The Interaction Between Burn Injury and Vitamin D Metabolism and Consequences for the Patient

Gordon L. Klein*

Professor, Department of Pediatrics, University of Texas Medical Branch and Shriners Burns Hospital, Galveston, TX 77555-0352, USA

Abstract: The stress and inflammatory responses to burn injury are associated with bone loss. The stress response entails production of large amounts of endogenous glucocorticoids that decrease osteoblasts on the mineralization surface of bone and decreases differentiation of marrow stromal cells into osteoblasts, thereby decreasing the amount of bone formation. Deficiency of osteoblasts also blocks osteoclastogenesis thus leading to low bone turnover and bone loss. The inflammatory response generates cytokines such as interleukin 1-beta and interleukin-6, which normally increase osteoclastogenic bone resorption via stimulation of osteoblast production of RANK ligand. However, in the absence of osteoblasts as a target we postulate that they attack the parathyroid gland chief cells and up-regulate the calcium-sensing receptor. The consequence of this upregulation is the lowering of the circulating calcium necessary to suppress parathyroid hormone production and the development of hypocalcemia and urinary calcium wasting. It is the parathyroid hormone suppression that causes us to postulate acute deficiency of 1,25-dihydroxyvitamin D and the consequence of this for post-burn metabolism could include derepression of the gene that controls renin production, leading to elevated levels of angiotensin II, which can contribute to insulin resistance, as can vitamin D deficiency itself .Moreover, the skin from burned patients cannot synthesize vitamin D normally. Thus vitamin D supplementation is the only means by which to ensure vitamin D sufficiency for burn victims. The proper requirement for vitamin D in acutely burned patients remains unknown.

INTRODUCTION

In undertaking to write about the effect of burn injury on vitamin D metabolism and the consequences for post-burn pathophysiology, it is necessary to understand what happens to the body following a large burn. For sake of clarity we will consider burn injuries affecting at least 40% of total body surface area with the majority of the involved area being full-thickness burns. It is this type of injury that has been most extensively studied and which is now survived by an increasing number of victims. For this reason, long-term effects on rehabilitation and re-integration of burn victims into society have been identified as important goals for the burn patient and because of recent studies new information has become available. Moreover, understanding the effects of burns on the body can help shed light on the ways vitamin D metabolism and sufficiency are affected by the injury and this knowledge may be extrapolated to other conditions in which similar body responses are at play.

OVERVIEW OF PERTINENT PATHOPHYSIOLOGIC RESPONSES TO BURNS

Among the body's responses to a burn injury are the *stress response*, the *systemic inflammatory response,* and the *catabolic response* we will deal with each separately.

The *stress response* causes an immediate increase in endogenous glucocorticoid and catecholamine production. It is not certain how quickly it occurs but it is almost certainly present within the first twenty-four hours following injury. There is a rapid egress of intravascular fluid into the extravascular compartment with ensuing shock and death unless rapid and aggressive fluid resuscitation is undertaken. One of the most common means to provide fluids acutely to severely burned patients is to use Ringer's Lactate, a balanced electrolyte solution that contains no magnesium, thus producing magnesium depletion in virtually every patient admitted to the Shriners Burns Hospital in Galveston [1,2].

Urine catecholamine excretion is intermittently elevated over the first several weeks post-burn but these elevations are not sustained. In contrast, urine free cortisol is elevated up to 8-fold [3,4] over the first few weeks post-burn and gradually falls over the next six to nine months [5], reaching a level of approximately twice normal by one year post-burn.

These elevated levels of endogenous glucocorticoids have substantial effects on post-burn metabolism, most prominently negative nitrogen balance secondary to increased muscle catabolism leading to peripheral muscle wasting.

These effects have negative consequences for bone. The decreased muscle mass decreases skeletal loading with resultant decrease in signaling to the osteocytes, which are the pressure transducers of the bone and stimulate production of additional osteoclast-stimulated bone renewal. Moreover, the endogenous glucocorticoids themselves initially stimulate osteoblasts on the bone surface to produce a protein known as the *Ligand* for the *receptor activator* of the nuclear transcription factor *NFKB* (RANK Ligand, or RANKL). RANKL in turn stimulates the bone marrow precursor cells, or stromal cells, to differentiate into osteoclasts, leading to a transient increase in bone resorption and consequent bone loss. Reduced skeletal loading, such as immobilization or interoperative bed rest also produces an increase in osteoclastic

*Address correspondence to this author at the Children's Hospital Rm 3.270, University of Texas Medical Branch, 301 University Boulevard, Galveston TX 77555-0352, USA; Tel: (409) 772-1689; Fax: (409) 747-2213;
E-mail: gklein@utmb.edu

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bone resorption. This mechanism may be mediated by the sympathetic nervous system, according to recent studies in mice by Karsenty and others [6].

