



## Progress in Understanding Calcium and Vitamin D Endocrinology Following Burn Injury in Children

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### ABSTRACT

This review covers the progress made by our group over the past two decades in understanding the effects of severe burn injuries on the endocrine system, especially in relation to calcium and vitamin D metabolism. We examine the phenomena of transient growth hormone deficiency and the dose-dependent effects of recombinant human growth hormone on bone and muscle mass. We move on to discuss the occurrence of post-burn secondary hypoparathyroidism caused by the up-regulation of the parathyroid calcium-sensing receptor, and finally, we discuss vitamin D status, the progressive nature of vitamin D deficiency post-burn, the causes of the progressive deficiency, and what must be done to prevent it. These conditions taken together, while not primarily responsible for post-burn bone loss, may impair the recovery of normal bone density and leave, especially younger populations of victims, vulnerable to a reduction in peak bone mass with subsequent elevated risk of developing osteoporosis as adults.

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### ► Implication for health policy/practice/research/medical education:

This article reviews the endocrine changes that occur as a result of burn injury and provides a basis for understanding these changes in the context of all the changes wrought by burn injury. It also provides a means to study possible measures to prevent and treat these abnormal changes.

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## 1. Introduction

The purpose of this review is to describe the progress made in our understanding of what happens to calcium and vitamin D following burn injuries and to observe how various hormonal influences following burn injuries influence the status of both calcium and vitamin D. Furthermore we would like to elucidate how this additional information can aid in both the understanding of what happens to the body following a burn injury and how this improved understanding can affect the care of burn victims. With regard to the method of selection of articles to include in this review, the work in this area has

been done almost entirely by our group at the Shriners Burns Hospital in Galveston, Texas. It is my intention to summarize the work performed by our group over the past two decades along with work carried out by others in order to support the hypotheses and assumptions that we have made. These works will be cited specifically and appropriately referenced.

This review will include the following areas: the effects of burn injury on calcium homeostasis, specifically changes in circulating calcium, urinary calcium excretion, parathyroid hormone (PTH) levels in serum, and circulating levels of both 25-hydroxyvitamin D (25 (OH) D) and 1,25-dihydroxyvitamin D (1,25 (OH)<sub>2</sub>D). It will also include the effects of burn injury on the calcium sensing receptor (CaR) of the parathyroid gland and in the heart and blood vessels. Finally we will discuss the effects of hormonal therapy to date on bone and muscle mass to see if we can draw any conclusions regarding the roles

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of hormones in the body's metabolism following a burn injury.

## 2. The Effect of Burn Injury on Circulating and Urinary Levels of Calcium and Circulating Levels of PTH and Vitamin D

Because most of the following work was performed with victims sustaining a burn injury of 40% or more of their total body surface area, the remarks below pertain primarily to patients who have sustained severe burn injuries. With regard to circulating calcium in children (1) and adults (2), ionized calcium levels are acutely low following a burn injury. While the lower limit of normal is generally regarded as 1.08 mM, the majority of severely burned children have ionized calcium levels less than that value and many are as low as 0.8 mM (3). It is interesting to note that, urinary calcium excretion in severe burns can be as high as 8 mg/kg/24hr (3) while the pediatric normal urine calcium excretion is 3 mg/kg/24hr. Thus, these patients exhibit hypocalcemia and hypercalciuria. It should be noted that these effects persist despite the administration of calcium, both orally and intravenously of at least 3 g/meter square body surface area/day. Given that the current pediatric calcium Daily Reference Intake (4) is between 0.8 and 1.2 g per day, the amount of calcium administered clearly exceeds the normal daily requirements. So, why does this occur? To answer this question we need to look at circulating levels of PTH. These levels have been studied primarily in children and they have all been shown to be inappropriately low, not only as absolute values, but also in relation to circulating levels of ionized calcium (3). Moreover, subcutaneous administration of a single dose of PTH to these burn children produces a blunted rise in urinary cyclic AMP and phosphate excretion (3), giving us a picture not only of hypoparathyroidism, but also of PTH resistance. One possible explanation for the hypoparathyroidism, which could explain the hypocalcemia and hypercalciuria, is magnesium depletion. Magnesium depletion is a cause of PTH resistance and in as much as burn victims in the United States are acutely resuscitated with Ringer's Lactate, a magnesium-free solution, iatrogenic magnesium depletion remains a distinct possibility. In fact all of our patients were initially magnesium depleted when a magnesium loading test was performed (1, 5). Administration of magnesium during acute hospitalization for a burn injury, an average of 15 mg/kg/day both orally and intravenously, led to magnesium depletion in only half of the patients (5). When reexamining the intact PTH response to ionized calcium levels in both magnesium depleted and magnesium replete patients, we found no difference in PTH or ionized calcium levels from the previous study (3, 5), indicating that magnesium depletion was not the cause of the hypocalcemic hypoparathyroidism observed in these patients.

