

Chapter 2

Novel Findings in Bone Biology: Impact on Bone Health for Women

Susan A. Bloomfield and Corinne E. Metzger

Abstract The maintenance of bone integrity throughout life is critical for minimizing the risk of debilitating fractures, which most frequently occur in those with low bone mass (osteopenia) and frank osteoporosis. Bone dynamically adapts to mechanical stresses placed on it, as with increased exercise, but this adaptation may be modified by changes in circulating estrogen, altered oxidative status, and nutritional factors. This review addresses novel findings of the last decade as they affect bone health in women. Specific topics discussed include the negative impact of low energy availability due to prolonged caloric restriction, the surprising role of estrogen receptor-alpha in bone mechanotransduction, and how oxidative stress may be an important mechanism contributing to bone loss with aging and estrogen insufficiency.

Keywords Skeleton • Estrogen receptor • Dietary energy • Caloric restriction • Oxidative stress

Introduction

The skeleton's key functions are to provide levers for locomotion, protect vital organs, and serve as a calcium reservoir. These are rarely appreciated functions until, of course, one incurs a bone fracture and appreciates anew the mobility afforded by the intact skeleton. "Bone health" is an oft-used phrase reflecting the mechanical integrity of bone and its ability to resist fracture in all but extreme

S.A. Bloomfield, Ph.D. (✉) • C.E. Metzger, M.S.
Department of Health and Kinesiology, Texas A&M University, MS 4243,
College Station, TX 77843, USA
e-mail: sbloom@tamu.edu

loading situations. The most commonly used clinical measure of bone health is bone mineral density assessed by dual-energy X-ray absorptiometry (DEXA), with which most postmenopausal women are very familiar. This technique measures only the mineral content of bone, which is also composed of organic matrix (collagen and multiple non-collagenous proteins). Since BMD levels are reasonably predictive of fracture rates, this simple noninvasive test provides the handiest tool to track bone health in the general population, with a minimal radiation dose.

There are many varieties of metabolic bone disease (tumor metastasis to bone, disorders of osteoclast biology, osteomalacia with, e.g., renal failure). The best known and most prevalent is osteoporosis, which is simply a lower bone mass than expected for one's age and affects an estimated ten million Americans over 50 years of age [1]. Although the majority of these are female, aging men (and those on certain medications causing bone loss, such as corticosteroids) are also at risk. Intermediate bone loss that does not reach the criterion mark for osteoporosis is termed osteopenia, analogous to glucose intolerance that may precede a diagnosis of Type 2 diabetes. Osteopenia is very common in middle-aged women and in select populations of young women, particularly those who restrict dietary energy intake for prolonged periods. The accelerated bone loss observed in the osteoporotic patient dramatically diminishes bone strength and contributes towards 1.5 million so-called fragility fractures and health care costs of \$18 billion/year in the USA [1]. The survival rate 5 years after a hip or a vertebral fracture is ~80 % of that in men and women with no fractures [2], with higher mortality rates in men and in the more aged [1].

Bone cells behave quite similarly in men and women, but what is unique to women is, of course, the hormonal milieu and the dramatic changes in estrogen status at mid-life (or, on occasion, in younger women with amenorrhea or surgical menopause). Bone loss occurs fairly rapidly with the onset of estrogen deficiency due to a multitude of cellular mechanisms [3]. Briefly, bone resorption activity by osteoclasts, normally inhibited by circulating estrogen, accelerates dramatically with estrogen withdrawal. In addition, both differentiation and activity of mature bone-forming osteoblasts are impaired in the absence of estrogen. There is a dearth of evidence about the impact of estrogen deficiency on osteocytes, those cells embedded within bone matrix that are thought to serve as the essential mechano-sensors.

This short review discusses some novel findings in the bone biology field over the last 10 years that have direct relevance for bone health in women. The first section of this review considers how dietary energy intake, the critical "other bone nutrient," can impact bone mass and fracture risk in both young athletes and in the frail elderly. Low circulating estrogen levels can also impact negatively the expression of estrogen receptor- α (ER- α) in bone cells and on mechanotransduction, which may provide the mechanism for the lower responsiveness of bone to exercise in postmenopausal women; this topic is covered in the second section. The final topic briefly presents the growing evidence for an alternate mechanism for age-related and estrogen-deficiency bone loss: oxidative stress.

Impact of Reduced Dietary Energy Intake on Bone Integrity

Most individuals, when queried, will name calcium and vitamin D as the two most important nutrients supporting optimal bone health. Millions of older Americans focus on getting adequate intake of these two nutrients, which do indeed help to minimize (but cannot abolish) age-related bone loss. However, relatively few are aware of the impact that reduced energy (calorie) intake has on bone mass. In a recent NHANES survey, nearly 60 % of American women (and 40 % of men) are pursuing some weight loss regimen [4]; given the rising prevalence of obesity, the fraction of Americans who will pursue serious weight loss regimens can only increase. Others at risk for bone loss coincident with restricted energy intake include those with involuntary caloric restriction (frail elderly or those without regular access to adequate caloric intake, e.g., the food-insecure poor); active military personnel in the field; and athletes in training who voluntarily restrict food intake. On average, each 10 % decrement in body weight is associated with a 1–2 % decline in BMD [5]. The consequences of a 2 % decline in bone mass, particularly with reference to fracture risk, vary significantly across these different populations.

Both epidemiological evidence and controlled intervention trials provide evidence for significant bone loss and elevated fracture risk in individuals losing weight. A history of >10 % weight loss in community-dwelling older women [6] and men [7] increases the risk of hip fractures. Even more convincing are results from controlled randomized intervention trials, most frequently performed in obese postmenopausal women, in whom losses in bone mass consistently occur with reductions in body weight [5]. Notably, the bone lost may not be regained after the end of a weight loss period. Serum markers of bone resorption remained elevated [8] and BMD values remained lower after the end of an exercise-based weight loss intervention in obese individuals, even in the face of significant weight regain [9]. A similar lack of bone recovery was found in premenopausal women who had followed a very-low-calorie diet for 3 months; a full 33 months later, decreases in lumbar spine BMD were not restored even in those subjects who regained all bone lost. (However, in this case, trochanter BMD (at proximal femur) did recover with weight regain [10].) These data suggest that a burst of remodeling activated during a period of negative energy balance (at least in vertebral bone) does not switch off as soon as energy balance is resumed, resulting in continued loss of bone mineral even after the individual resumes usual caloric intake.