Osteoblasts and osteocytes, the mechanical force transducers in bone, are also adversely affected by the increased endogenous glucocorticoid production. By two weeks after the burn injury in children there are virtually no surface osteoblasts seen on iliac crest bone biopsy [3,7]. Study of glucocorticoid receptor messenger RNA (mRNA) in bone tissue by real time polymerase chain reaction (RTPCR) showed a trend toward reduction of the glucocorticoid receptor mRNA and a significant reduction of type I collagen mRNA in iliac crest bone biopsies of burned patients compared to controls [4]. Similar results were obtained when marrow stromal cells from the biopsies were grown in culture and assayed for the production of biomarkers of osteoblast differentiation. Thus, stromal cell production of type I collagen, alkaline phosphatase, core binding factor alpha 1 (cbfa 1), and bone morphogenetic protein (BMP)-2 were all lower in the marrow stromal cells of burned patients than in normal controls [4] suggesting that glucocorticoid production did reduce osteoblast differentiation as well. Also consistent with excessive glucocorticoid production is the retardation of growth velocity in children during the first year post-burn [8]. This timing coincides with the elevation of urine free cortisol excretion [5].

These findings with endogenous glucocorticoids are also seen in patients receiving exogenous steroids for inflammatory conditions. Therefore, it is likely that endogenous glucocorticoid production has the same effect on the body as do glucocorticoids administered as treatments for inflammatory conditions. Conditions of stress must be considered capable of producing similar effects on the body as pharmacologic treatments with exogenous steroids. It is not clear precisely what triggers the stress response in the patient but one candidate would be the *systemic inflammatory response* to be discussed below.

The *systemic inflammatory response* results from the destruction of the skin as a protective barrier to the entrance of microorganisms into the body. The marrow generates a significant inflammatory cell response, which is likely the explanation for the failure of the above-mentioned RTPCR studies of the glucocorticoid receptor in bone to demonstrate significant suppression [4]. Thus marrow inflammatory cells also possess the glucocorticoid receptor and it is not possible for RTPCR to sort out marrow from cancellous bone. The inflammatory cells generate large quantities of cytokines, at least two of which, interleukins (IL)- 1 β and IL-6 can stimulate the osteoblasts to produce RANKL to stimulate marrow osteoclastogenesis and increased resorption [9]. Therefore, in combination with the initial increase in corticosteroid-promoted RANKL production it is likely that there is a significant resorptive stimulus to bone within the first 24 hours following a burn to the advent of increased corticosteroid-induced osteoblast apoptosis which in children should be by two weeks post-burn. Sampling of markers of bone resorption have not been obtained during this time period; therefore, to this point in time, the increased resorption has not been documented except by the 2% loss of total body bone mineral content and the 8% loss of lumbar spine bone mineral content from hospital admission to discharge [10] for treatment of the burn. However, this period also includes corticosteroid-induced osteoblast deficiency thereby preventing precise attribution of bone loss to either mechanism but must be a product of both. By time a 24 hour urinary sample was collected for determination of deoxypyridinoline, fragments of type I collagen breakdown which serve as a biomarker for bone resorption [3], at approximately two weeks post-burn, these biomarkers were low, providing a picture of low-turnover bone loss, or, in current parlance, adynamic bone [3].

It should be pointed out that both glucocorticoid-induced osteoblast apoptosis and inflammation-induced bone resorption have played significant roles in bone loss in other conditions, such as in exogenous steroid-associated bone loss and in chronic inflammatory bowel disease or arthritis. Therefore, both of these mechanisms are becoming increasingly recognized as causes for secondary bone loss accompanying a variety of chronic conditions.

One reason that urinary calcium excretion cannot be used as a marker for increased bone resorption following burn injury is because of a relatively unique set of findings that may be applicable only to a limited set of conditions, burns being one of them, that result in urinary calcium wasting. We currently believe that the inflammatory cytokines up-regulate the parathyroid gland calcium-sensing receptor [11-13]) leading to a decrease in the amount of circulating calcium needed to suppress parathyroid hormone (PTH) secretion. This results in hypoparathyroidism and urinary calcium wasting [1].

Burn patients have been reported to have hypocalcemia, hypercalciuria and inappropriately low circulating PTH levels for the ionized calcium concentration [1]. Study of a sheep model of 40% full-thickness surface area burn reproduced the hypocalcemia and hypomagnesemia seen with burns in humans [11]. By 48 hours post-burn the sheep were sacrificed and the parathyroids evaluated by northern blot for calcium-sensing receptor mRNA and by immunoperoxidase staining for appearance of the intact receptor protein on the membrane of the parathyroid chief cells. In the burned sheep compared to the sham-burned control, densitometric analysis demonstrated a 50% up-regulation of the calcium-sensing receptor mRNA [11]. Thus the scenario in sheep and human was analogous and a cogent argument could be made that it is the up-regulation of the calcium-sensing receptor that produces the effects that are most consistent with clinical and experimental findings.