Unfortunately, circulating levels of 25 (OH) D and 1,25

(OH)<sub>2</sub>D in the acute stage following a burn injury are difficult to interpret and therefore their contribution to this acute derangement in calcium homeostasis cannot be fully assessed. The reason for this difficulty is that the levels of various constitutive proteins are depressed following a burn injury. These include vitamin D binding protein (3) and albumin (6). As assays of free 25 (OH) D and free 1,25 (OH)<sub>2</sub>D are no longer performed we cannot judge whether these levels are low because of the depressed metabolism of vitamin D or because the levels of binding proteins are low in the serum. We will have more to say about the circulating levels of vitamin D metabolites as we follow children further away from the acute burn period.

## 3. The Role of the Calcium-Sensing Receptor Following Burns

Genetic mutations of the parathyroid calcium sensing receptor (CaR) that have resulted in its up-regulation are associated with hypocalcemia, hypercalciuria, and hypoparathyroidism. This is because as the CaR is up-regulated, the amount of circulating calcium required to suppress PTH secretion by the parathyroids necessarily decreases. Thus even hypocalcemic levels may be sufficient to suppress PTH secretion (7). Magnesium depletion could not explain the hypocalcemic hypoparathyroidism exhibited by burn patients, therefore examination of the parathyroid CaR following burn injury should be a way of addressing the possibility of CaR involvement in the pathogenesis of hypocalcemic hypoparathyroidism in these patients.

Because sampling of human parathyroid tissue following burns is not feasible, we resorted to a sheep model of controlled burn injury that has been in use for over 20 years, to study the body's responses to burn injuries from the standpoints of the respiratory and cardiovascular systems as well as sepsis. This model has long had the approval of the Institutional Animal Care and Use Committee of the University of Texas Medical Branch, primarily because following the administration of a flame burn of 40% of the total body surface area under halothane anesthesia, the pain nerve endings are destroyed and thus the animals are in no pain. Once given fluid resuscitation for the burn they are ambulatory, eating, and in no discomfort. Sacrifice of the animals normally takes place 48 hr post-burn. In these studies we were able to reproduce acute hypocalcemia and hypomagnesemia in the sheep and at sacrifice the parathyroid glands were isolated and removed. After snap-freezing in liquid nitrogen, the specimens were subjected to northern blotting to assess CaR mRNA and then to immunoperoxidase staining to assess the amount of CaR on the parathyroid chief cell membranes (8). Through the use of gel densitometry, we detected an up-regulation of the parathyroid CaR by 50%, and in a semi-quantitative manner, a demonstrable increase in CaR membrane-bound protein (8). This dem-

onstrated that the CaR was up-regulated in the parathyroid gland following burn injury and likely provides the explanation for the hypocalcemic hypoparathyroidism. Given this finding one can ask whether this abnormality could be reversed by administering a calcilytic agent, that is, a pharmaceutical that antagonizes the CaR to prevent its up-regulation. To date there is very limited evidence on even the experimental use of calcilytic agents. Furthermore, as there are CaRs in other systems of the body, most prominently the endocardium of the heart and endothelial lining of blood vessels, which do not change following burn injury, at least in the sheep model (9), the specificity of a calcilytic agent for the parathyroid gland must be demonstrated before use in humans following a burn injury.