Many obese individuals enter a period of caloric restriction with higher than average BMD values [11]. Hence losing 1–3 % of this elevated bone mass may result in a minimal impact on fracture resistance as compared to the same proportional bone loss in a near-normal weight population. Military recruits in basic training and those in intense combat operations often incur rapid weight loss [12]; in this case, the negative energy balance is more likely due to the enormous increase in energy expenditure with hours of vigorous activity each day. A classic illustration of this is provided by the data of Friedl et al. [13], who monitored caloric intake and energy expenditure during an 8-week field training experience by Army Rangers,

during which average energy intake was 1,000–1,200 kcal/day below average energy expenditure and body weight loss averaged 12 kg.

In the extreme instance, individuals with eating disorders or frank anorexia nervosa incur significant bone loss; fracture rates are sevenfold higher than expected for these (usually) young individuals [14]. A wealth of literature now documents the impact of the prolonged and severe negative energy balance on bone health in these populations, which may be complicated by shifts in neuroendocrine function. Less severe restriction of energy intake occurs frequently in those on the other end of the age spectrum: the frail elderly. Reduced eating may be voluntary (lack of appetite) or involuntary (food insecurity, inability to prepare food) in this population, but the result can be accelerated bone loss beyond that observed in well-nourished individuals of the same age [15]. Fracture risk in this group is further exacerbated by loss of muscle mass with inadequate energy intake, which reduces both the mechanical loading delivered to bone as well as the energy absorption effect of the overlying soft tissue should a fall occur.

Another unique population at risk of bone loss are those athletes who over-restrict energy intake over prolonged periods (often years) to maintain extremely lean physiques and lower body weights. Early findings of Drinkwater et al. [16] confirming spinal BMD values in amenorrheic varsity rowers equivalent to those typical in 51-year-old women galvanized the research community into explaining how bone mass could *decline* with vigorous physical activity. A plethora of studies in the subsequent decades confirmed these findings in athletes in weight-classed sports (like rowing), in sports (e.g., distance running) where low body weights provide a competitive advantage, and in the aesthetic sports that demand an extremely lean appearance (dance, gymnastics, figure skating). Recently published data using high-resolution CT scans in adolescent amenorrheic athletes provide evidence of impaired cancellous bone micro-architecture (reduced trabecular number, increased trabecular spacing), which can independently elevate fracture risk [17].

Whether bone loss due to reduced energy intake is more specific to cortical or cancellous bone seems to vary some with the bone site examined. Human studies generally rely on DEXA measures of bone mass, which focus on the clinically relevant sites of lumbar spine and proximal femur. These are “mixed” bone sites, with a dense cortical shell surrounding a core of cancellous bone, which forms a lattice-work of connected rods and struts that strengthen the structure. Several intervention trials have documented a twofold higher rate of bone loss at anatomic sites with more cancellous bone (e.g., distal radius and proximal femur trochanter) over that observed in total body scans [9] or in cancellous compartments within the distal tibia, as separated out by peripheral quantitative computed tomography (pQCT) [18]. Bone “quality,” if not bone quantity, may be protected in older adults practicing caloric restriction but getting adequate protein and other nutrients, in contrast to the amenorrheic adolescent athletes mentioned earlier. Even in the face of significant reductions in BMD, middle-aged adults consuming a healthy diet with ~35 % fewer calories exhibit no changes in indicators of trabecular bone micro-architecture as measured by high-resolution MRI [19]. A number of animal studies utilizing peripheral quantitative or micro-computed tomography document that cancellous

bone is preferentially affected in adult female rats [20, 21]; however, the primary deficits in young male mice subjected to similar reductions in energy intake are limited to cortical bone [22]. It remains unclear whether these disparate results reflect species and/or gender differences.

The functional concern related to loss of bone mass with chronic energy restriction is two-pronged. The first is a long-term health issue: should this bone loss occur in a younger individual, it may result in “premature” osteoporosis, some years before that individual might have otherwise reached osteoporotic BMD levels. Hence the risk of fragility (low-trauma) fractures can be significant at a relatively young age. The second functional consequence is of more immediate concern to younger individuals, particularly military personnel and athletes: an increased risk of stress fracture [23, 24]. The military in particular has a vested interest in reducing the incidence of stress fractures in recruits, since the required 6 weeks’ healing time can seriously disrupt basic training schedules.

What are the systemic or the cellular mechanisms for these losses of bone mass with reduced energy availability? Early research on low BMD values in amenorrheic women athletes presumed that reduced circulating serum estrogen associated with cessation of normal menstrual cycles was the culprit, creating a “premature menopause” endocrine profile conducive to accelerated bone loss. However, the limited success of oral contraceptives in restoring lost bone mass in amenorrheic athletes or in anorexia nervosa patients [25] suggests that estrogen-independent mechanisms are also important contributors to this bone loss.

Chronic negative energy balance impacts two primary endocrine axes: the metabolic hormones that are sensitive to energy intake and the reproductive hormone axis, each of which can impact bone cell activity leading to loss of bone mass. An elegant illustration of this derives from tightly controlled studies of nonathletic women subjected to combined treadmill running and graded energy restriction, resulting in 10–30 kcal/kg LBM/day reductions in energy availability [26]. (In this context, “energy availability” was computed as total energy intake minus exercise energy expenditure.) After only 5 days of this regimen, reductions in circulating IGF-I and leptin, evident even at the milder levels of reduced energy availability, were highly predictive of reductions in serum biomarkers of bone formation activity. Conversely, elevations in bone resorption biomarkers (but only at the most extreme reduced energy availability) tracked closely with reductions in serum estrogen.

Although prolonged hypoestrogenemia almost certainly contributes to bone loss in women with prolonged amenorrhea, metabolic hormones related to energy status such as IGF-I and leptin may play an important role in mediating bone loss, particularly in those individuals experiencing milder (subclinical) changes in estrogen status, such as luteal phase defects [27]. More recent data indicate that estrogen-deficient exercising women who are energy-replete exhibit few perturbations of bone resorption or formation, reinforcing the key role of energy status in modulating bone cell activity and hence bone mass [25]. These data provide the mechanistic endocrine link between the reduced BMD frequently observed in amenorrheic athletes and their reduced energy intake, which together describe the phenomenon labeled the female athlete triad (for a comprehensive review [28]).

