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UPDATES IN SPINE SURGERY - TECHNIQUES, BIOLOGICS, AND NON-OPERATIVE MANAGEMENT (B FEELEY, SECTION EDITOR)

Sex-Based Difference in Bone Healing: A Review of Recent Pre-clinical Literature

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Abstract

Purpose of Review Recent literature has sought to understand differences in fusion failure, specifically considering how patient sex may play a role. Overall, there exists inconclusive data regarding any sex-based differences in bone healing.

Recent Findings In vitro studies examining the roles of sex hormones, 5-LO, IGF-1, VEGF, osteoclasts, and OPCs seem to show sexually dimorphic actions. Additionally, donor characteristics and stem cell environment seem to also determine osteogenic potential. Building on this biomolecular basis, in vivo work investigates the aforementioned elements. Broadly, males tend to have a more robust healing compared to females. Taking these findings together, differences in sex hormones levels, their timing and action, and composition of the inflammatory milieu underlie variations in bone healing by sex.

Summary Clinically, a robust understanding of bone healing mechanics can inform care of the transgender patient. Transgender patients undergoing hormone therapy present a clinically nuanced scenario for which limited long-term data exist. Such advances would help inform treatment for sports-related injury due to hormonal changes in biomechanics and treatment of transgender youth. While recent advances provide more clarity, conclusive answers remain elusive.

Keywords Bone healing · Sexual dimorphism · Animal model

Introduction

Sexual dimorphism in biological mechanisms has been well established in the literature. Recent investigations into cellular mechanisms of immune regulation have shown a sexually dimorphic response specifically among mainstays of the innate immune system: toll-like receptors (TLRs) [1]. Studies have shown that expression of TLRs 4, 7, and 8 shows dimorphic responses with higher levels in females compared to males [2–4]. Additionally, inflammatory cytokine regulation by sex hormones may contribute to a dimorphic immune response. Testosterone upregulates IL-10 while downregulating IFN-γ. In contrast, estrogen increases IFN-γ, TNFα, and IL-12 production while decreasing production of IL-10 [5, 6]. Such differences have clinical ramifications: females have higher rates of autoimmune diseases such as Graves' disease, systemic lupus erythematosus, and rheumatoid arthritis [7]. This also connotes a biological advantage in females via lower rates of infection by bacteria, fungi, and parasites [8].

Sexual Dimorphism Within Bone Healing

Spinal fusion has been utilized for treatment of a variety of disorders including congenital deformity, trauma, spondylolisthesis, and degenerative disease [9]. Its use has increased in the USA, driven by advances in fixation devices, bone grafting materials, and a growing elderly population. While spinal fusion demonstrates benefit for some patients, studies show that this benefit may be obscured by long-term outcomes [10, 11]. Recent literature has sought to understand differences in fusion failure, specifically considering how patient sex may play a role. Overall, there exists inconclusive

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data regarding any sex-based differences in fusion rates. Ekman et al. found that female patients tend to have significantly worse outcomes in terms of pain and functional ability after posterolateral lumbar fusion (with and without instrumentation) and posterior lumbar interbody fusion [12]. Other studies corroborate this finding, stating that male patients have significantly better outcomes following anterior cervical decompression and fusion as well as in posterolateral lumbar fusion [13, 14]. Conversely, Schmitt et al. found that female patients had a significantly higher fusion rate following thoracic to sacral fusion to correct adult spinal deformity [15].

Bone healing following a fracture is dependent on the biomechanical properties of the site: conventionally, this is regarded as primary (direct) or secondary (indirect) bone healing [16, 17]. Primary healing occurs with fracture stabilization or low movement environments, whereas secondary healing occurs in high movement settings and is characterized by callus formation (Fig. 1). Multiple orthopedic studies have considered the role of sex on bone healing. Chang et al. studied outcomes following vascularized bone graft for scaphoid nonunion, concluding that female sex was a significant univariate predictor for graft failure [18]. Further literature seems to contradict these findings: a retrospective analysis of proximal interphalangeal joint arthrodesis and a large meta-analysis analyzing tibial non-unions found that male sex is a significant predictor of fracture nonunion [19–21]. Inconclusive data on how sex may play a role in bone healing clinically obfuscates clinician patient selection as well as optimal outcomes following treatment.