However, an important question that remains is what triggers the parathyroid calcium-sensing receptor up-regulation and is this a generalized phenomenon in all tissue affected by a large burn. Work done by Nielsen and colleagues in Boston working with bovine parathyroid cells [12], Toribio *et al.* at Ohio State [13] using equine parathyroid cells, and Canaff and Hendy in Montreal [14] working with rats all suggested that the pro-inflammatory cytokines, IL-1β. IL-6, and tumor necrosis factor (TNF) α trigger an up-regulation of the parathyroid calcium-sensing receptor *via* activation of NFκB or other mediators.

What is also clear is that after the osteoblasts have been reduced by the endogenous glucocorticoids following burn injury, serum levels of IL-1β, which are three-fold elevated and IL-6, which are one hundred-fold elevated [3] cannot alter the state of adynamic bone. Therefore, we postulate that as osteoblasts are no longer a target for cytokines, the parathyroid chief cells constitute a secondary target, resulting in a reduced set-point for inhibition of PTH secretion by circulating calcium. It is also known that there is not a generalized post-burn up-regulation of the calcium-sensing receptor because in the same sheep model, cardiac calcium sensing receptor is located in the enodcardium, epicardium, and vascular endothelium, as well as the aorta, and is not altered following burn injury [15]. At the time of this writing other tissues are also under study with regard to alterations in the calcium-sensing receptor following burns.

Other inflammation-related metabolic changes that affect the skeleton after burn injury include suppression of constitutive plasma proteins, especially those that are negative acute phase reactants, such as albumin [3]. Vitamin D Binding Protein is also low acutely [16].

A metabolic abnormality that has several implications for the pathophysiologic mechanisms post-burn is hyperglycemia and insulin resistance. Its development is likely multifactorial but among the contributing factors are catecholamine breakdown of fats [17] secondary to the stress response and decreased beta oxidation within muscle and reduced liver secretion of fats secondary to inflammation-associated loss of lipoproteins [17]. These processes lead to accumulation of ectopic triglyceride storage in muscle and liver as postulated by Cree and Wolfe [17]. These ectopic intracellular lipid stores would then contribute to abnormalities of insulin signaling [17].

Also of interest and pertinent to the insulin resistance is a possible role of bone especially the osteoblastic peptide osteocalcin in the regulation of energy metabolism. Studies by an international group of collaborators led by Karsenty [18] showed that mice lacking osteocalcin have decreased pancreatic β cell proliferation, glucose intolerance, and insulin resistance. Further, they found that osteocalcin can stimulate insulin expression by pancreatic β cells and expression of adiponectin, an insulin-sensitive adipokine, in adipocytes. Thus, in a setting such as burn injury, where osteoblast apoptosis is promoted by endogenous glucocorticoids, a regulator of insulin and glucose homeostasis, i.e. osteocalcin, is lost or reduced, possibly contributing to the post-burn insulin-resistant, hyperglycemic state.

Insulin resistance-hyperglycemia also has implications for the renin-angiotensin-aldosterone axis and indeed post-burn alterations are also seen here. The renin-angiotensin-aldosterone system has been associated with the development of insulin resistance [19] and is activated post-burn [20] although the mechanism involved in the activation is not certain. Recent work of Kasper in association with rats [20] demonstrated that following a burn of 30% body surface area, use of losartan, an angiotensin II receptor antagonist abolished post-burn insulin resistance in these animals.

To recapitulate, post-burn we see a metabolically interlinked set of reactions to the injury including a stress reaction involving significant output of endogenous glucocorticoids and catecholamines and a systemic inflammatory response involved in the production of large quantities of pro-inflammatory cytokines, especially interleukins- 1β and -6. These responses of the body to the burn trauma give rise to bone loss *via* postulated increased resorption and documented reduced bone formation, including apoptosis of surface osteoblasts and reduced differentiation of marrow stromal cells into osteoblasts. Reduction of osteoblasts and consequent reduced osteocalcin production along with ectopic intracellular triglyceride accumulation at least in part due to inflammation-associated reduction in lipoprotein biosynthesis, contribute to post-burn insulin resistance, as may also the idiopathic activation of the renin-angiotensin system. These changes are shown schematically in Fig. (**1**) below. Moreover, there is a postulated inflammation-associated up-regulation of the parathyroid gland calcium-sensing receptor leading to hypocalcemic hypoparathyroidism with associated urinary calcium wasting. Thus the stage is now set to examine the effects of burn injury on vitamin D metabolism and the possible consequences of alteration of vitamin D metabolism on the pathophysiology of the post-burn metabolic state.