Research in relevant mammalian models provides valuable insight into tissue-level mechanisms for bone loss with restricted energy intake. One often unrecognized contributor is the reduced absorption of dietary calcium with estrogen deficiency. Ovariectomized (OVX) rats exhibit a fourfold lower net calcium absorption when subjected to a 40 % restriction of energy intake; this effect can be reversed with estrogen replacement [29]. The impact of energy insufficiency on reduced bone formation and/or increased resorption activity can derive from reduced activity of mature osteoblasts or increased vigor of mature osteoclasts, respectively. Alternatively, chronic restriction of dietary energy can alter the differentiation of stem cell populations, decreasing the availability of mature osteoblasts [30], and expanding adipocyte populations in bone marrow [30, 31]. This impact on stem cell populations is coincident with reductions in circulating IGF-I and leptin, reinforcing the notion that these metabolic hormones that reflect energy balance are important mediators of bone loss with energy restriction.

An important related question is the following: Does concurrent exercise mitigate bone loss with restricted energy intake? Most physicians recommend increased caloric expenditure (most frequently, a walking program) in addition to reduced caloric intake for patients pursuing weight loss. Although it makes intuitive sense that the metabolic shift that occurs with regular exercise would enhance the weight loss with energy restriction alone, the evidence that this results in significantly greater weight loss is surprisingly weak, even in well-controlled randomized interventions [32]. Even if exercise during a caloric restriction regimen does not improve weight loss outcomes, it is reasonable to hypothesize that the mechanical loading effect of an exercise regimen might mitigate the negative impact of reduced energy intake on bone mass. Circumstantial evidence is provided by multiple cross-sectional studies comparing amenorrheic sedentary and athletic populations, in which the athletes have higher BMD values than their nonathletic peers [33]. More solid experimental evidence for this derives from well-controlled clinical trials comparing BMD losses in obese populations seeking weight loss by caloric restriction alone vs. exercise-induced weight loss. For example, middle-aged men and women achieving weight loss by increased exercise expenditure only experienced no loss of BMD, whereas those restricting energy intake by up to 20 % experienced significant (2 %) declines in spine and hip BMD [34]. Increases in serum sclerostin (an osteocyte-specific protein that inhibits bone formation) and negative changes in hip geometry observed in those losing weight through calorie restriction alone are prevented with exercise training concurrent with the restricted energy intake [35]. At least one published study in dieting humans demonstrated that exercise during a period of reduced energy intake increased serum IGF-I and reduced markers of inflammation while mitigating bone loss [36].

It is far easier to strictly control energy intake and expenditure in animal studies, particularly for long-duration experiments. Recent work testing the impact of reductions in energy availability achieved by reducing dietary energy intake alone vs. combining smaller reductions in energy intake with increased energy expenditure in adult female rats provides several interesting findings. If energy availability is reduced by 25 %, significant bone loss does occur in cancellous-rich bone

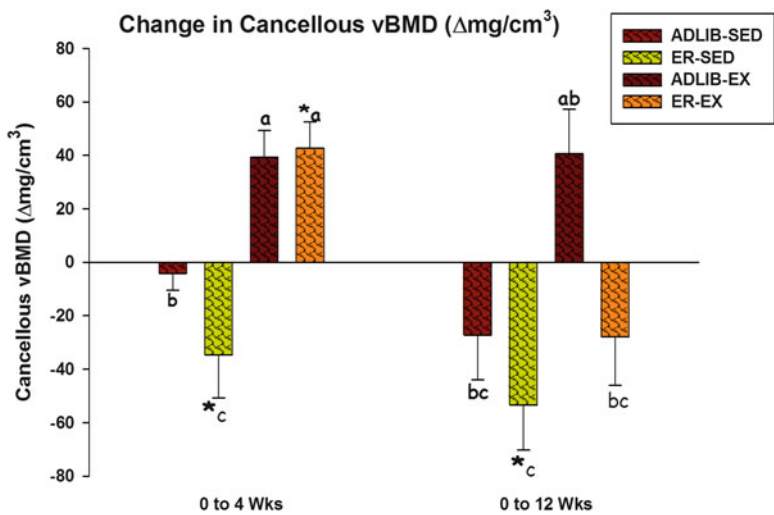


Fig. 2.1 Short-term protection observed at 4 weeks against loss of cancellous bone mass in rats achieving reduced energy availability with exercise and dietary energy restriction (ER) disappears after 12 weeks. Decline in volumetric bone mineral density (vBMD) at 12 weeks in ER-exercised animals is still half that observed in ER-sedentary (SED) rats. Delta values with the same letter superscript are not significantly different; * $p < 0.05$ vs. baseline value on day 0. Data from Swift et al. [21]

compartments in sedentary animals after 4 weeks but, interestingly, not in rats subjected to treadmill running (see Fig. 2.1) [21]. However, the implied protective benefit of exercise disappears after 12 weeks of reduced energy availability, by which time even exercising animals incur a 9 % deficit in volumetric BMD (vs. a 17 % deficit in sedentary rats). This pattern of bone loss may be related to alterations in serum IGF-I, declines in which are mitigated in exercising animals [37]. If indeed the anabolic effect of exercise attenuates negative changes in metabolic endocrine factors during energy restriction, this may provide some protection over short-term dieting periods. It appears, however, that should energy availability be reduced on a chronic basis, the impact of energy insufficiency overwhelms metabolic or mechanical loading advantages concurrent exercise might provide.

