



## Review

# Why Does Inflammation Result in Resorptive Bone Loss? What the Study of Burns Teaches Us

Gordon L. Klein

Department of Orthopaedic Surgery and Rehabilitation, University of Texas Medical Branch at Galveston, Galveston, TX 77555-0165, USA; gordonklein@ymail.com

**Abstract:** Burn injury serves as an example of a condition with a robust systemic inflammatory response. The elevation of circulating interleukins (IL)-1 $\beta$  and -6 in children and adolescents with severe burn injury upregulates the parathyroid calcium-sensing receptor (CaSR), resulting in hypocalcemic hypoparathyroidism accompanied by urinary calcium wasting. This effect protects the body from the hypercalcemia that results from bone resorption, liberating calcium into the circulation. Extracellular calcium can exacerbate and prolong the inflammatory response by stimulating mononuclear cell chemokine production as well as the NLRP3 inflammasome of the innate immune system, resulting in increased IL-1 production by monocytes and macrophages. Interestingly, the CaSR upregulation in response to inflammatory cytokines disappears with age, potentially trapping calcium from bone resorption in the circulation, allowing it to contribute to increased inflammation and possibly increased calcium deposition in small arteries, such as the coronaries, as conditions with increased chronic inflammation, such as spinal cord injury, osteoarthritis, and rheumatoid arthritis have an incidence of cardiovascular disease and coronary artery calcium deposition significantly higher than the unaffected age-matched population.

**Keywords:** inflammation; calcium-sensing receptor; burns; chemokines; NLRP3 inflammasome



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## 1. Introduction

Inflammation results in the release of various cytokines from the body's immune cells, most notably interleukin (IL)-6, IL-1 $\beta$ , and tumor necrosis factor (TNF)- $\alpha$ . In addition, various immune cells produce chemoattractant cytokines, called chemokines. These substances attract immune cells to a site of inflammation within the body, increasing the intensity and/or duration of the inflammatory response.

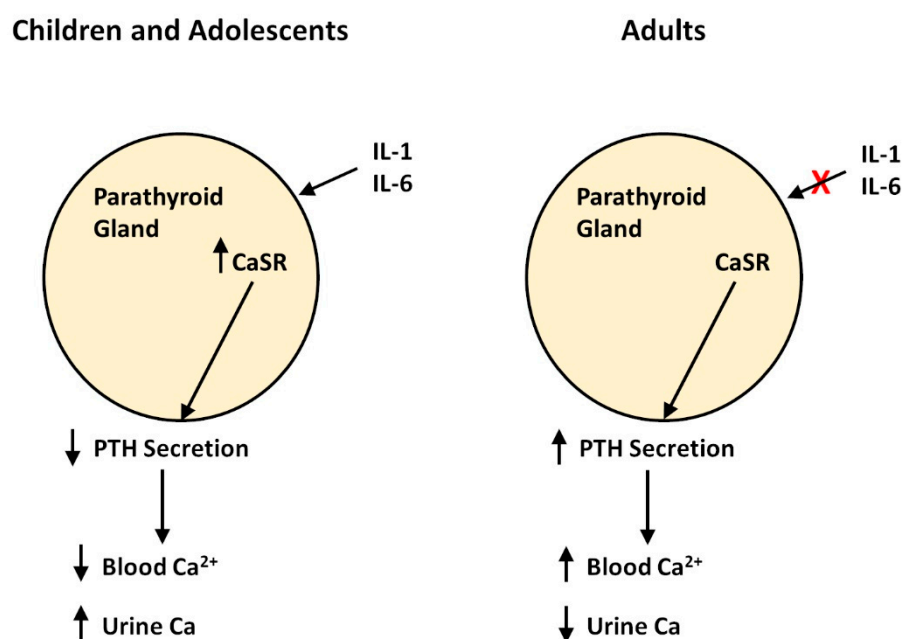
Burn injury is an example of a condition in which a robust systemic inflammatory response is manifested by elevated circulating concentrations of IL-1 $\beta$  and IL-6 by 3-fold and 100-fold, respectively [1].