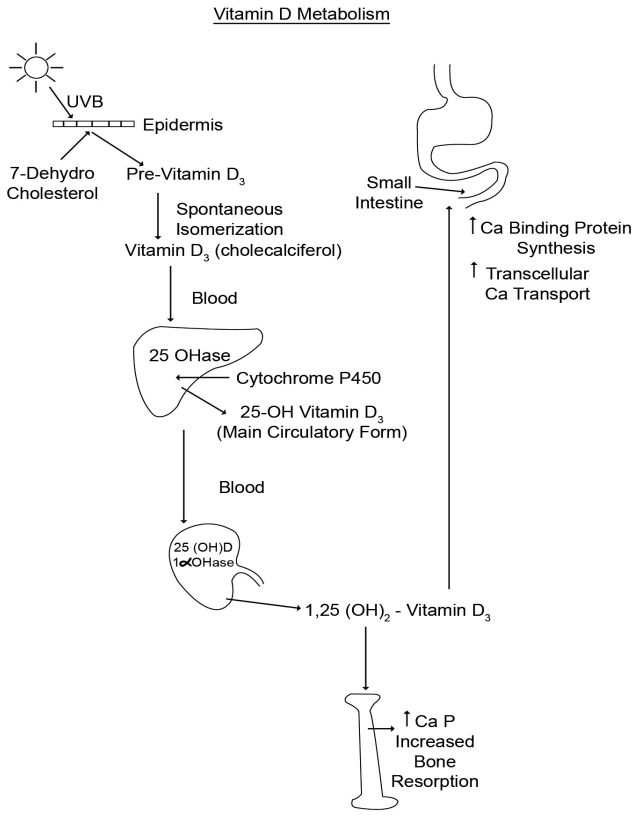

Fig. (1).

THE NATURAL HISTORY OF VITAMIN D METABOLISM POST-BURN

It is difficult to evaluate the immediate effects of burn injury on serum levels of vitamin D metabolites because the binding proteins for 25-hydroxyvitamin D (25(OH)D), the

main circulating form of the vitamin, and for 1,25-dihydroxy-vitamin D ($1,25(OH)_2D$) are low. Thus vitamin D Binding Protein [16] and albumin [3], two constitutive plasma proteins, are reduced, presumably as a result of the inflammatory response to the burn. Assays for the unbound 25(OH)D or $1,25(OH)_2D$ are no longer routinely performed.

By six months post-burn, serum albumin and total protein are normal [21] although the length of time it takes serum vitamin D Binding Protein to return to normal has not been studied.

While no serial studies have been done examining serum levels of 25(OH)D and $1,25(OH)_2D$, cross-sectional studies of burn patients at 14 months [22] and two and seven years post-burn [23] have shown that at 14 and 24 months post-burn the great majority of serum 25(OH)D levels measured were low while circulating concentrations of $1,25(OH)_2D$ were normal. In contrast, by seven years post-burn, not only were all the serum levels of 25(OH)D below normal range but half the circulating levels of $1,25(OH)_2D$ were low as well, suggesting a *progressive* deficiency. Why this deficiency could occur and its possible consequences will be explored next.

Burn victims do not spend a great deal of time outside because sweat glands are destroyed by the injury and the patients develop heat intolerance. Furthermore, there is concern amongst burn specialists that direct exposure to sunlight will cause hyperpigmentation of the burn wound. These two factors in conjunction with the failure of burn specialists to provide routine supplementation of vitamin D upon hospital discharge could contribute to the development of hypovitaminosis D. However, one additional observation also likely plays a role and that is the failure of sunlight exposure to produce normal levels of circulating vitamin D_3.

In children suffering a burn injury of $\geq$ 40% total body surface area, samples of burn scar and adjacent normal-appearing skin were assayed for the vitamin D_3 precursor 7-dehydrocholesterol, or 7-DHC, as well as for the percentage conversion of 7-DHC to vitamin D_3 following exposure to a standard dose of ultraviolet B radiation, The absolute amount of 7-DHC in the skin of the burn victims was significantly reduced compared to normal controls not only in the burn scar but also in the normal-appearing adjacent tissue [22]. Moreover, the percentage conversion of 7-DHC to vitamin D_3 was reduced to 25% of normal controls not only in the burn scar but also in the normal-appearing skin adjacent to the scar.

Thus the skin of recovered burn patients is biochemically abnormal, not just the burn scar but also the adjoining skin that appears to be normal. The failure of not only the burn scar but the normal adjacent skin to synthesize normal quantities of vitamin D_3 suggests that even a greater skin surface than that identified by the percentage surface area burn is incapable of synthesizing normal amounts of vitamin D_3 following ultraviolet B irradiation [22]. This finding helps us to understand why vitamin D supplementation is absolutely essential following burn injury. No studies have yet been done to establish the quantity of vitamin D necessary to achieve normal levels of serum 25(OH)D in this burn population.

