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Review Article

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Alterations in skeletal and mineral metabolism following thermal injuries

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Abstract

Severe burns and other chronic inflammatory diseases are associated with altered skeletal metabolism that result in an increased incidence of osteopenia. In thermally injured children and adults there is a dramatic decrease in bone formation accompanied with an increase or maintenance of bone resorption. Children also exhibit a growth delay and subsequently fail to reach a predicted stature. Animal models, including the thermal injury mouse model, are being used to understand the mechanisms behind the uncoupling of bone formation and resorption that occurs following a major burn. The model has numerous commonalities with the human condition such as reduced bone formation, increased bone resorption, and decreased endochondral growth. The mechanisms that modulate calcium and skeletal metabolism following a thermal injury are complex and likely involve a number of endocrine, cytokine, and immune factors. Specifically, the potential roles of glucocorticoids, growth hormone, insulin-like growth factor-1, parathyroid hormone, interleukin-1 and -6, and tumor necrosis factor alpha are addressed. Subsequent to the increased survival rate of burn victims, there has been a heightened focus on therapeutic interventions that prevent or decrease the impact of thermal injuries on the skeletal system. These include exercise programs, exogenous recombinant human growth hormone, insulin, and oxandrolone.

Keywords: Burn, Osteopenia, Inflammation, Histomorphometry

Introduction

The connection between systemic inflammation and bone loss has received increasing attention since osteopenia is associated with numerous chronic inflammatory conditions such as: rheumatoid arthritis[1], chronic obstructive pulmonary disease (COPD)[2-6] and traumatic injuries, such as a burn[7-10]. The pathophysiological mechanisms linking systemic inflammation and osteopenia are currently not clear. However, there appear to be some common phenotypical skeletal changes. Specifically, there is evidence both clinically and through the use of animal models that the inhibition of bone formation plays a role in association with maintained or increased levels of resorption[7,9,11,12]. Additionally, there is accumulating evidence for roles of the endocrine system, immune system and mechanical factors associated with skeletal under-loading in the skeletal changes that occur following burn injuries and other systemic inflammatory conditions. It is the goal of this review to examine the specific skeletal changes that are associated with thermal injuries, as well as discuss possible mechanisms.

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Skeletal and mineral metabolism in humans following thermal injury

In thermally injured children and adults, there are losses of bone mass, or failure to gain bone mass during growth that may predispose the patient to an increased incidence of fractures and potentially life-long osteoporosis[8,10]. The potential for long-term alterations to the musculoskeletal system in children following a thermal injury is demonstrated by the persistence of a hypermetabolic and muscle catabolic state for nine months following the injury[13] and reduced longitudinal growth for up to two years following the initial injury[8]. Additionally, sustained changes in calcium homeostasis have also been described that include hypocalcemia, hypoparathyroidism, and low vitamin D levels[14-17].

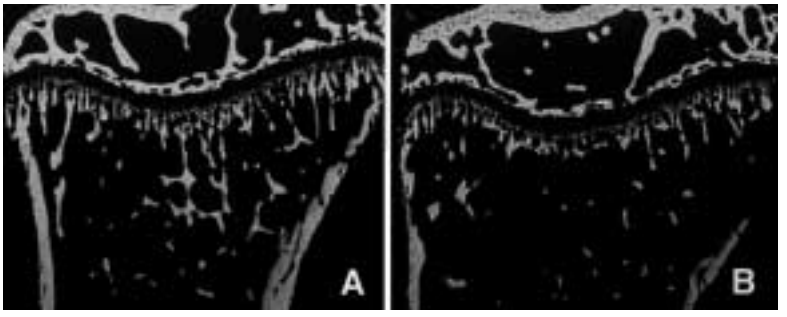

Figure 1. Backscattered electron image of frontal sections of the proximal tibial metaphysis 10 days post-burn from the sham (A) and burn (B) group. There is a reduced bone density in the metaphyseal region of the burn group compared with the sham group. Magnification 25X.

From these observations it has been generally concluded that following a burn injury, there are sustained disturbances in mineral and skeletal metabolism. These changes may have significant and sustained longer-term clinical consequences in both children and adults.