Estrogen Receptor-Alpha and Mechanotransduction in Bone

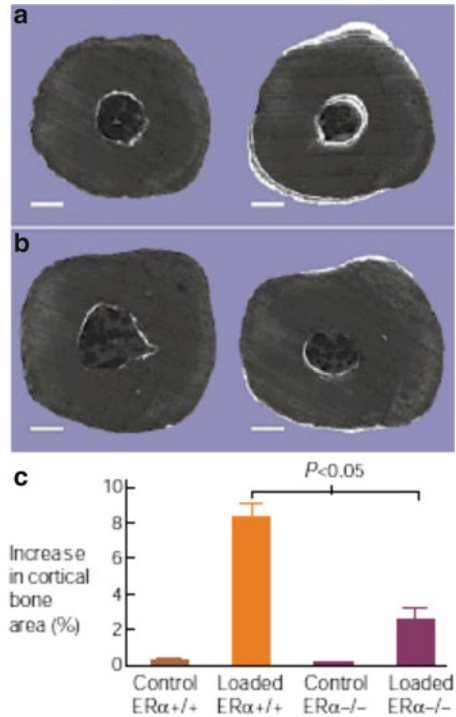
Recently, a fascinating new line of research has examined the effect of reduced circulating estrogens may have on bone’s ability to adapt to mechanical strains and exercise. It has long been known that bone structure adapts to the typical loading patterns to which it is subjected. Current recommendations focus on weight-bearing exercise and resistance training as effective means of increasing bone mass across most of the life-span. However, bone is less responsive to even optimal exercise

modes with increasing age [38]. Interestingly, the sensitivity of bone's response to loading is also reduced in individuals with estrogen deficiency, including postmenopausal women, amenorrheic athletes, and men with progressive estrogen deficiency [39]. This observation led to the hypothesis that low serum estrogen or a closely related factor is responsible for this diminished sensitivity of bone to mechanical loading; key candidates were the intracellular estrogen receptors, of which there are two: ER α or estrogen receptor- β (ER β). ER α became a likely candidate for this role as it is expressed in osteoblasts, osteoclasts, and osteocytes [39]. Also, it has been previously shown in humans as well as animals that the estrogen receptor expression varies with levels of circulating estrogen [40, 41]. Therefore, a decline in circulating estrogen would lead to fewer estrogen receptors expressed in bone cells. If these receptors have any influence on key signaling pathways important to the regulation of bone formation activity in osteoblasts, an individual with low estrogen status would be predicted to have an attenuated bone response to exercise.

The first clue regarding ER α 's response in mechanical loading of bone was provided by *in vitro* experiments in which osteoblast-like cells were exposed to mechanical strain and treated with the estrogen receptor modulators ICI-182,780 and tamoxifen [42]. These two compounds, both estrogen receptor antagonists, eliminated or reduced osteoblast proliferation to the mechanical strain, suggesting that bone's adaptive response to mechanical strain is mediated through a mechanism which involves ER α . To confirm these findings *in vivo*, Lee et al. [43] studied the response to mechanical loading of bone in ER α -null mice. This commonly used model provides axial loading of the ulna using a servomotor device to cyclically deform the bone in an anesthetized animal; the key advantage of this model is to provide very precise control over the loading signal delivered (strain magnitude and strain distribution), but it does not involve muscle contraction or the integrated physiological response to voluntary exercise. While wild-type mice exhibited an 8 % increase in cortical area at the midshaft ulna, the response in ER α -null mice was nearly fourfold lower (2.4 % gain in bone area) (see Fig. 2.2). Primary osteoblast cultures from the ER α knockout mice exhibited no proliferative response when exposed to strain *in vitro*, while cells derived from wild-type littermates increased in number by 58 %. These data demonstrate some critical role for ER α activity in the adaptive response of bone to strain-related signals. The loss of bone and reduction in ability to respond to exercise in estrogen deficiency could be explained through the estrogen receptor's role in adaptation to loading.

The role of ER β is not as well established as that of ER α , but it, too, might have a role in the loading response of bone. The fact that there are two estrogen receptors present in bone has made determining the role of each individual receptor a greater challenge. ER β is also present in osteoblasts, but early research was inconclusive as to its impact on the loading response. With *in vivo* loading of ER α knockout mice and ER β knockout mice, similar changes in bone are seen when the estrogen receptors are individually silenced. ER β knockout mice exhibit only half the increase in cortical area due to periosteal expansion as do ER β -intact (wild-type) mice [44]. This impact of the missing ER β on the cortical bone gain is about half of that

Fig. 2.2 Mechanotransduction is impaired in the absence of estrogen receptor- α (ER α). Transverse sections of unloaded control (*left*) and loaded (*right*) ulnae from (a) ER α wild-type and (b) ER α knockout mice. White fluorochrome label on bone surfaces represents newly formed bone. (c) Quantification of increased cortical bone area in the loaded versus control wild-type and knockout mice. From Lee et al. [43]

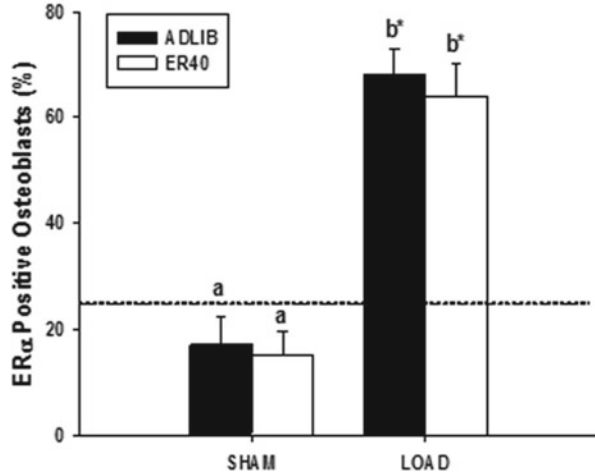


observed in ER α -null mice (nearly a fourfold decrease) [43], but it appears that the absence of either ER α or ER β results in attenuation of the osteogenic response to loading.

To complicate this story, more recent research demonstrates that there may be differential roles for ER α and ER β in males and females. With ER α or ER β deleted, the different effects of either reduced loading on tibial bone (via sciatic neurectomy) or increased loading were investigated in male and female mice [45]. In both genders ER α -null, but not ER β -null, mice exhibited an attenuated loss of cancellous bone after prolonged disuse, as compared to their wild-type counterparts. Those female mice lacking ER α had a diminished osteogenic response to loading on cortical bone but, interestingly, no effect was observed for cancellous bone surfaces. By contrast, ER α knockout males had a *greater* osteogenic response (vs. wild-type littermates) to increased loading on both cortical and cancellous bone surfaces. Deletion of ER β , on the other hand, resulted in an increased osteogenic response on cortical bone surfaces in both male and female mice, suggesting a tonic inhibition of periosteal osteoblast response to loading in the intact animal [45]. The precise role of these two estrogen receptors in mechanotransduction may be more complex than initially thought, given these gender-specific responses.