Having provided evidence for progressive vitamin D deficiency following burn injury, we must place this in the context of resolving bone problems by one year post-burn, including the resumption of bone remodeling [7] and the resolution of hypocalcemia and hypoparathyroidism. Thus the progressive vitamin D deficiency post-burn may be responsible for failure of bone mass to return to a more normal range [24].

We stated earlier that it was not possible to evaluate adequacy of vitamin D status in the acute post-burn period due to reduction in serum levels of vitamin D Binding Protein and albumin. We furthermore know that pediatric patients at the Shriners Burns Hospital in Galveston received at least

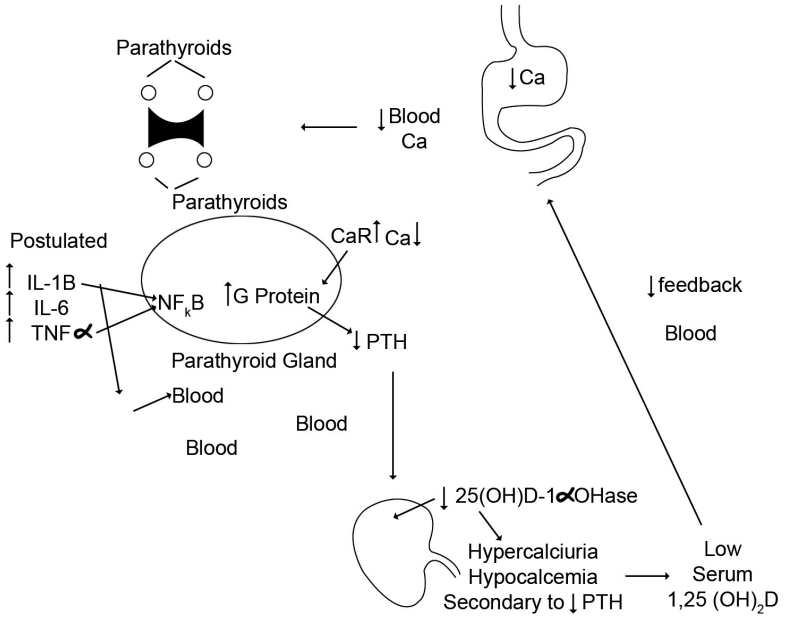

Fig. (2). Relation between parathyroid hormone (PTH) and $1,25(OH)_2$ – Vitamin D with up regulation of the parathyroid Ca sensing receptor.

Table 1. Selected Non-Calciotropic Actions of 1,25-Dihydroxyvitamin D_3

ANTI-INFLAMMATORY
COX-2 inhibition
IL-2 inhibition
CD4+ T-cell apoptosis
REPRESSION OF RENIN GENE
Decreases angiotensin II production
Improves hypertension
Reduces insulin resistance
STIMULATION OF MUSCLE CONTRACTILITY
Skeletal
Cardiac

400 IU, 10 μg, per day of vitamin D_3 (Klein 2008, unpublished data). While it could be argued that this regimen proves vitamin D sufficiency in the acute stages following burn injury, it could equally be argued that the vitamin D requirements of a burn-injured child are not presently known and therefore this quantity may be inadequate. Moreover, as postulated by Canaff and Hendy [14], a hypoparathyroid state would lead to decreased 25(OH)D-1 α hydroxylase activity in the kidney resulting in lower serum concentrations of $1,25(OH)_2D$ regardless of the confounding variable of reduced circulating albumin, the binding protein for $1,25(OH)_2D$. Thus it is likely that before six months post-burn, patients are deficient in at least the biologically active steroid hormone calcitriol ($1,25(OH)_2D$).

Operating under that assumption, then, suggests that vitamin D deficiency exists in the acute phase following burn injury. If we pursue this line of reasoning, let us see how some of the metabolic problems occurring post-burn may be explained by vitamin D deficiency. In particular, three areas will be examined: vitamin D and the calcium-sensing receptor, vitamin D and insulin resistance, and vitamin D and the renin-angiotensin-aldosterone axis.

With regard to the relationship between vitamin D and the calcium-sensing receptor we are aware of an up-regulation of the calcium sensing receptor resulting in reduced serum levels of PTH and a consequent reduction in calcitriol levels. Is there any evidence that vitamin D has an effect on the calcium-sensing receptor? Presently there are no reports that indicate that $1,25(OH)_2D$ affects calcium receptor expression in any tissue.

