Skeletal muscle and bone: effect of sex steroids and aging

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IN THE PAST SEVERAL DECADES, the relevance and importance of female and male sex hormones for the health and well being of skeletal muscle and bone have become recognized. Although there are multiple sex hormones, those that have been studied the most are estrogen and testosterone. Both estrogen and testosterone are present in men and women, and both hormones exert direct and indirect effects on skeletal muscle and bone in men and women. As would be expected, testosterone values are high in men and low in women, and estrogen values are high in women and low in men.

Aging results in a highly significant loss of estrogen in women and testosterone in men. Aging also reduces the level of testosterone in women and estrogen in men. The pattern of decline differs by sex, with women showing a precipitous loss of estrogen during menopause and men losing testosterone continuously throughout life, starting in the third decade. Indeed, many men are hypogonadal by the eighth decade with free testosterone levels below 320 pg/dl, the accepted minimum (11). Women become postmenopausal typically by the sixth decade, thus spending approximately one-third of their lifetime in an estrogen-deficient state (8).

In young men and women, there are a number of conditions that cause sex hormone levels to drop to nearly undetectable levels, such as trauma, spinal injury, brain injury, and bed rest (e.g., Ref. 23). There is emerging evidence that a sedentary lifestyle and associated obesity are also associated with low sex hormone levels in men, which may indicate that hypogonadism is on the rise in our society. The long-term consequences of low hormone levels at a young age have yet to be clearly defined.

Because sex hormones are markedly reduced with age, and we are living longer, there has been recent interest in restoring hormone levels to “normal” levels in aging men and women. As expected, bringing testosterone levels above 320 pg/dl in hypogonadal old men has an anabolic effect on skeletal muscle. Significant gains in muscle mass and strength have been realized; however, testosterone hormone replacement in older men is not without penalty. Likewise, providing estrogen to older women has an anabolic effect on bone, and possibly muscle, but there may be negative consequences of giving estrogen to women in their 60s and 70s. A summary of the effects of estrogen and testosterone is shown in Table 1.

**Direct Estrogen Effects on Skeletal Muscle in Women**

There appear to be direct effects of estrogen on skeletal muscle. Skeletal muscle has estrogen receptors (ERs) on the cell membrane, in the cytoplasm, and on the nuclear membrane. It is believed that estrogen exerts direct effects on skeletal muscle through ERα, but there is a possibility that estrogen influences the maintenance and well being of skeletal muscle using other pathways such as the IGF-1 receptor-mediated pathway. Recently, a second ER type was discovered in skeletal muscle (ERβ), but its function is largely unknown, particularly in humans.

In young women and young rats, estrogen has an effect on muscle metabolism. Indeed, women have more endurance than men in long-distance running events, using less glycogen and more fat as fuel than men. Male rats given estrogen can run longer distances on a treadmill. Estrogen has also been shown to protect younger women from muscle injury apparently by stabilizing the muscle membrane (28). Whether the glycogen-sparing effects of estrogen (muscle endurance) and the protection of muscle from injury are lost with menopause is largely unexplored. It does appear anecdotally that postmenopausal women are more susceptible to muscle injury.

Is there an accelerated loss of skeletal muscle with menopause? Cross-sectional studies have suggested that women may have a faster rate of loss in muscle mass and strength during the perimenopausal years (Fig. 1). Additionally, skeletal muscle has a higher proportion of ERα on type II or fast twitch muscle fibers, which may be one reason for the greater loss in type II muscle fiber size with age. Type I or slow fibers maintain their size for most of a woman’s lifetime, whereas type II fibers begin to show atrophy in the fifth decade. It is tempting to speculate that the loss of estrogen is associated with the decline in muscle mass with aging, but the literature is difficult to interpret. There are a number of studies that support an association of muscle mass and estrogen and others that find no association between estrogen and muscle mass and strength. An example of a study in which there was no association of estrogen and muscle mass was reported by Hansen et al. (13). Women in this study were given 20-mg doses of estrogen for 64 wk, and the increase in muscle mass was not significant ($P = 0.09$). It should be noted, however, that fat mass decreased and bone mass increased significantly in subjects that received estrogen. Conversely, Sørensen et al. (26) performed a 12-wk double-blind crossover study where estrogen or placebo was administered, there was a 16-wk washout period, and women were then given whichever drug (estrogen or placebo) they had not received during the first 12 wk. There was a significant increase in lean mass during the estrogen phase.
Overall, the strength of evidence in support of an anabolic effect of estrogen on skeletal muscle via meta-analysis outweighs the evidence of no effect (2, 20).

One question that has surfaced recently is whether the accelerated loss in muscle mass with menopause is due to a decline in testosterone (total and free) rather than a decline in estrogen. Testosterone does decrease with age, but serum values of testosterone show the greatest decline before menopause and little additional change during menopause (8). Thus, there does not appear to be a relationship between muscle mass loss and lower levels of testosterone with menopause.