Another intriguing question is if mechanical loading or exercise is capable of independently affecting estrogen receptor expression in bone cells. Osteoblast and

Fig. 2.3 Short-term mechanical loading (LOAD) upregulates ER- α expression in (a) osteoblasts in the distal femur metaphysis, even in animals subjected to 12 weeks of 40 % reduction in energy intake (ER-40). Dashed line denotes baseline control (BC) animals' values. Those groups not sharing the same letter are significantly different from each other ($p < 0.05$). * $p < 0.05$ versus baseline control mean value. Data from Swift et al. [46]



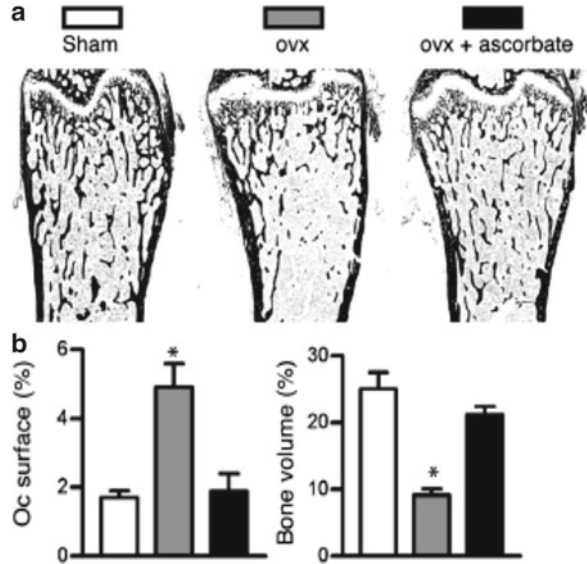
osteocyte expression levels of ER- α protein are 6- and 26-fold greater, respectively, in female rats after 1 week (3 bouts) of loading achieved by active muscle contraction (see Fig. 2.3) [46]. Interestingly, 12 weeks of energy restriction preceding the week of imposed loading, which usually diminishes circulating estrogen, does not diminish these increases in ER- α expression, but does blunt the bone formation response to mechanical loading. Hence, in this case, the anabolic response of osteoblasts to exercise does not appear to be caused by a down-regulation in ER- α protein in osteoblasts or osteocytes.

While more research needs to be conducted to fully understand the roles of each of the estrogen receptors, there may be interesting implications of this work. For example, could a pharmacological agent that manipulates estrogen receptors improve mechanosensitivity and diminish the negative effects of estrogen deficiency on bone? In conjunction with an appropriate exercise program, could manipulation of estrogen receptors increase the effectiveness of exercise and improve bone mass? Novel treatments for postmenopausal osteoporosis and age-related bone loss may capitalize on the role of estrogen receptors in bone's adaption to mechanical loading.

Oxidative Stress: New Mechanism for Aging-Related Bone Loss?

Oxidative stress has been a leading theory of aging for decades [47], but only very recently has it been advanced as a mechanism for age-related bone loss [48]. The primary mechanism leading to oxidative stress is the formation of reactive oxygen species (ROS) by electrons escaping from the electron transport chain during aerobic metabolism which then join with oxygen to form superoxide ($O_2^{\cdot -}$), hydrogen

Fig. 2.4 Antioxidant treatment can reverse the impact of estrogen deficiency on cancellous bone mass and osteoclast surfaces. **(a)** Photomicrographs of distal femurs of mice subjected to sham ovariectomy (*white bar*), ovariectomy (*gray bar*), and ovariectomy with ascorbate treatment (*black bar*). Mineralized bone is in *black*. **(b)** Bone volume normalized to tissue volume (%BV/TV) and **(b)** Percent osteoclast surface (Oc.surf %) and cancellous bone volume normalized to tissue volume (%BV/TV) in the three groups. Adapted from Lean et al. [52]



peroxide (H_2O_2), and the hydroxyl radical ($OH^\bullet$) [48]. ROS cause damage to proteins, lipids, and DNA, initiating cell death and also trigger important signaling pathways, such as the p66^{shc} pathway initiating cell apoptosis.

For several decades, it has been known that ROS in bone tissue are involved in the formation and activation of osteoclasts, thereby increasing bone resorption [49]. This led to an interesting hypothesis that oxidative stress could enhance, or even provide an alternate mechanism for, bone loss associated with menopause and aging. In a large-scale study of postmenopausal women, that subset with a diagnosis of osteoporosis had a higher oxidative status and lower antioxidant status as assessed from serum markers. There was a significant negative correlation between a key indicator of oxidative stress in this population and bone mineral density in the lumbar and femoral neck region [50].

Recent research has shown that estrogen may play a key role in protecting bone tissue against the damage caused by ROS. Estrogens have been identified as antioxidants in many tissues, effectively functioning as radical scavengers and suppressing peroxidation reactions [51]. Lean et al. [52] hypothesized that this antioxidant function of estrogen might be an important mechanism for its well-known salutary effect on bone health. Glutathione and thioredoxin, two major oxidative defense enzymes, are much reduced in rodent bone marrow after ovariectomy. A single dose of 17β -estradiol rapidly normalizes these antioxidant enzyme levels. More convincingly, administration of exogenous antioxidants, *N*-acetyl cysteine (NAC) and ascorbate, prevents bone loss in OVX mice (see Fig. 2.4) [52]. To further validate the effect of oxidative stress on bone, administration of L-buthionine-(S,R)-sulfoximine, an inhibitor of glutathione, resulted in similar loss of bone as observed in OVX animals. These data show, first of all, that ROS can lead to bone loss and, secondly, estrogens help to prevent bone loss by increasing intracellular oxidative defense

mechanisms. The loss of estrogens at menopause could lead to a substantial decrease in the ability of the bone cells to defend against ROS and thereby contribute to increased osteoclastic activity and bone loss. Hence, the loss of estrogens in women at menopause may amplify the oxidative stress accounting for aging-related bone loss.

Besides antioxidant enzymes (glutathione, thioredoxin, and superoxide dismutase), there are other pathways that lead to protection against ROS. The FoxO genes, so named for their unique structure with a special winged DNA helix known as a Forkhead box, are upregulated by ROS. Once FoxOs are activated and translocated into the nucleus of the cell, they lead to the transcription of free radical scavenging enzymes (MnSOD, catalase) and DNA repair genes (such as Gadd45). The FoxOs are also able to induce apoptosis in cells damaged by ROS [48, 53]. Mice null for FoxOs exhibit increased oxidative stress and a loss of both cortical and cancellous bone due to deficient bone formation [54]. If FoxO1 is conditionally deleted in osteoblasts, osteoblast proliferation and bone formation are significantly impaired. Administration of the antioxidant NAC normalizes osteoblast number, bone formation rate, and bone volume in mice lacking FoxO1 [55] and rates of osteoblast apoptosis in mice with osteoblast-specific deletions of FoxO1, O3, and O4 [56]. These studies further highlight the effects of oxidative stress on bone loss and the importance of defense mechanisms against an increase in ROS. Also of importance, exogenous antioxidant compounds are able to counteract the deleterious effects of the deletion of oxidative defense genes.