Clinically, it has been observed that individuals that suffer from inflammatory conditions exhibit an increased incidence of osteopenia. Individuals with rheumatoid arthritis, COPD, and thermal injuries all demonstrate a reduced BMD compared to age-matched controls[2,3,8]. Sixty percent of severely burned children and 31% of moderately burned children had a reduction in skeletal mass of greater than a z-score of -1[8]. Additionally, children with severe burns, greater than 40% total body surface area (TBSA), have an apparent increase in fractures after discharge, suggesting long-term skeletal effects, including a failure to attain peak bone mass[8]. The failure to attain peak bone mass may partially be the result of a reduction in longitudinal growth, since there is a significant reduction in growth velocity of children with greater than 40% TBSA[10]. Thus, growth delay and a failure to reach a predicted stature has been reported in children with severe burns[10].

The osteopenia present in patients with chronic inflammatory conditions such as thermal injuries are associated with a reduction in bone formation. For example, a study of adult patients with greater than 50% TBSA burn injuries showed that this population exhibited lower bone formation rates (BFR) than age-matched controls and volunteers subjected to 7 days of bed rest[9]. This suggests that the loss of bone post-burn is due to more than the skeletal under-loading that results from sustained bed rest. The reduction in bone formation can be dramatic. In one investigation, only one of 18 children with a greater than 40% TBSA took up fluorescent labels in their cancellous bone, as obtained from an iliac crest biopsy[7]. It appears that in patients with thermal injuries there is a reduction in BFR but not mineral apposition rate (MAR), suggesting that moderate to severe thermal injury decreases the number of osteoblasts, but not the activity of individual osteoblasts.

Skeletal and mineral metabolism in experimental models following thermal injury

Animal models of thermal injuries, including mice, rats, and sheep, are currently being utilized to better understand the mechanisms involved in the skeletal alterations observed post-burn. A commonly used model is the thermal injury mouse model, which has been utilized to examine systemic immunosuppression[18,19] and skeletal alterations[11,12] that occur following a burn injury. There appear to be numerous commonalities between the thermal injury mouse model and patients having suffered a burn. The animals receive about a 20% TBSA full thickness scald injury[11,12]. A full thickness burn is equivalent to a third degree burn, where most cutaneous nerve structures are destroyed. Suppressed longitudinal growth and bone formation accompanied with losses of skeletal mass have been observed through the use of the thermal injury mouse model in our laboratory[11,12], as well as clinically[7] (Figure 1). Recent preliminary data from our laboratory also observed a decreased combined weight for the gastrocnemius, soleus, and plantaris at 10 days post-burn, which supports research showing an increase in muscle protein degradation in the thermal injury mouse model post-burn[20].

Upon examination of fluorochrome labeling patterns at early times (4-7 days) after thermal injury, the indices of cancellous bone formation were substantially reduced, whereas those in the cortical bone were essentially nonexistent on both the periosteal and endosteal surfaces (Figure 2)[11,12]. The rapid and profound suppression of bone formation is accompanied by greatly increased bone resorption. The mouse thermal injury model exhibits increased osteoclast numbers in cancellous bone and an increase in the endocortical eroded surfaces (Figure 2)[11,12]. Since there is a dramatic decrease in bone formation within this experimental model, some of the observed resorption surfaces may have been inactive. Additionally, an "uncoupling" of bone remodeling (increased resorption with decreased formation) exists following a thermal injury. In other physiological osteopenic models (e.g., ovariectomy, lactation, antler formation), bone formation and resorption are normally "coupled", suggest-

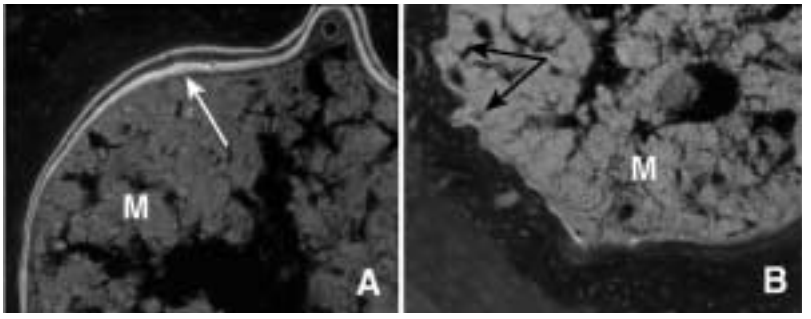

Figure 2. Fluorescent light microscope image of sham (A) and burn (B) groups. There is more fluorochrome double label (white arrow) in the sham mouse on the endocortical surface at the tibia-fibula junction compared to the burn mouse. There is also a large region of eroded surface (black arrows) in the burn mouse image. M: Marrow Magnification 10X.

ing different mechanisms.

