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# 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.<sup>1</sup> 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.<sup>1</sup> 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.<sup>2</sup> Elderly women are the most likely demographic group to develop the disease, although it can develop in anyone at any point in life.<sup>3,4</sup> 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.<sup>5,6</sup> 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.<sup>7,8</sup>

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.<sup>9</sup> The fracture rates were positively correlated ( $r = 0.841$ ) with milk consumption and positively correlated with lactase persistence ( $r = 0.735$ ).<sup>9</sup> East Africans adopted cattle domestication between 2,700–6,800 years ago, and have been consuming milk from cattle ever since.<sup>10</sup> 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.<sup>11–13</sup>

Current guidelines on osteoporosis encourage maximum calcium consumption.<sup>14</sup> 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 mech-

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anism for calcium homeostasis, resulting in susceptibility to developing degenerative bone diseases like osteoporosis.

### DEVELOPMENT OF LACTASE PERSISTENCE

For ninety-nine percent of human history, humans would become lactose intolerant around early adulthood.<sup>15–17</sup> Milk was primarily consumed during infancy and over time there became no need to digest milk.<sup>15</sup> Around 10,000 years ago, mutations developed in the genes regulating lactase, the enzyme that breaks down the lactose sugar.<sup>10, 11</sup> Without the ancestral need to digest the milk into adulthood the lactase non-persistent (LNP) trait naturally downregulates lactase production.<sup>16</sup> 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.<sup>10,11,15</sup>

Upon the domestication of cattle, milk became an easy source of calories for those who could digest the lactose sugar.<sup>18</sup> Consuming lactose in the absence of lactase can create painful abdominal cramps and gas, discouraging those who are lactose intolerant from consuming milk.<sup>18</sup> 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.<sup>19</sup> This selective advantage allowed those who expressed the LP trait to pass on their genes.<sup>20</sup> This selecting force, along with other cultural and environmental factors, allowed LP to replace lactose intolerance in certain parts of the world.<sup>10,18</sup> 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.<sup>10,11</sup> In Africa, several other variants of the LCT gene have been identified as causes of LP trait expression.<sup>10</sup>

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.<sup>21</sup> 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.<sup>21</sup> One such hormone is parathyroid hormone (PTH).

PTH is synthesized in the parathyroid glands located posterior to the thyroid gland in the lower neck.<sup>22</sup> 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.<sup>22</sup> PTH also binds to cell surface receptors on the kidney tubules to cause reabsorption of calcium into the plasma.<sup>22</sup> 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<sub>3</sub> in the kidney to turn Vitamin D<sub>3</sub> into its biologically active form, 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>, also known as calcitriol. PTH accomplishes this by increasing production of the enzyme 1- $\alpha$ -hydroxylase, which converts 25-hydroxyvitamin D<sub>3</sub> 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.<sup>23</sup> In a correctly 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.<sup>24</sup> 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.<sup>24</sup> 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.<sup>25</sup> Once in the bloodstream, the body utilizes this calcium for life sustaining functions.<sup>21</sup> 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- $\kappa$ B (RANK), and osteoprotegerin (OPG) are key signaling-proteins involved in osteoblast and osteoclast regulation.<sup>26</sup> 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.<sup>26</sup> OPG is a signaling-protein that functions as a

**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

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.<sup>26</sup> OPG is vitally important in maintaining a healthy BMD.

**THE EVOLUTION OF THE DERIVED CALCIUM HOMEOSTASIS**

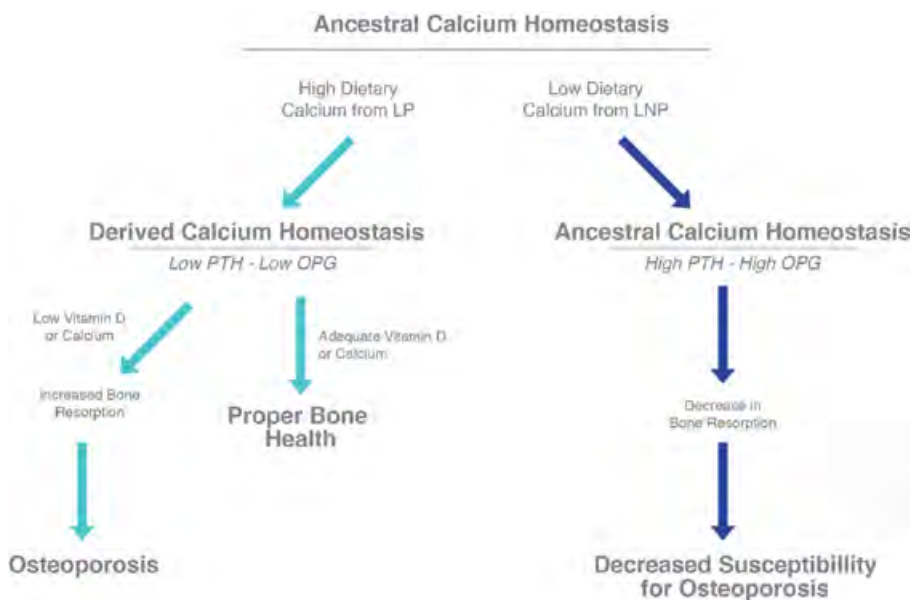
Preagricultural diets had extremely low dietary calcium levels, which can be partially attributed to the absence of milk.<sup>27-29</sup> Therefore, this ancestral calcium homeostasis would have been extremely advantageous for our early ancestors before dairy agriculture was present. We theorize that 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.<sup>22</sup> 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.<sup>22</sup> 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.<sup>22,30,31</sup> 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.<sup>32</sup>

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.<sup>18</sup> This large influx of dietary calcium led to high levels of calcium in the bloodstream, which would decrease the relative amount of PTH.<sup>22,33,34</sup> 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

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.<sup>35</sup> Without the protective effects of OPG, osteoclast activity is minimally inhibited and outpaces osteoblast activity.<sup>26</sup> 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.<sup>26</sup>



**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.”<sup>14</sup> 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.<sup>36–38</sup> 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.<sup>39,40</sup>

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.<sup>39</sup> 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.<sup>41,42</sup> 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.<sup>43,44</sup> 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.<sup>45–47</sup> These potential health and lifestyle consequences combined with the immense cost of treating osteoporosis indicate the dire need for future osteoporosis-related research.

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