As Lean et al. hypothesized, the antioxidant abilities of estrogens may be critical to bone health due to its capacity to defend against oxidative stress [52]. Further research has shown that loss of estrogens and androgens accelerate the effects of aging by decreasing defenses against oxidative stress [48]. The loss of estrogen results in increased lipid peroxidation and hydrogen peroxide, and a decrease in oxidative defense enzymes (SOD, glutathione peroxidase, and glutathione *S* transferase) in femoral bone tissue of OVX mice, resulting in increased oxidative stress [57]. Almeida et al. compared normally aging mice (up to 31 months of age) of both genders with mice that underwent gonadectomy at 5 months of age [58]. Similar changes in ROS, glutathione reductase, and phosphorylation of p53 and p66^{shc} were observed within 6 weeks of gonadectomy as observed over 31 months of aging. The administration of NAC protected against all measures of oxidative stress damage and was as effective at preserving spinal BMD as administration of either estrogen or testosterone. This exogenous antioxidant also mitigated the increase in osteocyte and osteoblast apoptosis caused by gonadectomy and maintained cancellous bone mass [58]. Later studies by the same laboratory confirm that antioxidants are as effective at maintaining bone integrity as sex steroid replacement [48, 54]. Taken together, these studies demonstrate the importance of oxidative defense mechanisms in bone tissue and demonstrate the powerful antioxidant effect of sex steroids.

Other potentially important mechanisms for estrogen's antioxidant function relate to osteoblast and osteocyte apoptosis and to immune cell function. (Osteocytes are those cells embedded inside bone that are essential to signaling targeted remodeling of damaged bone, as well as sensing the mechanical loads placed on bone.)

Pretreatment of MLO-Y4 osteocyte cells with either 17 β -estradiol or selective estrogen receptor modulators (SERMs) prevents osteocyte apoptosis [59]. This suggests that estrogen deficiency may lead to a loss of osteocytes due to an inability to protect against ROS-induced damage. Oxidative stress also impacts osteoblast apoptosis. Aging or loss of androgens or estrogens leads to activation of nuclear factor- κ B (NF- κ B), and phosphorylation of p66^{shc}, which increases the production of ROS and stimulates apoptosis. Administration of 17 β -estradiol effectively inhibits ROS-stimulated activation of NF- κ B and p66^{shc} by acting on PKC β , thereby attenuating osteoblast apoptosis [54]. OVX mice also exhibit an increase in ROS in bone marrow, which enhances the activity of bone marrow dendritic cells that activate T cells, leading to a signaling cascade resulting in bone loss. Bone loss is effectively prevented by supplying either an antioxidant or an inhibitor of the CD80/CD28 pathway, which is involved in T cell activation [60]. Taken together, these studies demonstrate that the loss of estrogen leads to a decreased ability to defend against ROS acting on key bone and immune cells important for maintaining bone integrity.

The evidence that oxidative stress leads to bone loss and the effect antioxidants exert on reversing this damage could lead to novel concepts for treating bone disorders like osteoporosis. Could a diet high in antioxidants help prevent the increase in ROS seen with aging? Could administration of antioxidants prevent the sharp decline in oxidative defense seen with postmenopausal osteoporosis and, therefore, preserve bone mass or attenuate its decline? In a cross-sectional study of postmenopausal women, Rao et al. [61] demonstrated a strong inverse correlation between serum levels of the antioxidant lycopene (found in tomatoes, red bell peppers, watermelon, and other red fruits and vegetables) with N-telopeptides (NTx), a well-accepted serum marker of bone resorption. There is also strong epidemiological evidence for the beneficial impact of flavonoids, polyphenolic compounds found in many plant foods that have anti-inflammatory and antioxidant properties, on bone health [62]. However, intervention trials provide less definitive conclusions to date.

Conclusion

The preceding provide a fine example of how integrative physiology informs the field of bone biology, since nutritional status, endocrine regulation, and oxidative stress are all important modulators of bone cell differentiation and activity and, ultimately, bone structural integrity and fracture risk. It has become clear that prolonged negative energy balance is an important contributor to bone loss in a variety of populations. Given the large number of Americans repeatedly attempting to lose weight, useful interventions to minimize this loss of bone should be pursued, especially as it remains uncertain if bone loss associated with dietary energy restriction is reversible. Estrogen deficiency clearly impacts estrogen receptor function, which may explain why exercise appears to have a diminished osteogenic effect in postmenopausal women. The interaction of estrogen withdrawal at menopause and

oxidative stress, with its myriad effects on bone cell function, may lead to interesting new therapeutic or dietary interventions for improving antioxidant defenses in aging individuals. Defining the interplay of these factors in animal models and in human intervention trials provides a stimulating challenge to integrative physiologists interested in optimizing bone health for all women.

