2014): 1137-1146.
35. Kumar, R., and J. R. Thompson. "The Regulation of Parathyroid Hormone Secretion and Synthesis." *Journal of the American Society of Nephrology* 22, no. 2 (December 16, 2010): 216-24.
36. Levis, Silvina, and George Theodore. "Summary of AHRQ's Comparative Effectiveness Review of Treatment to Prevent Fractures in Men and Women with Low Bone Density or Osteoporosis: Update of the 2007 Report." *JMCP Journal of Managed Care Pharmacy* 18, no. 4 Supp B (May 2012): 1-15.
37. Looker, A. C., T. B. Harris, J. H. Madans, and C. T. Sempos. "Dietary Calcium and Hip Fracture Risk: The NHANES I Epidemiologic Follow-Up Study." *Osteoporosis International* 3, no. 4 (1993): 177-184.
38. Rozenberg, Serge, Jean-Jacques Body, Olivier Bruyère, Pierre Bergmann, Maria Luisa Brandi, Cyrus Cooper, Jean-Pierre Devogelaer, Evelien Gielen, Stefan Goemaere, Jean-Marc Kaufman, René Rizzoli, and Jean-Yves Reginster. "Effects of Dairy Products Consumption on Health: Benefits and Beliefs—A Commentary from the Belgian Bone Club and the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases." *Calcified Tissue International Calcif Tissue Int* 98, no. 1 (October 07, 2015): 1-17.
39. Tella, Sri Harsha, and J. Christopher Gallagher. "Prevention and Treatment of Postmenopausal Osteoporosis." *The Journal of Steroid Biochemistry and Molecular Biology* 142 (2014): 155-70.
40. Eriksen, Erik F., Adolfo Díez-Pérez, and Steven Boonen. "Update on Long-term Treatment with Bisphosphonates for Postmenopausal Osteoporosis: A Systematic Review." *Bone* 58 (2014): 126-35.
41. "Denosumab (Prolia and Xgeva)." *Aetna*. Accessed July 9, 2016. http://www.aetna.com/cpb/medical/data/800_899/0804.html.
42. "ProliaPlus and Medicare Insurance." *ProliaPlus and Medicare Insurance*. http://www.proliahcp.com/proliaplus/medicare-insurance/.
43. Mundi, Simran, Bharadwaj Pindiprolu, Nicole Simunovic, and Mohit Bhandari. "Similar Mortality Rates in Hip Fracture Patients Over the Past 31 Years." *Acta Orthopaedica* 85, no. 1 (2014): 54-59.
44. Schnell, Scott, Susan M. Friedman, Daniel A. Mendelson, Karilee W. Bingham, and Stephen L. Kates. "The 1-Year Mortality of Patients Treated in a Hip Fracture Program for Elders." *Geriatric Orthopaedic Surgery & Rehabilitation* 1, no. 1 (2010): 6-14.
45. Tajeu, G. S., E. Delzell, W. Smith, T. Arora, J. R. Curtis, K. G. Saag, M. A. Morrisey, H. Yun, and M. L. Kilgore. "Death, Debility, and Destitution Following Hip Fracture." *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 69A, no. 3 (2013): 346-53.
46. Tsai, C.-H., C.-L. Lin, H.-C. Hsu, and W.-S. Chung. "Increased Risk of Coronary Heart Disease in Patients with Hip Fracture: A Nationwide Cohort Study." *Osteoporosis International* 26, no. 6 (2015): 1849-855.
47. Carpintero, Pedro. "Complications of Hip Fractures: A Review." *World Journal of Orthopedics* 5, no. 4 (2014): 402.