#### 4. What the Therapeutic Use of Growth Hormone in Burn Victims Has Taught us

Immediately following a severe burn injury there is a hypermetabolic response with resting energy expenditure rising 1.2-1.8 times from normal (10). This hypermetabolic response is associated with a negative nitrogen balance and wasting results in a decrease in skeletal loading and consequent potential bone loss. In fact bone density in pediatric burn victims has been demonstrated to be low (1) and biopsies from adults (2) and children (6) have repeatedly demonstrated reduced bone formation. It has also been demonstrated that the annual extrapolated fracture incidence in boys and girls recovering from a severe burn injury is greater than in age and sex-matched normals (1). Other body adaptive responses are also likely to play a role in the reduced bone density, namely the stress and inflammatory responses. While these latter two adaptive responses are outside the scope of this discussion, it would be helpful to mention that the stress response results in the production of large quantities of endogenous glucocorticoids, which can have the same effect on bone as the exogenous administration of steroids (11). The inflammatory response produces the cytokines that are indirectly capable of resorbing bone as they act directly through the cytokines. Therefore, both of these post-burn adaptive responses are likely to make a significant contribution to post-burn bone loss.

Recombinant human growth hormone (rhGH) is not only an anabolic agent; it is also a glucocorticoid antagonist. It was originally used in the treatment of severe burns in children because of the reduced growth velocity seen in the first year post-burn (12). This reduction in growth velocity was also thought to be linked to transient growth hormone deficiencies given the initial low serum levels of insulin-like growth factor (IGF)-1. Administration of rhGH to children following burns quickly improved circulating levels of IGF-1 (13). Of interest is that the effects of rhGH on the bone appear to be dose-dependent. Children given a standard amount of rhGH (0.05 mg/kg/day) by subcutaneous injection for one year

demonstrated an increase in lean body mass, including muscle, by 9 months post-burn, however bone mineral content only increased at 12 months post-burn (14). The implication of this observation is that rhGH builds muscle mass through IGF-1 and it is the resultant increase in skeletal loading that causes an increase in bone mineral content, with new bone formation possibly being mediated by osteocytes. Also of note, the increase in bone mineral content is not accompanied by an increase in bone mineral density. This implies that as bone density is the quotient of bone mineral content and bone area, there is a proportionate increase in bone mineral content and bone area, thus making the bone bigger and biomechanically stronger (14). Lest one think that the effect of rhGH/IGF-1 on the skeleton is only indirect, if the dose of rhGH is increased four-fold, to 2.0 mg/kg/day for one year there is an increase in bone resorption despite a continued increase in muscle mass (15). Therefore, whether rhGH/IGF-1 primarily affects muscle or bone appears to be dose-dependent. Moreover, it should be noted that the more common complications of giving rhGH to children, premature closure of the epiphyses and hyperglycemia, were not reported (14, 15). However, the use of rhGH has fallen out of favor because of a recent report of increased mortality with its use in critically ill patients (16). Accordingly, another anabolic agent, the anabolic steroid oxandrolone, has been used for approximately the last decade instead of rhGH. Oxandrolone has several advantages over rhGH. These include oral intake rather than subcutaneous injection, lower cost, and similar effects on lean body mass and bone mineral content (17). While this drug is still under study in burned children, there have been no reports of premature epiphyseal closure, but there is approximately a 3% incidence of clitoral hypertrophy, which appears to be reversible when the drug is discontinued. The explanation as to why IGF-1 is initially low following a burn injury and why it responds so quickly to rhGH is not certain. Transient growth hormone deficiency has been the explanation offered, but the nature and pathogenesis of this phenomenon have not been adequately studied.