The robust systemic inflammatory response is due to the destruction of skin as a barrier to infection, and burn patients are all presumed to be septic due to wound infection. In conjunction with relative immobilization and elevated endogenous glucocorticoids [2], resorptive bone loss is observed in children with over 40% of total body surface area burned. The result is a loss of 7% of total trabecular bone density of the lumbar spine over the first six weeks following burn injury and a 3% loss of total body bone mineral density, mainly cortical bone, over the first six months post-burn.

In the laboratory, in vitro studies of bovine parathyroid cells [3] and equine parathyroid cells [4] incubated with IL-1 $\beta$  and IL-6 [5] demonstrate the upregulation of the parathyroid calcium-sensing receptor (CaSR). The CaSR is found in many organs across the body, including kidneys [6], bone [7], intestine [8], and cardiovascular endothelium [9,10]. In the parathyroid, the CaSR is a G-protein-coupled membrane-bound receptor on the parathyroid chief cells that detects extracellular calcium and signals the cell the amount of PTH to

be secreted into the circulation. The coordination of body CaSRs requires further investigation, and this paper deals specifically with the parathyroid CaSR. The upregulation of the parathyroid CaSR has the effect of lowering the amount of circulating calcium necessary to suppress parathyroid hormone (PTH) secretion, leading to hypocalcemic hypoparathyroidism. This is what we have observed in a sheep model of burn injury [11] as well as in pediatric patients with injury of  $\geq 40\%$  total body surface area burn [12].

Another observation in the same sheep model of burn injury and over the same time frame, i.e., the first five days following burns, was that backscatter scanning electron microscopic study of iliac crest demonstrated scalloping, a hallmark of resorption, plainly visible by day 5 [13]. In addition, urine C-telopeptide of type I collagen (CTX), a biochemical marker of bone resorption, was elevated on day one. Notably, the coincidence of the cytokine-mediated upregulation of the parathyroid CaSR and the onset of bone resorption, stimulated by inflammation, immobilization, and increased endogenous steroid production, likely serves as a way to facilitate the excretion of excess calcium entering the circulation following acute bone resorption (see Figure 1). Concomitantly, the inflammation-induced upregulation of the CaSR suppresses PTH secretion [14], allowing increased urinary calcium excretion, thus protecting the body from hypercalcemia. A question that arises is what does this have to do with inflammatory cytokines causing resorptive bone loss?



**Figure 1.** Parathyroid gland response to pro-inflammatory cytokines in children and adolescents and in adults following burn injury. In children and adolescents, the cytokines upregulate the membrane-bound G-protein coupled calcium-sensing receptor, causing a reduction in the amount of circulating calcium necessary to suppress parathyroid hormone secretion by the gland. The result is hypocalcemic hypoparathyroidism with increased urinary calcium excretion. In adults, this ability for the calcium-sensing receptor to upregulate in response to inflammatory cytokines appears to be lost.

## 2. Extracellular Calcium and Inflammation

In an unrelated in vitro study we performed, we isolated peripheral blood mononuclear cells from normal adult volunteers and incubated them in media containing varying amounts of calcium [15]. We found very tight, direct, and inverse correlations between various chemokines produced by these mononuclear cells and the amount of calcium in the medium. It is not clear why chemokines such as Regulated on Activation Normal T and Secreted (RANTES) and Monocyte Inhibitory Protein (MIP)-1  $\alpha$  were strongly stimulated, whereas Monocyte Chemoattractant Protein (MCP-1) was equally strongly inhibited by