In Vitro data

Estrogen and Androgens

There exists significant complexity in the numerous biomolecular factors in bone healing. The role of estrogen in bone healing seems clearer in females. In males, however, estrogenic effects on bone healing have only been recently investigated [22]. In both males and females, estrogen acts via two receptors, estrogen receptor-alpha and estrogen receptor-beta [23]. While downstream targets remain opaque, estrogen causes release of osteoprotegerin (OPG) which binds to RANK-L, thereby inhibiting osteoclast maturation [24].

The role of estrogen in the growing skeleton is crucial in both males and females allowing for bone growth and maturation; dysfunction during childhood results in decreased bone mass [25]. Initially, it was thought that estrogen and androgens modulate bone mass in females and males, respectively. In vivo studies helped reinforce this paradigm; however, recent studies seem to reduce the emphasis of androgens on the male skeleton [26, 27]. Studies reporting on males with estrogen deficiency (via receptor mutation or aromatase deficiency) resulted in osteopenia and no epiphyseal closure [26, 27]. While estrogen is important in male bone formation and regulation, evidence also exists for the function of androgens in the male skeleton [28]. A study by Callewaert et al. shows androgen receptor prominence in males. Disruption of the androgen receptor decreased trabecular bone mass, but estrogen-α receptor disruption had no additional effect on the AR-dependent trabecular bone loss. Conversely, inactivation of both androgen and estrogen-α receptors reduced cortical bone and muscle mass compared with inactivation of either receptor alone (Fig. 2) [29]. Furthermore, Venken et al. shows that testosterone rescues orchidectomy-induced bone loss, confirming the importance of androgen receptor signaling in male skeleton regulation [30].

Despite the emphasis of estrogen on both male and female bone regulation and formation, androgens also play a role in the sexual dimorphism of bone growth and development. Recent studies point to the manipulation of growth hormone (GH) and IGF-1 levels by way of sex hormones. While estrogen has long been known to modulate linear bone growth, testosterone more recently demonstrated regulation potential [31, 32]. A study examining the relationship between testosterone and bone regulation with a hypogonadal mouse model concluded that the perinatal androgen surge in males primes the IGF-1/GH axis either via aromatization or by direct action of testosterone [32]. Perinatal imprinting effects seem to predict adult bone growth [33].

Dehydroepiandrosterone (DHEA)

Dehydroepiandrosterone (DHEA) is an adrenal-produced precursor sex hormone found in both males and females [34]. Declining serum levels of DHEA into the sixth decade of life, coinciding with bone mineral density (BMD) loss, has led some to hypothesize that supplementation of DHEA would improve bone health [35]. Interestingly, DHEA may share cellular machinery with IGF-1 signaling pathways. A meta-analysis examined randomized controlled trials testing supplementation of DHEA found that BMD increased at two locations in women but found no BMD effects in men [36]. In addition to these clinical findings, an in vivo study has further elucidated the role of DHEA by examining its role on osteoblasts. Wang et al. found that DHEA caused both proliferation of osteoblasts and inhibited their apoptosis through a mitogen-activated protein kinase signaling pathway in a post-menopausal model with ovariectomized mice, questioning the prior theory that DHEA exerts its effects on bone by way of conversion to sex hormones [37].

5-Lipoxygenase (5-LO)

5-LO catalyzes the formation of leukotrienes from arachidonic acid. Leukotrienes are inflammatory mediators released most commonly by activated mast cells [38]. In context of an acute