The relationship between vitamin D, the renin-angiotensin-aldosterone axis and insulin is not entirely clear but several lines of evidence indicate that $1,25(OH)_2D$ can suppress the renin gene while elevated levels of angiotensin II lead to insulin resistance. Thus deficiency of $1,25(OH)_2D$, which we postulate occurs acutely post-burn may fail to suppress the renin-angiotensin-aldosterone axis, thus contributing to the post-burn increase in angiotensin II and insulin resistance [25].

There is substantial epidemiologic evidence linking vitamin D deficiency states with increased insulin resistance, although the precise mechanism has not been elucidated. Thus Inomata *et al.* [26] showed that treatment of patients with type II diabetes with 1α hydroxyvitamin D improved insulin secretion and reduced the concentration of serum free fatty acids. Pereira and colleagues [27] reported that increasing serum 25(OH)D concentrations from 10 to 30 ng/ml improved insulin sensitivity by 60% in obese subjects, and that this improvement was greater than those reported with either metformin or troglitazone. Other studies [28, 29] have also shown an inverse correlation between vitamin D and insulin resistance in non-diabetic populations.

With regard to potential mechanism by which vitamin D could play a role in insulin resistance, several studies have indicated that vitamin D may affect insulin secretion by the pancreas. Reports from Norman's laboratory [30] and from DeLuca's Laboratory [31] in the 1980's indicate that in animals vitamin D deficiency inhibits pancreatic insulin secretion and that vitamin D supplementation increases insulin release and glucose clearance independent of calcium and phosphorus. However, the effect of vitamin D on insulin secretion is not related to insulin resistance.

Perhaps of greater relevance is the indirect effect of $1,25(OH)_2D$ on development of insulin resistance by inhibiting expression of the renin gene by an unelucidated mechanism. Deficiency of $1,25(OH)_2D$ in a condition such as acute burn injury leads to derepression of the renin gene and increased levels of angiotensin II [32]. Exactly how the elevated levels of angiotensin II lead to insulin resistance is not clear but Sowers [33] suggests that angiotensin II receptor type 1 (AT1) regulates insulin signaling in vascular tissue in skeletal muscle by means of protein kinase B activation by inositol tris phosphate (IP_3) kinase and inactivation of the insulin receptor substrate.

The proposed mechanisms by which angiotensin II produces insulin resistance must be interpreted with caution, however, because in a series of experiments reported by Klein, Mills, and Wilson in 1971 [34], direct infusion of angiotensin II into the renal arteries of dogs *in vivo* resulted in the rapid development of tachyphylaxis to the pressor effects of angiotensin II. Therefore, it is possible that at least the vascular effects of angiotensin II are not sustained. Consistent, however, with the postulated mechanism, Kasper *et al.* [20] reported pre-treatment of burned rats with theAT1 receptor blocker losartan improved insulin sensitivity as indicated by performance on a glucose tolerance test. Other studies by Sowers [35] and by Dahlof *et al.* [36] are consistent with this hypothesis. Thus insulin resistance may be due to several factors including ectopic deposition of lipids as postulated by Cree and Wolfe [18], the lack of osteocalcin-producing osteoblasts, and deficiency of $1,25(OH)_2D$ as postulated by Canaff and Hendy [14] following calcium receptor up-regulation in the parathyroid gland with subsequent hypoparathyroidism, thereby decreasing activation of the renal enzyme 25(OH)D 1 α hydroxylase, an enzyme that produces $1,25(OH)_2D$.

In light of the massive inflammatory response already discussed, brief mention should be made of whatever we know of the role of $1,25(OH)_2D$ in the inflammatory response. It is reported by Krishnan *et al.* [37] and Aparna *et al.* [38]

that calcitriol has anti-inflammatory activity by at least three actions: a) blocking the synthesis and action of pro-inflammatory prostaglandins by inhibiting expression of cyclo-oxygenase 2 (COX2), the enzyme critical for prostaglandin synthesis, b) inducing 15-prostaglandin dehydrogenase, the enzyme which inactivates prostaglandins, and c) decreasing expression of the receptors for prostaglandins E and F, which mediate prostaglandin signal transduction. Another report by Pedersen *et al.* [39] studying mice notes that in experimental autoimmune encephalomyelitis, a model of multiple sclerosis, $1,25(OH)_2D$ induces apoptosis of CD4+ T lymphocytes, thus removing the driving force for continuing inflammation in these animals. Still others [40] provide evidence that $1,25(OH)_2D$ suppresses IL-2. In a state of $1,25(OH)_2D$ depletion it is possible that either the calcitriol deficiency play a permissive role in the severity of the inflammatory response or calcitriol deficiency derepresses the inflammatory response in some way.

There remain other effects of $1,25(OH)_2D$ on various aspects of post-burn metabolism that have not been explored, such as cardiovascular function and muscle strength. These roles are pertinent especially in light of the systemic catabolism and peripheral muscle wasting which follow burn injury.