We have recently completed a longer-term study and have determined that the restoration of bone formation rates (BFRs) coincide with the healing of the skin at the burn site. Figure 3 shows that relative BFRs at 10 days are greatly suppressed, but by 30 days the relative BFRs have rebounded and exceed that of the sham group. This does not tell the complete story, since there is still a suppression of endochondral growth and a persistent osteopenia. The persistent osteopenia is explained by the fact that entire areas of cancellous bone are completely lost and there is no physiological process whereby these can grow back.

We have also recently obtained biomechanical data and have found that the osteopenia observed in a thermal injury mouse at 10 days also results in alterations in the mechanical properties of the femur. One of the largest differences was in energy absorption, with the thermally injured mouse femur absorbing 20% less energy at fracture compared to the sham burned mouse ($p<0.01$) (Figure 4). There was also a trend for an inverse linear relationship between breaking load and burn size ($p = 0.08$, $r = 0.57$), suggesting the severity of the injury could influence the degree of skeletal alterations, which is supported by data in severely burned children[8].

The rat has also been used to examine the muscular and skeletal changes that occur following a thermal injury[21-23]. The rats are typically subjected to a 30% TBSA full-thickness flame or scald burn injury. Skeletally, there was a decrease in trabecular number and area, a decrease in longitudinal growth, and a limited amount or absence of fluorochrome label on the endocortical surface[21]. Muscularly, there is an increase in muscle protein breakdown, with a greater degree of degradation of fast twitch muscle, extensor digitorum longus, compared to slow twitch muscle, soleus[22]. Contrary to the mouse and rat model, the sheep model exhibited no reduction in bone formation 2 weeks post-burn, 40% full thickness flame burn injury, but the sheep model exhibits hypocalcemia[17,24]. Table 1 summarizes the commonalities and differences between the animal models of thermal injury and a human with a thermal injury.

The mechanisms that modulate calcium and skeletal metabolism following thermal injury are undoubtedly complex and likely involve a number of endocrine, cytokine, and immune factors. The rest of this review will focus on the possible mediators as well as current therapeutic interventions. However, the precise contribution of these and other factors to the observed skeletal alterations have yet to be fully elucidated.

Endocrine mediators

There are numerous possible endocrine mediators in osseous tissue changes following injury, as well as disruptions in normal diurnal and circadian rhythms of all hormones. Prolonged high circulating levels of glucocorticoids are observed in burn injuries[7,25]. The specific effects of higher glucocorticoid levels on skeletal tissue include suppression of bone formation and growth, but increased or sustained bone resorption[26]. Glucocorticoids are also known to suppress the absorption of calcium and overall can cause the organism to enter a negative calcium balance. Research by several investigators suggests a major role of glucocorticoids in the increased number of apoptotic cells in the thymus and spleen[27-29], suggesting glucocorticoids may be involved in the systemic immune suppression following a thermal injury.

Growth hormone, a potent anabolic agent, is decreased in burn patients[30]. Growth hormone is important in the regulation of longitudinal growth, and patients deficient in growth hormone are characterized by short stature. Increased height and bone mineral density are attained in individuals with growth hormone deficiency following injections with growth hormone[31]. The importance of growth hormone in the altered skeletal system with burn patients is supported by the fact that long-term growth hormone therapy increases bone mineral content[30].