Most of the clinical trials that support an anabolic estrogen effect on skeletal muscle in women have tended to have younger subjects. There may be less of an effect of estrogen with advancing age, but the strength of evidence to support this contention is minimal. Two studies (5, 15) bring into question the effect of estrogen in older women. In one study, the incidence of sarcopenia (age-related muscle mass loss) was investigated in older women who had been on hormone replacement therapy (HRT) for at least 2 yr. Women on HRT had a 23% incidence of sarcopenia, whereas those not on HRT had a 22% incidence. As is the case with most studies of older women, the dose of estrogen differed among subjects, and there were a number of variables not accounted for, such as activity levels, diet, and medications. Sarcopenia takes years to develop, and it is possible that the potential benefit of HRT had not begun to show in just 2 yr of intervention. In another exercise study, older women were aerobically trained for 9 mo. Half of the subjects began HRT at the beginning of training. At the end of 9 mo, strength gains for women on HRT were the same as the gains made by women who were not on HRT (16% vs. 17%). Women in this study performed weight-bearing exercise, however, not resistance exercise, and it could be argued that the exercise stimulus was not sufficiently intense to augment the estrogen effect. In summary, possible estrogen effects on skeletal muscle in older women are not well understood.

To amplify the potential anabolic effect of estrogen on skeletal muscle, we chose a rat model in which we could combine loss of ovarian hormones with inactivity. The reasoning behind this approach is that if ovarian hormones, notably estrogen, influence muscle mass and function, then the combination of ovariectomy (Ovx) and inactivity should result in greater muscle atrophy than either condition alone. We also hypothesized that recovery from a bout of inactivity would be delayed in those rats without ovarian hormones. To induce inactivity, we used a technique called hindlimb unweighting, which simulates bed rest (Fig. 2). The hindlimbs are free to move, as in bed rest, but not bear weight. Rats were kept in this simulated bed rest condition for 28 days. Some rats were studied as controls and some were studied following the unweighting, and the remaining rats were studied following 2 wk of recovery from 4 wk of unweighting. Results for a representative hindlimb muscle, the gastrocnemius, are shown in Fig. 2. Briefly, rats with intact ovaries and those that were Ovx experienced the same degree of muscle atrophy with unweighting. In Ovx rats, however, atrophy persisted during recovery, with no evidence of an increase in muscle mass (6). To verify this finding, we unweighted another group of Ovx rats for 4 wk and allowed half of the rats to recover for 2 wk. In both Ovx groups, we restored estrogen values to within normal limits using estrogen implants. Gastrocnemius regrowth in the Ovx-estrogen-supplemented rats showed the same magnitude of muscle mass regrowth as intact rats, suggesting that estrogen was necessary to stimulate muscle regrowth following atrophy (Fig. 2B). To further pursue this possibility, we examined the Akt/mammalian target of rapamycin pathway, which is critical for muscle protein synthesis. In the Ovx-recovery rats, there was a failure to turn on the Akt pathway (Fig. 2C, no phosphorylation of Ser473), suggesting that estrogen is necessary to regrow muscle that has undergone atrophy. These results suggest that estrogen stimulates muscle regrowth, possibly through the ER or possibly through estrogen stimulation of the IGF-1 receptor (25). If these findings of failure to reverse muscle atrophy are representative of what happens in women who undergo periods of bed rest due to illness, there is cause for concern. Women lose ~50% of available muscle mass with normal aging, which is barely enough muscle mass and strength to function throughout the normal lifespan. Life events that potentially increase age-related muscle loss put women at even greater risk for loss of independence. Whether rehabilitation exercise can stimulate complete regrowth of atrophic muscle in the absence of estrogen is unknown.

Recently, a more basic approach to studying estrogen-deprived muscle was taken using Ovx mice. Using electron paramagnetic spin microscopy, a new technique, Moran and colleagues (18) determined that the muscle mass and quantity of contractile protein were unchanged in Ovx animals but muscles from Ovx mice generated less contractile tension because the interaction between actin and myosin, the essential contractile elements, was altered.

In summary, there is considerable confusion in the literature related to direct estrogen effects on muscle mass and strength, but it can be reasonably concluded that estrogen does influence muscle mass in younger women and loss of estrogen negatively impacts contractile function. A summary of the direct effects of estrogen on skeletal muscle in women is shown in Table 2.