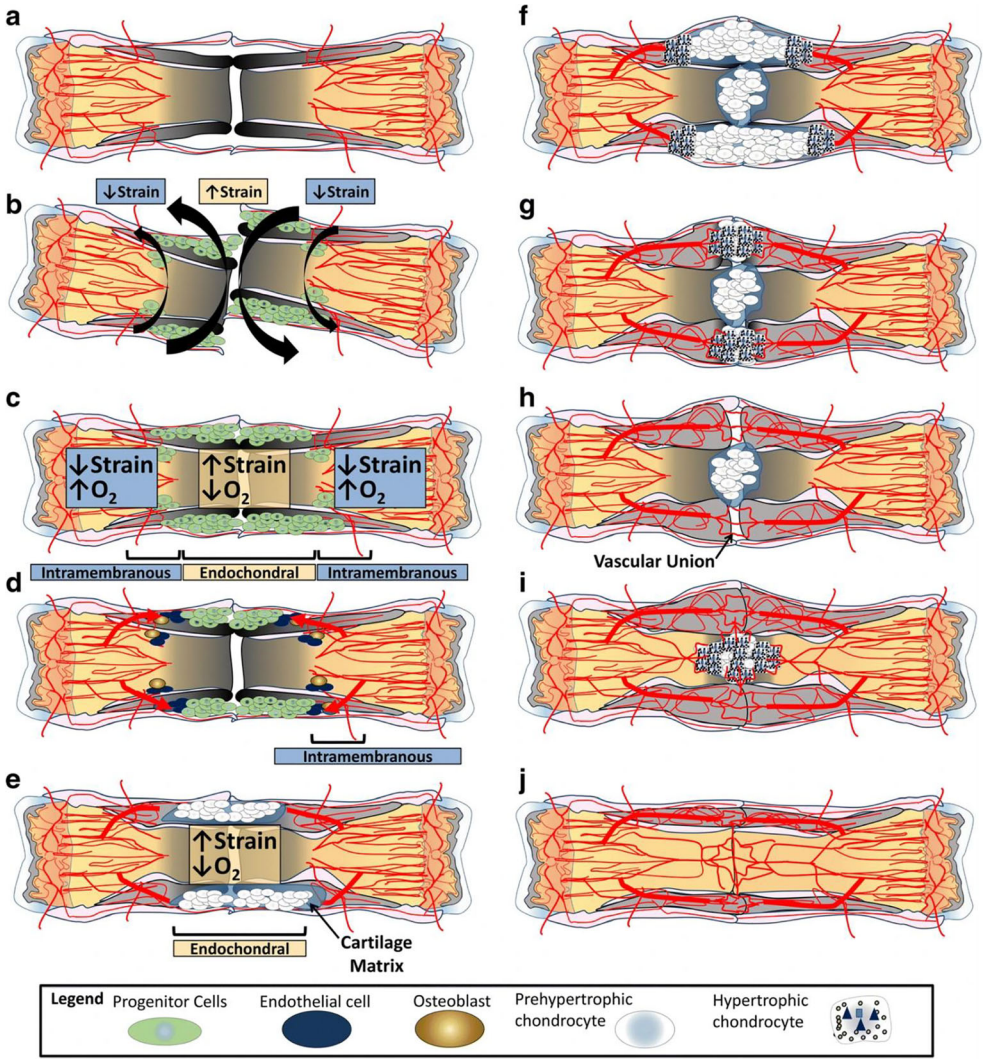

Fig. 1 Secondary bone healing. In the majority of fractures, the structural integrity of the bone and the vascular supply to the fracture site are disrupted, leading to (**a**) hypoxia and interfragmentary motion. Under these conditions, (**b**) progenitor cells are drawn to the fracture site and, depending on the conditions of strain and oxygen tension, either (**c**) intramembranous or endochondral ossification will ensue. (**d**) At the periphery of the fracture (relatively preserved oxygen supply and low strain), progenitor cells in close association with the bone's intact blood supply differentiate into osteoblasts and begin the process of intramembranous ossification. Within the center of the fracture site (high strain and low oxygen tension) (**e**), the progenitor cells develop into pre-hypertrophic chondrocytes, proliferate in response to strain, and resolve strain by forming a biomechanical extracellular matrix. When strain is sufficiently resolved, (**f**) these chondrocytes undergo hypertrophy and become hypertrophic chondrocytes that direct angiogenesis and osteogenesis. (**g**) Hypertrophic chondrocytes promote vascular invasion and osteogenesis by releasing BMP, VEGF, and hydroxyapatite. (**h**) Vascular union always precedes bony union at the fracture site, as the endothelial cells are necessary for ossification. (**i**) With bony union of the fracture callus, the fracture is stabilized, and the remaining chondrocytes become hypertrophic. (**j**) The fracture is now healed and remodeling proceeds. Figure attribution: Bone Fracture Acute Phase Response—A Unifying Theory of Fracture Repair: Clinical and Scientific Implications. Author: Courtney E. Baker et al. Publication: Clinical Reviews in Bone and Mineral Metabolism. Publisher: Springer Nature. Date: Dec 29, 2018. Copyright © 2018, The Author(s). Creative Commons. This is an open access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. You are not required to obtain permission to reuse this article. To request permission for a type of use not listed, please contact Springer Nature