Glucocorticoids along with growth hormone also play an important role in the regulation of insulin-like growth factor (IGF-1)[32,33]. A decrease in IGF-1 has been demonstrated to occur early after thermal injury and to remain reduced[34,35]. IGF-1 is implicated in playing a role in decreased bone formation parameters, as well as the pronounced catabolic response in skeletal muscle[23,36,37]. While systemic administra-

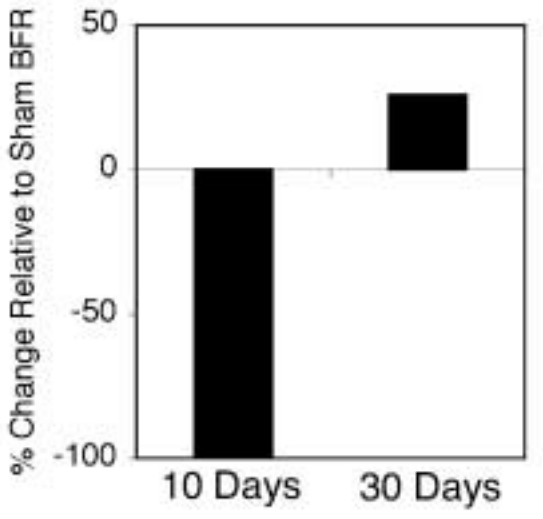

Figure 3. Bar graph comparing the relative bone formation rates (BFRs) on the endocortical surface of the tibia-fibula junction at 10 and 30 days post-burn.

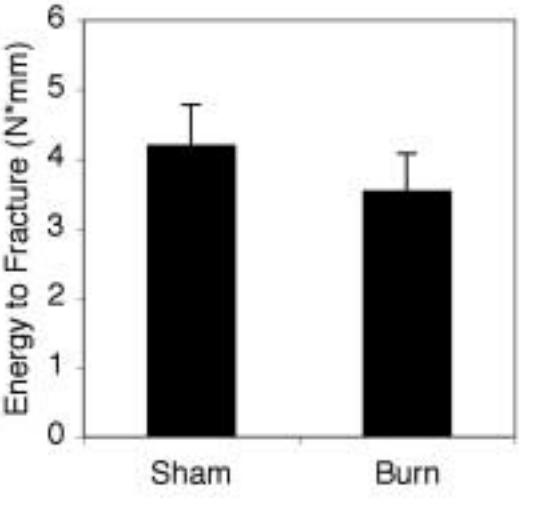

Figure 4. Bar graph comparing the energy to fracture from a three-point bending test of the femur in the sham and burn group at 10 days post-burn. The burn group absorbed less energy prior to fracturing than the sham group ($p < 0.05$).

tion of recombinant human growth hormone increases the circulating levels of IGF-1 in severely burned children there was no observed change in serum markers of bone formation[33]. It was hypothesized that the lack of skeletal response may be attributed to the roles of IGF-binding proteins, such as IGFBP-4 which may influence the bioavailability of IGF-1. Systemic administration of IGFBP-4 has been shown to increase bone formation parameters in mice by increasing IGF bioavailability in the circulation[36,37].

Measurement	Mouse	Rat	Sheep	Human
Bone Mass	↓[11,12]	N.D.*	N.D.	↓[8]
Bone Formation	↓[11,12]	↓[21]	N.C.+	↓[7,9]
Bone Resorption	↑[11,12]	N.D.	N.D.	↑/N.C. [7,9]
Bone Strength	↓**	N.D.	N.D.	↓++ [10]
Longitudinal Growth	↓[11,12]	↓[21]	N.D.	↓[8,10]
Muscle Mass	↓**	↓[22]	N.D.	↓[13]
Calcemia	N.D.	N.D.	↓[17]	↓[9,15]
Calcuria	N.D.	N.D.	N.D.	↑[9,15]
Parathyroid Hormone	N.D.	N.D.	N.D.	↓[14,15]
Vitamin D	N.D.	N.D.	N.D.	↓[16]
Glucocorticoid	↑[28,29]	↑[27]	↑[24]	↑[7,25,35]
Growth Hormone	N.D.	N.D.	N.D.	↓[30,35]
Insulin-like Growth Factor	N.D.	↓[32]	N.D.	↓[34]
Androgens	N.D.	N.D.	N.D.	↓[38,39]
Immunosuppression	↑[18,19]	↑[67]	N.D.	↑[70]
Interleukin-6	↑[68]	↑[67]	N.D.	↑[7]
Interleukin-1	N.D.	↑[67]	N.D.	↑[7,48]
Tumor Necrosis Factor Alpha	N.C.[68]	↑[67]	N.D.	↑[47,48]

*N.D.: No data
+N.C.: No change
**Unpublished data
++Increased incidence of fracturing

Table 1. Comparison of some of the alterations to the musculoskeletal, endocrine, and immune system following a thermal injury in the mouse, rat, sheep, and human.