TREATMENT OF VITAMIN D DEFICIENCY

Mention must also be made of the recommended treatment or prevention of vitamin D deficiency accompanying burn injury. There are no recommendations at present because no adequate studies have been performed to date that demonstrate appropriate means of either prevention or treatment of vitamin D deficiency in this patient population.

As previously mentioned, patients at Shriners Burns Hospital in Galveston receive at least 400 IU (10 μg) per day and it is likely insufficient because all the abovementioned problems have been studied while the patients received this quantity of the vitamin. Note that 400 IU (10 μg) per day is currently *twice* the reference daily intake as recommended by the most recent publication of the Food and Nutrition Board of the Institute of Medicine [41]. Importantly, while there is debate about the daily vitamin D requirements in healthy individuals, the vitamin D requirement in illness or injury is completely unexplored and much more needs to be done to evaluate the vitamin D needs in chronic disease, acute illness, and trauma.

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REFERENCES

[1] Klein GL, Nicolai M, Langman CB, Cuneo BF, Sailer DE, Herndon DN. Dysregulation of calcium homeostasis following severe burn injury in children. Possible role of magnesium depletion. J Pediatr 1997; 131: 246-51.

[2] Klein GL, Langman CB, Herndon DN. Persistent hypoparathyroidism following magnesium repletion in burn-injured children. Pediatr Nephrol 2000; 14: 301-4.

[3] Klein GL, Herndon DN, Goodman WG, *et al.* Histomorphometric and biochemical characterization of bone following acute severe burns in children. Bone 1995; 17: 455-60.

[4] Klein GL, Bi LX, Sherrard DJ, *et al.* Evidence supporting a role of glucocorticoids in short-term bone loss in burned children. Osteoporos Int 2004;l 15: 468-74.

[5] Norbury WB, Herndon DN, Albright WB, Jeschke MG. Hypercortisolemia is associated with increased infection rates following severe burn injury. J Burn Care Res 2008; 29 (Suppl): S74, abstract 62.

[6] Kondo H, Nifuji A, Takeda S, *et al.* Unloading induces osteoblastic cell suppression and osteoclastic cell activation to lead to bone loss via sympathetic nervous system. J Biol Chem 2005; 280: 30192-200.

[7] Przkora R, Herndon DN, Sherrard DJ, Chinkes DL, Klein GL. Pamidronate preserves bone mass for at least 2 years following acute administration for pediatric burn injury. Bone 2007; 41: 297-302.

[8] Rutan RL, Herndon DN. Growth delay in post-burn patients. Arch Surg 1990; 125: 392-5.

[9] Ross FP. Osteoclast biology and bone resorption. In: Favus MJ Ed, Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism Sixth ed, American Society for Bone and Mineral Research, Washington DC, 2006; 30-5.

[10] Klein GL, Wimalawansa SJ, Kulkarni G, Sherrard DJ, Sanford AP, Herndon DN. The efficacy of acute administration of pamidronate on the conservation of bone mass following severe burn injury in children: a double-blind, randomized, controlled study. Osteoporos Int 2005; 16: 631-5.

[11] Murphey ED, Chattopadhyay N, Bai M, *et al.* Up-regulation of the parathyroid calcium-sensing receptor after burn-injury in sheep: A potential contributory factor to post-burn hypocalcemia. Crit Care Med 2000; 28: 3885-90.

[12] Nielsen PK, Rasmussen AK, Butters R, *et al.* Inhibition of PTH secretion by interleukin-1 beta in bovine parathyroid glands in vitro is associated with an up-regulation of the calcium sensing receptor mRNA. Biochem Biophys Res Commun 1997; 238: 880-5.

[13] Toribio RE, Kohn CW, Capen CC, Rosol TJ. Parathyroid hormone (PTH) secretion, PTH mRNA and calcium-sensing receptor mRNA expression in equine parathyroid cells, and effects of interleukin-(IL)-1 beta, IL-6, and tumor necrosis factor alpha on equine parathyroid cell function. J Mol Endocrinol 2003; 31: 609-20.

[14] Canaff L, Hendy GN. Calcium-sensing receptor gene transcription is up-regulated by the proinflammatory cytokine, interleukin-1β. J Biol Chem 2005; 280: 14177-88.

[15] Klein GL, Enkhbaatar P, Traber DL, *et al.* Cardiovasclar distribution of the calcium sensing receptor before and after burns. Burns 2008; 34: 370-5.

[16] Klein GL, Herndon DN, Rutan TC, *et al.* Bone disease in burn patients. J Bone Miner Res 1993; 8: 337-43.

[17] Cree MG, Wolfe RR. Post-burn trauma insulin resistance and fat metabolism. Am J Physiol Endocrinol Metab 2008; 294: E1-9.