fracture, 5-LO is among many inflammatory mediators released. In vitro and in vivo studies have shown that inhibition or reduction of local 5-LO leads to increased bone formation [39•]. Notably, a recent study has that 5-LO inhibitors were more efficacious in females (both in vivo and in vitro) as androgens in males play a role in prohibiting assembly of genetic machinery downstream of 5-LO [40].

Growth Factors

IGF-1 has long been known to have a key role in bone growth. IGF-1 is produced mainly by stimulation of growth hormone (GH). GH is released from the anterior pituitary and acts on the liver to produce IGF-1 [41]. In the developing skeleton, IGF-1 plays a dominant role in both bone mass and

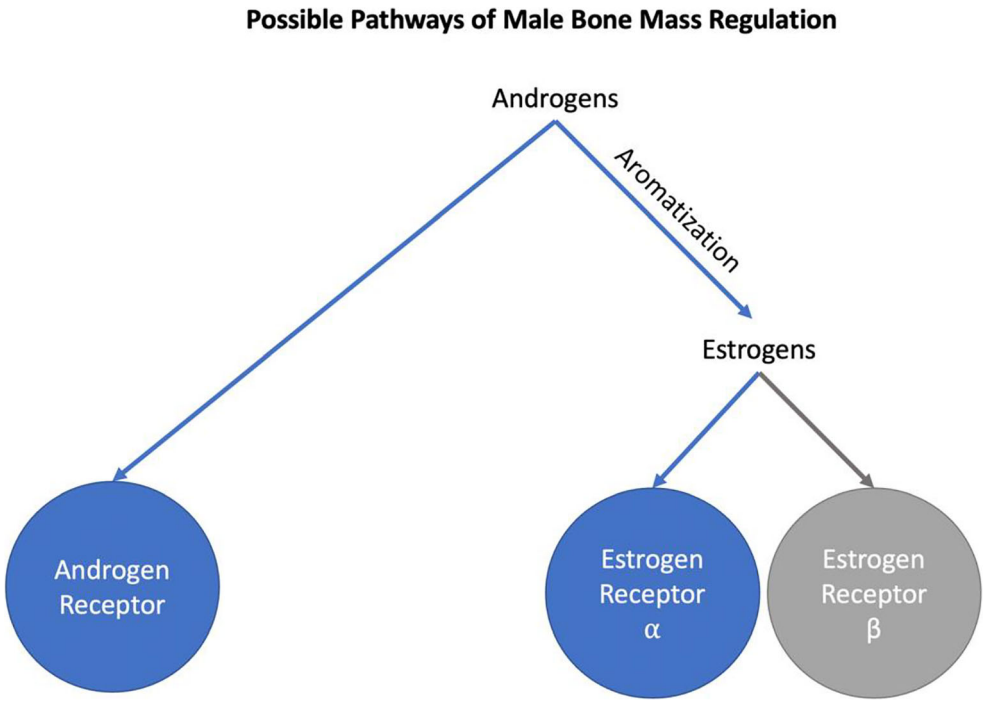

Fig. 2 Possible pathways of male bone mass regulation. Prior studies have demonstrated use of both the androgen receptor and the alpha estrogen receptor, but not the beta estrogen receptor

longitudinal bone growth attainment [42]. IGF-1 has multiple interactions with estrogen and androgens, suggesting sex-based differences. Estrogen at high doses reduces serum availability of IGF-1, whereas testosterone seems to both indirectly (by aromatization to estrogen) and directly increase IGF-1 [43–45]. IGF-1 may even have time-dependent and sexually dimorphic effects on bone health and structure [46]. Clinically, there seems to be conflicting data on how bone mineral density and IGF-1 are positively correlated; however, a low level of IGF-1 is a strong predictor of fracture independent of bone mineral density [47, 48].