References

1. Harvey N, Dennison E, Cooper C (2008) Epidemiology of osteoporosis. In: Rosen CJ (ed) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 7th edn. Amer. Society for Bone and Mineral Research, Washington, DC, pp 198–202
2. Cooper C, Atkinson EJ, Jacobsen SJ, O'Fallon WM, Melton LJ 3rd (1993) Population-based study of survival after osteoporotic fractures. *Am J Epidemiol* 137(9):1001–1005
3. Drake MT, Khosla S (2008) Role of sex steroids in the pathogenesis of osteoporosis. In: Rosen CJ (ed) *Primer on the metabolic bone diseases and disorders of mineral metabolism*, 7th edn. Amer. Society for Bone and Mineral Research, Washington, DC, pp 208–213
4. Yaemsiri S, Slining MM, Agarwal SK (2011) Perceived weight status, overweight diagnosis, and weight control among US adults: the NHANES 2003–2008 study. *Int J Obes (Lond)* 35(8):1063–1070
5. Shapses SA, Sukumar D (2012) Bone metabolism in obesity and weight loss. *Annu Rev Nutr* 32:287–309
6. Langlois JA, Mussolino ME, Visser M, Looker AC, Harris T, Madans J (2001) Weight loss from maximum body weight among middle-aged and older white women and the risk of hip fracture: the NHANES I epidemiologic follow-up study. *Osteoporos Int* 12(9):763–768
7. Mussolino ME, Looker AC, Madans JH, Langlois JA, Orwoll ES (1998) Risk factors for hip fracture in white men: the NHANES I epidemiologic follow-up study. *J Bone Miner Res* 13(6):918–924
8. Hinton PS, LeCheminant JD, Smith BK, Rector RS, Donnelly JE (2009) Weight loss-induced alterations in serum markers of bone turnover persist during weight maintenance in obese men and women. *J Am Coll Nutr* 28(5):565–573
9. Villalon KL, Gozansky WS, Van Pelt RE et al (2011) A losing battle: weight regain does not restore weight loss-induced bone loss in postmenopausal women. *Obesity (Silver Spring)* 19(12):2345–2350
10. Fogelholm GM, Slevänen HT, Kukkonen-Harjula TK, Pasanen ME (2001) Bone mineral density during reduction, maintenance and regain of body weight in premenopausal, obese women. *Osteoporos Int* 12(3):199–206
11. Zhao LJ, Jiang H, Papasian CH et al (2008) Correlation of obesity and osteoporosis: effect of fat mass on the determination of osteoporosis. *J Bone Miner Res* 23(1):17–29
12. Institute of Medicine (IOM) (1998) Assessing readiness in military women: the relationship of body composition, nutrition and health. In: Marriott BM (ed) *Committee on military nutrition research, food and nutrition board*. National Academy Press, Washington, DC
13. Friedl KE, Moore RJ, Hoyt RW, Marchitelli LJ, Martinez-Lopez LE, Askew EW (2000) Endocrine markers of semistarvation in healthy lean men in a multistressor environment. *J Appl Physiol* 88(5):1820–1830
14. Vestergaard P, Emborg C, Stoving RK, Hagen C, Mosekilde L, Brixen K (2002) Fractures in patients with anorexia nervosa, bulimia nervosa, and other eating disorders: a nationwide register study. *Int J Eat Disord* 32(3):301–308
15. Fried LP, Tangen CM, Walston J et al (2001) Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 56(3):M146–M157

16. Drinkwater BL, Nilson K, Chesnut CH 3rd, Bremner WJ, Shainholtz S, Southworth MB (1984) Bone mineral content of amenorrheic and eumenorrheic athletes. *N Engl J Med* 311(5): 277–281
17. Ackerman KE, Nazem T, Chapko D et al (2011) Bone microarchitecture is impaired in adolescent amenorrheic athletes compared with eumenorrheic athletes and nonathletic controls. *J Clin Endocrinol Metab* 96(10):3123–3133
18. Sukumar D, Ambia-Sobhan H, Zurfluh R et al (2011) Areal and volumetric bone mineral density and geometry at two levels of protein intake during caloric restriction: a randomized, controlled trial. *J Bone Miner Res* 26(6):1339–1348
19. Villareal DT, Kotyk JJ, Armamento-Villareal RC et al (2011) Reduced bone mineral density is not associated with significantly reduced bone quality in men and women practicing long-term caloric restrictions with adequate nutrition. *Aging Cell* 10(1):96–102
20. Baek K, Barlow AA, Allen MR, Bloomfield SA (2008) Food restriction and simulated microgravity: effects on bone and serum leptin. *J Appl Physiol* 104(4):1086–1093
21. Swift SN, Baek K, De Souza MJ, Bloomfield SA (2008) Treadmill exercise provides only short-term protection against cancellous bone loss with reduced dietary energy intake: endocrine mechanisms. *J Bone Miner Res* 23(suppl 1):M138
22. Hamrick M, Ding K, Ponnala S, Ferrari S, Isales C (2008) Caloric restriction decreases cortical bone mass but spares trabecular bone in the mouse skeleton: implications for the regulation of bone mass by body weight. *J Bone Miner Res* 23(6):870–878
23. Myburgh KH, Hutchins J, Fataar AB, Hough SF, Noakes TD (1990) Low bone density is an etiologic factor for stress fractures in athletes. *Ann Intern Med* 113(10):754–759
24. Lauder TD, Dixit S, Pezzin LE, Williams MV, Campbell CS, Davis GD (2000) The relation between stress fractures and bone mineral density: evidence from active-duty ARMY women. *Arch Phys Med Rehabil* 81(1):73–79
25. De Souza M, West S, Jamal S, Hawker G, Gundal C, Williams N (2008) The presence of both an energy deficiency and estrogen deficiency exacerbate alterations of bone metabolism in exercising women. *Bone* 43(1):140–148
26. Ihle R, Loucks A (2004) Dose–response relationships between energy availability and bone turnover in young exercising women. *J Bone Miner Res* 19(8):1231–1240
27. De Souza MJ, Williams NI (2004) Physiological aspects and clinical sequelae of energy deficiency and hypoestrogenism in exercising women. *Hum Reprod Update* 10(5):433–448
28. Nattiv A, Loucks AB, Manore MM, Sanborn CF, Sundgot-Borgen J, Warren MP, American College of Sports Medicine (2007) American College of Sports Medicine position stand. The female athlete triad. *Med Sci Sports Exerc* 39(10):1867–1882
29. Cifuentes M, Advis JP, Shapses SA (2004) Estrogen prevents the reduction in fractional calcium absorption due to energy restriction in mature rats. *J Nutr* 134(8):1929–1934
30. Delvin M, Cloutier A, Thomas N et al (2010) Caloric restriction leads to high marrow adiposity and low bone mass in growing mice. *J Bone Miner Res* 25(9):2078–2088
31. Baek K, Bloomfield SA (2012) Blocking β -adrenergic signaling attenuates reductions in circulating leptin, cancellous bone mass, and marrow adiposity seen with dietary energy restriction. *J Appl Physiol* 113:1792–1801. doi:[10.1152/jappphysiol.00187.2012](https://doi.org/10.1152/jappphysiol.00187.2012) (online publication preceding print)
32. Donnelly JE, Blair SN, Jakicic JM et al (2009) American College of Sports Medicine position stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc* 41(2):459–471
33. Wolman RL, Clark P, McNally E, Harries M, Reeve J (1990) Menstrual state and exercise as determinants of spinal trabecular bone density in female athletes. *Brit Med J* 301(6751):516–518
34. Villareal DT, Fontana L, Weiss EP et al (2006) Bone mineral density response to caloric restriction-induced weight loss or exercise-induced weight loss: a randomized controlled trial. *Arch Intern Med* 166(22):2502–2510
35. Armamento-Villareal R, Sadler C, Napoli N et al (2012) Weight loss in obese older adults increases serum sclerostin and impairs hip geometry but both are prevented by exercise training. *J Bone Miner Res* 27(5):1215–1221