Longer-term androgen deficiency has also been observed in burn injuries[38,39]. The low androgen levels likely play a role in the muscle catabolism, bone loss, and altered skeletal growth that occurs post-burn. There is also research suggesting that androgen deficiency may be a mitigator in the immunosuppression and increased cytokine levels in burn patients[40-42].

Severe burns are associated with dramatic changes in mineral homeostasis that include: hypocalcemia, hypercalciuria, hypoparathyroidism, and low vitamin D levels[14-17]. All of these symptoms are signs that there are major disruptions in calcium regulation. The parathyroid calcium sensing receptor (CaR) is an important mediator of the inhibitory actions of calcium levels on parathyroid hormone (PTH) secretion[43]. A major role of PTH is to increase calcium levels by increasing the release of calcium from bone, by increasing kidney absorption of calcium and by enhancing 1,25-dihydroxyvitamin D production in the kidneys. Previous research utilizing a sheep model observed that there is an upregulation of the CaR at 2 days following a thermal injury, along with a decrease in blood calcium levels at 4 hours[17]. The investigation did not determine if there were any concomitant changes to PTH levels or skeletal metabolism. Yet, research suggests that PTH plays an important role in bone formation and resorption[44-46].

There may also be a connection between PTH and local cytokine levels in bone. The removal of the parathyroid gland in patients with end stage renal failure, secondary hyperparathyroidism, results not only in a decrease in PTH and bone formation but also a decrease in the levels of TNF-α, IL-1β, and TGF-β within bone[46]. These results suggest the skeletal effects of varying PTH levels are partially mediated by local production of cytokines and growth factors, as well as the interconnected nature of the endocrine and immune systems.

Immune system cytokines

Cytokines mediate and regulate immune and inflammatory reactions, both acute and chronic. The skeletal changes that occur in conjunction with systemic inflammation may be influenced or caused by alterations in the production of cytokines. Elevated serum levels of interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and gamma interferon (γ-IFN) have all been measured in burn patients[7,47,48]. IL-1 and TNF-α have been shown to suppress bone formation[49] and IL-1, IL-6, and TNF-α stimulate bone resorption *in vivo* and *in vitro*[49-51]. Thermal injuries result in a decrease in bone formation and an increase in bone resorption, which is different from other osteopenic conditions, like ovariectomy and the reproductive cycle, which typically result in an increase in both bone formation and resorption. The inflammatory cytokines IL-1 and TNF-α, as mentioned above, suppress bone formation and stimulate bone resorption and are likely mediators of some of the observed skeletal changes.

There is also evidence for interactions between inflammatory cytokines and calcium regulation. Systemic administration of IL-1β results in a decrease in circulating levels of PTH[52]. *In vitro* work has also shown that IL-1β results in an upregulation of the CaR, as well as a decrease in PTH release[53]. Therefore, IL-1 may play a role in the hypocalcemia and hypoparathyroidism in burn patients by upregulating the CaR.

Therapeutic strategies to mitigate skeletal damage following thermal injuries

New therapeutic strategies have increased the survival rates of burned patients, therefore, it has become increasingly important to focus on the long-term care of burn survivors. Numerous pharmacological, as well as nutritional and exercise programs have been implemented to decrease the negative musculoskeletal alterations and expediate the return to pre-burn levels.

Severe burn injury results in persistent and extensive skeletal alterations including muscle and bone catabolism, which can be confounded by prolonged physical inactivity. Low endurance capacity and muscle strength are major obstacles in allowing a burn victim to return to school/work and to perform activities of daily living. Therefore, current research is focusing on the benefits of supervised resistance and aerobic exercise training programs. Children in one investigation were either enrolled in a supervised training program or a traditional home-based program[54]. The patients that participated in the supervised program showed marked increases in muscle strength, total work, and average power versus the home-based program. These functional changes were also accompanied with a greater increase in lean body mass, as measured by dual energy X-ray absorptiometry (DEXA). There were no data reported on BMC or BMD. There were no differences in the height of the children between the two groups, suggesting the supervised program was not able to prevent the decrease in linear growth that occurs following a thermal injury over a three-month period. A longer training duration may have gains in other areas besides muscle strength. Another benefit of the supervised exercise program was a reduction in the number of required surgeries to release burn scar contracture[55]. Despite these improvements in lean body mass and strength, the burned children had lower absolute peak torque values compared to age-, height-, and weight- matched non-burned controls, suggesting the program was not able to abate all of the muscle catabolism that occurs in these children.