[18] Lee NK, Sowa H, Hinoi E, *et al.* Endocrine regulation of energy metabolism by the skeleton. Cell 2007; 130: 456-69.

[19] Rammos G, Tseke P, Ziakka S. Vitamin D, the renin-angiotensin system, and insulin resistance. Int Urol Nephrol 2008 DOI 10.1007/s11255-007-9244-4.

[20] Kasper SO, Castle SM, Daley BJ, Enderson BL, Karlstad MD. Blockade of the renin-Angiotensin system improves insulin sensitivity in thermal injury. Shock 2006; 26: 485-8.

[21] Klein GL, Herndon DN, Chen TC, Holick MF. Standard multivitamin Supplementation does not improve vitamin D insufficiency after burns (abstract). Bone 2007; 40(Suppl 1): S56.

[22] Klein GL, Chen TC, Holick MF, *et al.* Synthesis of vitamin D in skin after burns. Lancet 2004; 363: 291-2.

[23] Klein GL, Langman CB, Herndon DN. Vitamin D depletion following burn injury in Children: a possible factor in post-burn osteopenia. J Trauma 2002; 52: 346-50.

[24] Klein GL, Herndon DN, Langman CB, *et al.* Long-term Reduction in bone mass after severe burn injury. J Pediatr 1995; 126: 252-6.

[25] Griffiths RW, Millar JG, Albano J, Shakespeare PG. Observations on the activity of The renin-angiotensin-aldosterone (RAA) system after low volume colloid Resuscitation for burn injury. Ann R Coll Surg Engl 1983; 65: 212-5.

[26] Inomata S, Kadowaki S, Yamatani T, Fukase M, Fujita T. Effect of 1 alpha (OH)-Vitamin D3 on insulin secretion in diabetes mellitus. Bone Miner 1986; 1: 187-92.

[27] Pereira MA, Jacobs DR Jr, Van Horn L, Slattery ML, Kartashov AI, Ludwig DS. Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA Study. JAMA 2002; 287: 2081-9.

[28] Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin Resistance and beta cell dysfunction. Am J Clin Nutr 2004; 79: 820-5.

[29] Ford ES, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin D and the Metabolic syndrome among U.S. adults. Diabetes Care 2005; 28: 1228-30.

[30] Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits Pancreatic secretion of insulin. Science 1980; 209: 823-5.

[31] Chertow BS, Sivitz WI, Baranetsky NG, Clark SA, Waite A, DeLuca HF. Cellular Mechanisms of insulin release: the effects of vitamin D deficiency and repletion on Rat insulin secretion. Endocrinology 1983; 113: 1511-18.

[32] Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-dihydroyxvitamin D(3) is a Negative endocrine regulator of the renin-angiotensin system. J Clin Invest 2002; 110: 229-38.

[33] Sowers JR. Insulin resistance and hypertension. Am J Physiol Heart Circ Physiol 2004; 286: H1597-1602.

[34] Klein GL, Mills IH, Wilson RJ. Changes in renal function associated with the Development of the renal vasculature to the arterial infusion of angiotensin. J Physiol (London) 1971; 215(1): 43P-44P.

[35] Sowers JR. Treatment of hypertension in patients with diabetes. Arch Intern Med 2004; 164: 1850-7.

[36] Dahlof B, Devereux RB, Kjeldsen SE, *et al.* Cardiovascular morbidity and Mortality in the Losartan intervention for endpoint reduction in hypertension study (LIFE): a randomised clinical trial against atenolol. Lancet 2002; 359: 995-1003.

[37] Krishnan AV, Moreno J, Nonn L, Swami S, Peehl DM, Feldman D. Calcitriol as a Chemopreventive and therapeutic agent in prostate cancer: role of anti-inflammatory Activity. J Bone Miner Res 2007; 22 (Suppl 2): V74-80.

[38] Aparna R, Subhashini J, Roy KR, *et al.* Selective inhibition of cyclooxygenase-2 (COX-2) by 1 alpha, 25-1C-ene-23-yne vitamin D_3, a less calcemic vitamin D analog J Cell Biochem 2008 [e-pub ahead of print].

[39] Pedersen LB, Nashold FE, Spach KM, Hayes CE. 1,25-dihydroxyvitamin D_3 reverses experimental autoimmune encephalomyelitis by inhibiting chemokine synthesis and monocyte trafficking. J Neurosci Res 2997; 85: 2480-90.

[40] Bemiss CJ, Mahon BD, Henry A, Weaver V, Cantorna MT. Interleukin-2 is one of Targets of 1,25-dihydroxyvitamin D_3 in the immune system. Arch Biochem Biophys 2002; 402: 249-54.

[41] Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride. Washington DC: National Academy Press, 1997; 1-448.

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