#### 5. Vitamin D Status

Lastly, while we have already mentioned the problem of the uninterpretable levels of 25 (OH) D and 1,25 (OH)<sub>2</sub>D in serum acutely following burn injury, the chief problem involving vitamin D, at least in burned children, is progressive vitamin D deficiency. Albumin, a binding protein for 1,25 (OH)<sub>2</sub>D, can return to normal levels in the serum as early as six months post-burn (18). When levels of vitamin D binding protein return to normal, has not been studied yet. However, both 25 (OH) D and 1,25 (OH)<sub>2</sub>D can bind to albumin in the absence of vitamin D binding protein. Thus, by 14-24 months post-burn, all serum levels of 25 (OH) D are low (19, 20) and all serum levels of 1,25 (OH)<sub>2</sub>D are normal (20). However, by 7 years post-burn not only are all values of 25 (OH) D low, but half

the patients have low serum levels of 1,25 (OH)<sub>2</sub>D as well (20).

Burn victims are not usually discharged with any vitamin D supplements and they normally do not spend much time exposed to sunlight, in part because there is concern that the burn scar will become hyperpigmented and in part because if the sweat glands are destroyed by the burn, the patients may develop heat intolerance. However, what was not apparent, but which has been reported (19), is that not only the burn scar but also the normal-appearing skin adjacent to the burn scar cannot make normal amounts of vitamin D<sub>3</sub> on exposure to ultraviolet B radiation. Not only is the conversion of vitamin D<sub>3</sub> precursor in the skin, 7-dehydrocholesterol (7 DHC), is only 25% of normal levels 14 months following a burn injury (19), but the quantity of the 7DHC substrate, not only in burn scar but in adjacent normal-appearing skin as well, is also significantly reduced in comparison to the normal (19). Therefore, even normal looking skin is biochemically abnormal following a burn injury, both in terms of its ability to synthesize vitamin D and, presumably, in its ability to synthesize cholesterol. The clinical implications of this progressive vitamin D deficiency in the burn patient are unclear at present. However, the vitamin D deficiency may be a reason for the continued failure of the bone density to recover to normal levels compared to age and sex-matched peers (1). In children, this failure to recover may lead to a reduction in their genetically-determined peak bone mass and therefore put them at greater risk for the development of osteoporosis in adult life. Therefore, whether or not the skin of burn patients is exposed to sunlight, it will not be able to synthesize normal quantities of vitamin D. Thus vitamin D supplementation of burn patients is mandatory. There has been very little work done to date on the vitamin D requirements of burn victims. However, a small cohort of children studied at the Shriners Burns Hospital in Galveston, Texas was discharged on a multivitamin supplement containing 400 international units (IU), or 10 micrograms, of vitamin D<sub>2</sub>. Six months later, with serum albumin levels normal, circulating levels of 25 (OH) D averaged 20 ng/mL (18). However, bone mineral density remained low in these children. The question, therefore, that must be raised is how much vitamin D supplementation is necessary in burned patients and for how long must the supplement remain at that high level? For life?

All of these studies have limitations. Long-term follow-up studies in children are always made more difficult by the dropout rate as the patients recover from the acute burn injury. Therefore, longer-term data are usually derived from a smaller number of children, even though statistical significance is maintained. Another limitation is that very little has been done to date to study the adult endocrine response to burn injury and the capabilities present to study long-term changes in adults are even more limited than those for the follow-up of children. Therefore, we are generalizing our conclusions based

on somewhat limited studies. It would be extremely valuable were these results to be confirmed, but larger and perhaps multi-center studies would be necessary in order to have greater confidence in how these severely ill patients are to be managed. In summary, severe burn injury results in an array of endocrine abnormalities ranging from transient growth hormone deficiency to acute secondary hypoparathyroidism resulting from up-regulation of the parathyroid calcium sensing receptor, to progressive and prolonged vitamin D deficiency. Future work must include ways to minimize sarcopenia and catabolism, prevent or treat the up-regulation of the parathyroid calcium sensing receptor, and to find the appropriate quantity of vitamin D supplementation to minimize chronic deficiency and, hopefully, shorten the course of hospitalization.

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