Growth hormone is a strong anabolic hormone that has been shown to increase muscle mass and bone mass, as well as other systemic effects. Exogenous recombinant human growth hormone (rHGH) is currently being utilized post-burn to promote anabolism. There have been conflicting reports of the ability of recombinant human growth hormone therapy to attenuate the osteopenia and muscle catabolism present following a thermal injury. Short-term intervention, up to two months, has resulted in little effect of

growth hormone administration on spine BMD, biochemical markers of bone formation, or muscle protein synthesis and breakdown[13,33]. However, long-term treatments, up to one year, have resulted in increased lean body mass, increased BMC, and increased height[30].

There have also been investigations conducted to determine if there is a synergistic effect when rHGH is combined with other anabolic therapies. One such program sought to determine the combined effect of a supervised exercise program in conjunction with rHGH[56]. After a 12-week intervention there was a similar gain in lean body mass with exercise, exercise + rHGH, and rHGH. However, those children enrolled in the exercise program also exhibited an increase in muscle strength that was not observed in the rHGH group.

Other anabolic agents besides rHGH have been used to promote catabolism after a burn, such as insulin. Continuous insulin profusion for seven days at a dosage that maintains maximal hyperinsulinemia increased protein synthesis in burned adults[57]. However, in order to maintain normal blood glucose levels, euglycemia, it was necessary to give patients approximately 5,000 extra calories per day. A subsequent study utilized submaximal dosages of insulin, thus reducing the amount of glucose required to maintain euglycemia and reducing the risk of hypoglycemia[58]. Again, as seen in the maximal insulin profusion investigation, there was an observed increase in muscle protein synthesis. The potential benefits of insulin profusion were confirmed in another investigation that provided insulin throughout the course of the hospital stay in children with severe burns[59]. The children were given insulin for approximately 45 days and exhibited an increase in healing rate, an increase in muscle mass, and an increase in bone mass compared to non-treated controls. There were no consistent concomitant changes in GH, IGF-1, IGF-binding protein 3, or PTH[59]. However, no measures of inflammatory cytokines were made, and previous research in thermally injured rats suggests insulin attenuates the inflammatory response by decreasing inflammatory cytokines including TNF-α, IL-1, and IL-6[60]. As noted previously, inflammatory cytokines have been shown to increase bone resorption and decrease bone formation.

Another anabolic agent that has been used to abate the muscle wasting following a burn is oxandralone, an oral synthetic testosterone[61-64]. Initial studies in thermally injured adults and children observed increases in weight gain, as well as a decrease in length of stay in the hospital[63,65]. It appears that oxandrolone increases protein synthesis while protein breakdown is unchanged[64]. There have not been any studies examining the influence of oxandrolone treatment on bone mass in children or adults.

Conclusion

Thermal injuries and other systemic inflammatory conditions are associated with osteopenia. The low BMD of adults and children with burns are likely the result of an increase in bone resorption accompanied with a decrease in bone formation. The uncoupling of bone remodeling is supported by data using the thermal injury mouse model, which also exhibits an increase in resorption and a decrease in bone formation. Additionally, thermal injuries are associated with an increased incidence of fractures in children and altered femoral mechanical properties in the mouse model. There are numerous potential mediators of the bone loss including: corticosteroids, PTH, growth hormone, IGF-1, and immune cytokines. It is likely that a combination of these mediators influence the catabolic state in burn victims. Current treatments including exercise programs, rHGH, insulin, and oxandrolone have focused more on abating the loss of lean body mass than on determining if they are effective treatments for preventing bone loss, but preliminary analysis suggests the potential benefit of these interventions.

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