extracellular calcium, although it is possible that the sequence and timing of appearance in the blood of the various chemokines are important to the inflammatory response. The implication of these findings is that extracellular calcium stimulates or suppresses certain chemokines, which can serve to attract more inflammatory cells to the areas of existing inflammation, thus intensifying and possibly prolonging the inflammatory response in burn patients. These observations were reinforced by the work of Rossol et al. [16], who demonstrated that extracellular calcium could stimulate the nod-like receptor (NLR)-P3 inflammasome of the innate immune system via the CaSR on monocyte membranes to stimulate monocytes and macrophages to produce more IL-1, further intensifying the inflammatory process. Subsequently, this group also demonstrated that circulating calcium and phosphate are converted by the serum protein Fetuin A to colloidal calciprotein particles in order to prevent ectopic calcification. These undergo pinocytosis by monocytes, a function of increased circulating calcium-triggering monocyte CaSR upregulation prior to the pinocytosis. This action triggers the activation of the NLRP3 inflammasome, resulting in the production of IL-1 $\beta$  [17]. Thus, it would appear that the calcium entering the circulation following bone resorption is capable of intensifying and prolonging the inflammatory response to burn injury and perhaps other conditions. Furthermore, Olszak et al. [18] demonstrated in vitro that extracellular calcium could increase the monocyte expression of various chemokine receptors, thus building even a stronger case for extracellular calcium stimulating the various components of the inflammatory response. Further evidence implicating calcium in the inflammatory response comes from Michalick and Kuebler [19], who point out that transient receptor potential vanilloid (TRPV)-type 4, or TRPV4, a mechanosensitive calcium channel, is involved in neutrophil activation and chemotaxis. TRPV4 has also been implicated in macrophage activation leading to lung injury following mechanical ventilation. These issues have also been reported in a rat model of immobilization and mechanical ventilation [20] in which it was shown that the pharmacologic immobilization of rats was accompanied by marked trabecular bone loss, thus liberating calcium into the circulation and possibly contributing to the inflammation described by Michalick and Kuebler [19].

### 3. Preservation of the Hypocalcemic Response in Small Burns

The data on the longitudinal decrease in bone density following burns were originally published in 1995 [21] and again in 2005 in conjunction with a study of antiresorptive agents and bone density [22] and were applicable to children with burns of  $\geq 40\%$  total body surface area. In the first of these two publications [21], we studied a cohort of children with burns of approximately 20% of total body surface area. Those children did not suffer significant resorptive bone loss and had no reduction in their bone mineral density. Therefore, there is a threshold effect regarding the metabolic response to burn such that the inflammatory response at 40% body surface area burn is sufficient to cause resorptive bone loss, but at 20% body surface area burn, even combined with the effects of immobilization on bone loss, there is no clinically significant bone loss, as determined by bone densitometry. From these data, we can infer that the additional amounts of calcium entering the circulation from bone resorption in the latter group was negligible. Therefore, we attempted to determine whether blood ionized calcium levels remained normal in children with small burns. Accordingly, we obtained blood ionized calcium concentrations from the records of 190 anonymized children with burns ranging from 1% to 20% total body surface area. Throughout hospitalization, their mean ionized calcium concentration was  $1.04 \pm 0.08$  (SD) mM, range 0.73–1.31, and normal range 1.12–1.37. The mean values were 8% below the lower limits of normal for age, while only 16.8% of the 190 pediatric patients with small burns had mean circulating ionized calcium concentrations within the normal range. In no setting of pediatric burn injury is the circulating ionized calcium normal. Since burn size plays a role in resorptive bone loss and reduced bone density, the hypocalcemic response to burns as small as 1% of total body surface area suggests that the upregulation of the parathyroid CaSR in response to acute inflammation is the prime purpose of the

reactive hypocalcemia rather than this response being uniquely related to resorptive bone loss induced by immobilization or endogenous glucocorticoid production. Unfortunately, neither serum PTH concentrations nor quantitative urinary calcium data were available in this anonymized population. In other words, even in small burns, the initial inflammatory response in children is sufficient to upregulate the parathyroid CaSR without the addition to the circulation of calcium from resorbing bone. In more severe burns, resorbed calcium entering the circulation would theoretically add to the urinary calcium load in pediatric burns. However, we do not have sufficient data at this time to document this statement.