VEGF is a key factor in regulating angiogenesis in various physiologic processes [49]. In the setting of an acute fracture, inflammatory cells and osteoblast precursor cells produce the majority of local VEGF [50]. Since angiogenesis is so tightly intertwined with osteogenesis, VEGF has been explored as a key mediator of potential sex-based differences in bone healing. Literature regarding the sexual dimorphism of VEGF is limited and recent; one study showed how conditional deletion of VEGF produced variation in bone porosity and geometry by sex [51].

Cell-Based Mechanisms

Osteoblasts and osteoclasts are the main cellular actors in bone homeostasis [52]. Osteoclasts are the main determinant of bone resorption. The production of osteoclasts is a complicated interplay between local cytokine milieu, RANK-L, and the presence of hematopoietic precursors [53]. Various sex-based differences in expression and differentiation of osteoclasts are known to exist [54]. Most recent literature argues that females tend to have decreased bone mass, concordant with increased levels of osteoclastogenesis, due to genetic sex-based differences in OCP differentiation as well as immune-mediated cross-talk [55].

The pluripotency of stem cells has elevated their role in many fields to address clinical pathology. Within orthopedic surgery, they have been applied to many problems ranging from healing the bone-tendon interface to functional gain in peripheral nerve surgery [56]. These treatments have yet to transition to widespread clinical use, however. Stem cells are broadly characterized by stage (pre-natal or post-natal) as well as source (location of harvest). Bone marrow MSCs and muscle-derived MSCs are most accessible and have been studied in context of sexual dimorphism [57]. From the limited literature exploring the impact of sex-based differences of these cells, it seems both local quantity and donor sex of stem cells modulate final osteogenic potential [58, 59].

Pre-clinical Data

In many instances, in vitro findings fail to translate to relevant animal or clinical models, losing external validity. The aforementioned findings have been explored in animal models as well. There seem to be many parameters that show sexual dimorphism, and each of these may play a role in explaining observed global differences.

Multiple studies laid the groundwork for further targeted investigations into the mechanisms of sex-based differences.

The first study reporting sex-based differences in bone healing an animal model was in 2011. Mehta et al. compared male and female rats following a femur osteotomy with rigid and semi-rigid fixation. Male rats had significantly higher biomechanical parameters and quicker, larger callus formation compared to female rats [60]. Haffner-Luntzer et al. also found that male mice had a significantly larger callus size on micro-CT as well as higher tissue mineral density [61]. While osteoblast counts did not vary by sex, osteoblast activity was significantly higher in male mice [61].

Recent findings from our laboratory have also implicated the role of sex in bone healing. Our study explored the response to a BMP-2 infused implant in a rat spinal fusion model [62•]. Female and male rats underwent posterolateral fusion with bilateral placement of a BMP-2 infused collagen sponge. At 8 weeks post-operative, we found that fusion rates differed significantly between sexes, with higher mean fusion scores noted in males compared to females. Micro-CT analysis showed significantly lower volumes of fusion masses in females compared to males, but significantly higher bone volume fraction and trabecular number in female rats [62•]. These findings suggest possible sex-based differences in bone healing. Various studies build on these to understand underlying mechanisms that may contribute to such sexual dimorphism.

Komrakova et al. describe the efficacy of efficacy between a selective androgen receptor modulator (SARM) and testosterone given as therapy or prophylaxis following osteotomy on an orchiectomized rat model [63]. Mechanistically, SARMs simplify the sex hormone interplay as they only agonize the androgen receptor. This provides clarity by reducing confounding effects of testosterone, which stimulates the androgen receptor but can aromatize into estrogen as well. Unsurprisingly, the orchiectomized group had impaired bone healing and significantly less callus formation in comparison to wild-type rats. Both prophylactic (prior to osteotomy) and therapeutic (following osteotomy) testosterone produced a stronger effect on bone healing than treatment with a SARM. As SARMs do not aromatize into estrogen, this provides evidence for the positive osteogenic effect of androgens. Testosterone promotes bone healing both directly and via aromatization. Interestingly, SARM treatment produced varying results based on the time at which it was initiated. Administration of a SARM prior to osteotomy improved callus parameters, like prophylactic testosterone treatments; short-term SARM treatment after osteotomy surprisingly reduced callus parameters. In vitro work shows that SARMs inhibit osteoblast differentiation therefore reducing bone resorption and remodeling, which is imperative for callus formation. The authors postulate that this could have contributed to a reduction in total callus size in the cohort treated with SARM after osteotomy; however, further work should clarify SARM responses. A similar study investigating the effect of SARMs in ovariectomized female rats found a dose-response within SARM treatment groups. Larger SARM doses produced the largest and most dense callus; however, this did not reach statistical significance [64]. Such findings may be in vivo evidence for dimorphism in downstream sex hormone action.