36. Silverman NE, Nicklas BJ, Ryan AS (2009) Addition of aerobic exercise to a weight loss program increases BMD, with an associated reduction in inflammation in overweight post-menopausal women. *Calcif Tissue Int* 84(4):257–265
37. Swift SN, Greene ES, De Souza MJ, Bloomfield SA (2009) Thresholds for bone loss with energy restriction change with exercise status. *FASEB J* 23:720.9
38. Ehrlich PJ, Lanyon LE (2002) Mechanical strain and bone function: a review. *Osteoporos Int* 13(9):688–700
39. Lee K, Lanyon LE (2004) Mechanical loading influences bone mass through estrogen receptor α . *Exerc Sport Sci Rev* 32(2):64–68
40. Hoyland JA, Baris C, Wood L et al (1999) Effect of ovarian steroid deficiency on oestrogen receptor α expression in bone. *J Pathol* 188(3):294–303
41. Lim SK, Won YJ, Lee HC, Huh KB, Park YS (1999) A PCR analysis of ER α and ER β mRNA abundance in rats and the effect of ovariectomy. *J Bone Miner Res* 14(7):1189–1196
42. Damien E, Price JS, Lanyon LE (1998) The estrogen receptor's involvement in osteoblasts' adaptive response to mechanical strain. *J Bone Miner Res* 13(8):1275–1282
43. Lee K, Jessop H, Suswillo R, Zaman G, Lanyon L (2003) Bone adaptation requires oestrogen receptor- α . *Nature* 424(6947):389
44. Lee K, Jessop H, Suswillo R, Zaman G, Lanyon L (2004) The adaptive response of bone to mechanical loading in female transgenic mice is deficient in the absence of oestrogen receptor α and β . *J Endocrinol* 182(2):193–201
45. Saxon L, Galea G, Meakin L, Price J, Lanyon LE (2012) Estrogen receptors α and β have different gender-dependent effects on adaptive responses to load-bearing in cancellous and cortical bone. *Endocrinology* 153(5):2254–2266
46. Swift SN, Swift JM, Greene ES, Bloomfield SA (2010) Mechanical loading increases estrogen receptor alpha expression in osteocytes and osteoblasts despite chronic energy restriction. *J Bone Miner Res* 25 (suppl 1). <http://www.asbmr.org/Meetings/AnnualMeeting/Abstracts10.aspx>. Accessed 11 March 2012
47. Harmon D (1956) Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 11(3):298–300
48. Manolagas SC (2010) From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. *Endocr Rev* 31(3):266–300
49. Garrett IR, Boyce BF, Oreffo RO, Bonewald L, Poser J, Mundy GR (1990) Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. *J Clin Invest* 85(3):632–639
50. Altindag O, Erel O, Soran N, Celik H, Seleik S (2008) Total oxidative/anti-oxidative status and relation to bone mineral density in osteoporosis. *Rheumatol Int* 28(4):317–321
51. Niki E, Nakano M (1990) Estrogens as antioxidants. *Methods Enzymol* 186:330–333
52. Lean J, Davies J, Fuller K et al (2003) A crucial role for thiol antioxidants in estrogen-deficiency bone loss. *J Clin Invest* 112(6):915–923
53. Manolagas S, Almeida M (2007) Gone with the Wnts: β -catenin, T-cell factor, forkhead box O, and oxidative stress in age-dependent diseases of bone, lipid, and glucose metabolism. *Mol Endocrinol* 21(11):2605–2614
54. Almeida M, Han L, Ambrogini E, Bartell S, Manolagas S (2010) Oxidative stress stimulates apoptosis and activates NF- κ B in osteoblastic cells via a PKC β /p66^{shc} signaling cascade: counter regulation by estrogens or androgens. *Mol Endocrinol* 24(10):2030–2037
55. Rached M, Kode A, Xu L et al (2010) FoxO1 is a positive regulator of bone formation by favoring protein synthesis and resistance to oxidative stress in osteoblasts. *Cell Metab* 11(2):147–160
56. Ambrogini E, Almeida M, Martin-Millian M et al (2010) FoxO-mediated defense against oxidative stress in osteoblasts is indispensable for skeletal homeostasis in mice. *Cell Metab* 11(2):136–146
57. Muthusami S, Ramachandran I, Muthusamy B et al (2005) Ovariectomy induces oxidative stress and impairs bone antioxidant system in adult rats. *Clin Chim Acta* 360(1–2):81–86

58. Almeida M, Han L, Martin-Millan M et al (2007) Skeletal involution by age-associated oxidative stress and its acceleration by loss of sex steroids. *J Biol Chem* 282(37):27285–27297
59. Mann V, Huber C, Kogianni G, Collins F, Noble B (2007) The antioxidant effect of estrogen and selective estrogen receptor modulators in the inhibition of osteocyte apoptosis in vitro. *Bone* 40(3):674–684
60. Grassi F, Tell G, Robbie-Ryan M et al (2007) Oxidative stress causes bone loss in estrogen-deficient mice through enhanced bone marrow dendritic cell activation. *Proc Natl Acad Sci U S A* 104(38):15087–15092
61. Rao LG, Mackinnon ES, Josse RG et al (2007) Lycopene consumption decreases oxidative stress and bone resorption markers in postmenopausal women. *Osteoporos Int* 18(1):109–115
62. Weaver CM, Alekel DL, We W, Ronis MJ (2012) Flavonoid intake and bone health. *J Nutr Gerontol Geriatr* 31(3):239–253

Integrative Biology of Women's Health

Spangenburg, E.E. (Ed.)

2013, X, 212 p. 28 illus., 17 illus. in color., Hardcover

ISBN: 978-1-4614-8629-9