#### **4. Developmental Disappearance of Calcium-Sensing Receptor Response to Inflammatory Cytokines**

Despite the apparent uniformity of findings in burn patients under the age of 19, studies in adults yield an entirely different picture of ionized calcium and PTH response to severe burn injury. In our patients [23], and in the adult burns reported in the literature [24,25], severely burned adult patients were normo- or mildly hypercalcemic and euparathyroid or mildly hyperparathyroid. In our patients, the mean blood ionized calcium concentration was  $1.08 \pm 0.03$  (SD) mM in children with the normal range being 1.12 to 1.37 [12], while in adults with similarly sized burns, the blood ionized calcium concentration was  $1.15 \pm 0.06$  mM, with normal range 1.00 to 1.15 mM. Similarly, serum PTH concentration in burned children was  $7 \pm 3$  pg/mL, with a normal range of 15 to 55, whereas in adults, the mean PTH for similarly sized burns was  $114 \pm 96$  pg/mL, with the normal range being 10 to 65. The mechanism explaining this difference is not apparent. However, it would appear likely that the ability of the parathyroid calcium-sensing receptor to respond to inflammatory cytokines by the upregulation and consequent development of hypocalcemic hypoparathyroidism is lost with age, sometime after the onset of puberty. By age 19, there is still no change in the childhood response pattern of parathyroid CaSR upregulation in response to inflammatory cytokines, thus likely causing the age at the loss of response of the parathyroid CaSR to inflammatory cytokines to be at least in the early 20s. This age approximates the time of acquisition of peak bone mass, and the relationship between these two developmental milestones requires further investigation. Likewise, sexual dimorphism was not apparent in the analysis of the results of circulating ionized calcium and PTH concentrations in children and adolescents past the onset of puberty, through the ages of 18–19 years.

#### **5. Potential Implications of the Disappearance of Calcium-Sensing Receptor Response to Inflammatory Cytokines**

The implications of the disappearance of the parathyroid CaSR response to inflammatory cytokines with age are at least two-fold. The first is that children and adults with similar body surface area burns have different clinical outcomes. Thus, children are able to respond to inflammatory cytokines by upregulating the calcium-sensing receptor, resulting in hypocalcemic hypoparathyroidism with accompanying increased urinary calcium excretion. The excess calcium lost from the skeleton and entering the circulation is excreted in the urine and therefore will not build up in the circulation and cause intensified or prolonged inflammation. In contrast, adults cannot upregulate the parathyroid CaSR in response to inflammatory cytokines, resulting in the failure to excrete the excess calcium introduced into the circulation in response to bone resorption, leading to intensified and/or prolonged inflammation. This difference is documented by the difference in mortality between children and adults experiencing severe burn injury. In a large multicenter study by Jeschke et al. [26] involving 226 children and 347 adults, children with greater than 60% of total body surface area burned were at greater risk for burn mortality, while adults burned greater than 40% of total body surface area were at greater risk for burn mortality, an extent of burn injury 20% lower than that in children.

In addition, long-term cardiac dysfunction complicates severe burn injury in children [27], including reduced left ventricular ejection fraction, systolic and diastolic dysfunction, and myocardial fibrosis, all possibly due to a putative effect of transforming growth

factor (TGF)- $\beta$  release from resorbing bone matrix adversely affecting cardiac smooth muscle as well as skeletal muscle catabolism [28–30] and fibrosis [31]. However, in adults, there is also the possibility of excess calcium from resorbing bone entering the circulation and remaining there as opposed to being excreted in the urine in significant quantities, leading to accumulation in end arteries, such as in coronary blood vessels, thus contributing to plaque formation. Alexander et al. [32] documented increased troponin levels in adults with burn injury of greater than 15% of total body surface area.

Campos-Obando et al. reported correlations between osteoporosis and heart disease, in aging adults [33] potentially involving mechanisms related to bone resorption. While the changes due to aging are clearly confounding variables, bone resorption and its consequent release of calcium into a circulation system that cannot adequately eliminate it could equally be a contributing factor.

## 6. Other Clinical Conditions

The bulk of this review focused on the body's response to burn injury as a model for understanding why bone resorption occurs with inflammation. However, there are many other conditions in which resorptive bone loss can play a role in exacerbating the systemic inflammatory response. Among them are spinal cord injury, rheumatoid and osteoarthritis, as well as hyper-resorptive conditions such as hyperparathyroidism, etc.