Levels of 5-LO have been shown to be sexually dimorphic [40]. Further studies have considered the impact of this in various in vivo studies. Manigrasso and O'Connor studied the osteogenic response in a 5-LO knockout mouse, finding significantly accelerated fracture healing in the 5-LO knockout mice compared to wild-type controls [65]. Cottrell et al. dosed female rats with an oral 5-LO inhibitor and found that it allowed for significantly faster and biomechanically robust callus formation compared to controls [66]. Cottrell et al. were able to replicate these results in another study using only local 5-LO administration technique [39•]. Although these studies failed to compare the efficacy of a 5-LO inhibitor in both male and female rat, such results may show a sexually dimorphic response in bone healing.

IGF-1 has been known to have crucial roles in the developing skeleton. A study by Ashpole et al. simulated age-related loss of IGF-1 at various time-points (early post-natal, early adulthood, late adulthood) in mice to understand its effects on bone [67]. They found that decreases in circulating IGF-1 within female mice at either early post-natal or early adulthood (5 months) were associated with increased vertebral bone parameters compared to wild-type controls. In contrast, male mice with age-dependent decreases in IGF-1 produced no change in bone volume fraction or trabecular number. Such findings may be evidence for sexual dimorphism in the effects of IGF-1 on bone.

Stem cells remain as exciting prospects translationally and clinically. Regarding the local environment in which MSCs function, two studies provide a basis for sex-based difference in bone healing. Strube et al. investigated sex-based differences following an osteotomy in both male and female rats [59]. Their group found worse callus parameters in female rats with significantly fewer colony-forming units of MSCs compared to males; there were no differences in functional characteristics of MSCs by sex, however. A study by Ueno et al. showed sexual dimorphism in the local inflammatory environment following a fracture [68]. An IL-4 overexpressing MSCs were implanted into a femoral bone defect. Micro-CT analysis showed that male mice had significantly larger defect healing compared to female mice [68].

Sex of MSDC hosts was similarly found to change the osteogeneic potential. Mezsaros et al. commented on the differences in ectopic bone formation dependent on MSDC host sex [69]. The authors compared BMP-4 expressing MSDCs implanted into males and female mice and found that male mice had quicker and more robust bone formation compared to female mice. Additionally, there was no difference in bone

formation between wild-type castrated or ovariectomized mice, showing that sex hormones did not appear to contribute to MSDC osteogeneic potential.

Conclusion

Further in vitro and in vivo work is needed to entangle the clinically ambiguous effects of sex on bone healing. In vitro studies examining the roles of sex hormones, 5-LO, IGF-1, VEGF, osteoclasts, and OPCs seem to show sexually dimorphic actions. Additionally, donor characteristics and stem cell environment seem to also determine osteogenic potential. Building on this biomolecular basis, in vivo work investigates the aforementioned elements. Broadly, males tend to have a more robust healing compared to females. Taking these findings together, differences in sex hormones levels, their timing and action, and composition of the inflammatory milieu underlie variations in bone healing by sex.

Clinically, a robust understanding of bone healing mechanics can inform care of the transgender patient. Transgender patients undergoing hormone therapy present a clinically nuanced scenario for which limited long-term data exist. Such advances would help inform treatment for sports-related injury due to hormonal changes in biomechanics and treatment of transgender youth [70•]. While recent advances provide more clarity, conclusive answers remain elusive.

Declarations

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of interest Steven Kurapaty and Wellington Hsu declare that they have no conflict of interest.

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