Notably, spinal cord injury bears many similarities to burn injury, including an increased inflammatory response, robust bone loss of up to one percent per week [34], skeletal muscle wasting, an increased incidence of cardiovascular disease and congestive heart failure [35], the suppression of PTH and FGF-23 in the case of burn [36], and increased tubular threshold for the excretion of phosphate in spinal cord injury, indicating the body's conservation of phosphate, possibly for a role in muscle metabolism. Del Rivero and Bethea [37] studied a mouse model of spinal cord injury and found acute and chronic bone loss associated with normal levels of PTH at one-week post-injury but a significant decrease in serum PTH concentration at four weeks, along with a rise in circulating calcium. These findings suggest that PTH does not induce a rise in bone resorption but is suppressed by the acute and continuous loss of bone with spinal cord injury. The progressive rise in serum calcium can be explained by the inflammation-induced bone resorption suppression of PTH. Additionally, as Bauman and Spungen [38] have noted, there is an increased risk of coronary heart disease in patients with spinal cord injury, currently attributed to immobilization and increased abnormalities in carbohydrate and lipid metabolism. However, the role of calcium retention in the body due to bone resorption has not been investigated as a contributing factor to coronary artery disease. In addition, Lieberman et al. [39], in a study of spinal cord-injured men aged 45–70 years, found correlations of coronary calcium score with the Framingham Risk Score and age, but there was a divergence of agreement of cardiovascular risk assessment for lipid-lowering treatments between Framingham Risk Scores and coronary calcium scores. Of the 20 of 38 patients who qualified for lipid-lowering treatment by either the Framingham Risk Score or the coronary calcium score, only 4 of the 20 patients, 20%, qualified by both assessments. While the numbers are small, the divergence leaves room for other factors, such as circulating calcium, to serve as a contributor to cardiovascular disease in this population.

Rheumatoid arthritis is another condition with a marked inflammatory response contributing to bone loss. In this condition, Karpouzas et al. [40] reported that cumulative inflammation in this chronic disease contributes to progressive coronary artery calcium accumulation, and Houri Levi et al. [41] confirmed an increased association between rheumatoid arthritis and ischemic heart disease in a case–control study of over 11,000 patients in Israel.

Osteoarthritis, another chronic inflammatory disease of joints associated with bone loss, has been shown in meta-analysis studies by Macedo et al. [42] in which patients with osteoarthritis were three times as likely to have atherosclerotic heart disease, compared with non-osteoarthritic cohorts. Wang et al. [43], in another meta-analysis, concluded that



patients with osteoarthritis had a 24% increase in risk for cardiovascular disease compared with the unaffected age-matched populations. Additionally, in yet another meta-analysis, Hall et al. [44] found that patients with osteoarthritis were nearly three times more likely to develop either heart failure or ischemic heart disease than the unaffected matched cohorts.

Calcium has been implicated as well in generating inflammation by combining with calmodulin to activate the enzyme,  $\text{Ca}^{2+}$ /calmodulin regulated kinase  $\delta$  in response to pressure overload. This enzyme then stimulates inflammatory gene expression and the activation of the NLRP3 inflammasome, which results in the increased production of IL-1 $\beta$  and IL-18 by the innate immune system. This has been demonstrated in cardiomyocytes [45]. A similar mechanism has been described for the initiation of inflammation in mechanical ventilation [20], and a related mechanism has been implicated following cardiac ischemia, also involving a  $\text{Ca}^{2+}$ /calmodulin regulated kinase [46]. It is unclear how much circulating Ca is necessary to trigger these latter effects, or if there is a threshold for circulating Ca to contribute to these effects. Nonetheless, they are also potential ways in which Ca can trigger an inflammatory response.

## 7. Conclusions

In summary, the release of calcium into the circulation following bone resorption is capable of contributing to the inflammatory response by affecting the peripheral blood mononuclear cell production of chemokines and stimulating the NLRP3 inflammasome, thus increasing the production of IL-1 by monocytes and macrophages of the innate immune system. In addition, extracellular calcium can play a role in the expression of inflammatory genes, which activate the NLRP3 inflammasome under conditions of ischemia or increased pressure. Children and adolescents may be capable of responding to systemic inflammation by dumping the excess calcium entering the circulation via bone resorption into the urine by means of the cytokine-stimulated upregulation of the parathyroid calcium-sensing receptor, a mechanism that developmentally disappears in adults at a time possibly coinciding with the acquisition of peak bone mass. The inability of adults to reduce their circulating load of calcium may contribute to increased mortality from burn injuries and coronary heart disease among other conditions. The expanded use of bisphosphonates and other antiresorptive agents in patients with severe inflammatory responses to illness may modify the effects caused by inflammation.

It is, therefore, incumbent upon us to investigate these potential effects of extracellular calcium in relation to the generation of inflammatory responses in adult populations and to determine if reducing bone resorption in these and other inflammatory conditions can modify their outcomes or prognoses.

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