![Bench Press](http://advan.physiology.org/) Fig. 1. Current world records for bench press for drug-tested women in the 148-lb weight class. Note the accelerated rate of strength loss that occurs during the menopause years even in women who are highly trained. [From the American Power Lifting Association.]
Indirect Effects of Estrogen on Skeletal Muscle in Women

It is possible that the indirect effects of estrogen have as much or more influence on skeletal muscle mass and function than the direct effects of estrogen. Estrogen is known to have a powerful behavioral effect on spontaneous activity, with low estrogen levels blunting the desire to be physically active. This behavioral effect is apparent from the study of Eckel et al. (10), who recorded daily spontaneous running distance in rats during each phase of the estrous cycle. There was a tripling of running distance during the estrous phase compared with the diestrous phase. Our laboratory has also recorded daily running distances in rats that were followed longitudinally when ovaries were intact, following Ovx, and following estrogen replacement therapy to restore estrogen levels to normal (Fig. 3). As shown in Fig. 3A, Ovx caused running distances to plummet within the first week, and running distance continued to decline during the entire 20 wk that rats were in an estrogen-deficient state. Estrogen replacement restored running distances to values that were normal for rats of 17 mo of age. These findings strongly suggest that women with low estrogen levels are more susceptible to inactivity-related declines in muscle mass and strength.

In addition to diminishing spontaneous physical activity, Ovx resulted in a significant weight gain (Fig. 3B) that was

Table 2. Summary of the direct effects of estrogen on skeletal muscle in women

Direct effects of estrogen on skeletal muscle in women:
- Probable maintenance of muscle mass in younger premenopausal women
- Protection from muscle injury in premenopausal women
- Probable alteration of contractile function with loss of estrogen
- Muscle metabolism (favors fat as a fuel, leading to enhanced endurance)
only partially due to a transient hyperphagia (Fig. 3C). Body weight went up almost 20% in Ovx rats, and this excess body mass was lost with estrogen replacement. One of the most prevalent complaints of women following menopause or hysterectomy is weight gain, particularly in the abdomen, even though dietary intake has not changed. These findings with rats suggest that women may not be gaining weight following menopause or with an estrogen-deficient state because of excess food intake. Estrogen regulates the amount and distribution of adipose tissue, and an increase in fat mass with low hormone values has been reported in a number of studies. Indeed, there is now strong evidence that low estrogen levels decrease energy expenditure, which would result in an increase in fat mass with no additional food intake. Day et al. (9) put 14 young females on a gonadatrophic-releasing hormone (GnRH) antagonist for 6 days to diminish endogenous estrogen release and found resting energy expenditure to decrease 5% in that short time.

In summary, findings for rats and humans suggest that low estrogen values are associated with decreased spontaneous physical activity, an increase in fat mass (particularly in the abdomen), and a reduction in resting energy expenditure. Inactivity likely exacerbates gains in fat mass, which, in turn, contributes to more inactivity. All of these factors predispose women in a low estrogen state to muscle atrophy, losses in muscle strength, and functional decline. A summary of the indirect effects of estrogen on skeletal muscle in women is shown in Table 3.

**Estrogen Effects on Bone in Women**

The positive association of estrogen and bone mass has been evident for decades. The total amount of bone mass in women is directly related to estrogen levels throughout their lifetime.
Table 3. Summary of the indirect effects of estrogen on skeletal muscle in women

Estrogen influences other systems that affect skeletal muscle mass:
- Estrogen affects the amount and distribution of fat mass. Low levels of estrogen are associated with an increase in fat mass.
- Estrogen exerts a strong behavioral effect of spontaneous activity. Low levels of estrogen are associated with low levels of physical activity.
- Increases in fat mass coupled with decreases in physical activity predispose women to lose muscle mass and strength secondary to inactivity.

There is a small amount of age-related bone loss between the ages of 25 and 50 yr, but during menopause, bone loss accelerates, with an ∼10% reduction in total bone mass in 5 yr. If estrogen is given during menopause, bone loss does not occur (Fig. 4), which is compelling justification for providing estrogen during menopause for women who are at high risk for osteopenia and osteoporosis in later years. Other factors that impact the decline in bone with age are dietary intake of calcium, exercise (those who are inactive are more likely to lose bone), medical conditions, and medications such as prednisone.

To provide a brief overview of bone, there are two primary cell types: the osteoblast, which produces bone, and the osteoclast, which breaks down bone. In a normal healthy organism, there is a balance (and communication) between the osteoblast and osteoclast such that the amount of bone being broken down is balanced with the amount of bone being secreted. Both the osteoclast and osteoblast respond to estrogen, with the osteoclast turning off bone breakdown and the osteoblast increasing bone formation.

Bone also atrophies in response to disuse. Women who engage in bone-loading exercise have more bone mineral content than women who are sedentary. Exercise results in stronger muscles, which, in turn, impose higher forces on bone that seems to stimulate bone growth. There is some evidence that estrogen and exercise have additive effects on bone in older women, which is important given the recent report that half of all women over the age of 50 yr are osteopenic. One investigator divided a sample of older frail women into two groups: one group that performed resistance and endurance exercise and one group that did home-based range of motion activities for 9 mo (controls). At the beginning of the study, all of the women began HRT. Those in the control flexibility exercise group showed a 1.5% increase in bone mineral density at the lumbar spine. Women on HRT who exercised had a 3.5% increase in bone mineral density at the lumbar spine. This study (29) demonstrates that older postmenopausal women are able to gain bone mass in response to HRT and also in response to exercise.

It should be mentioned that the Women’s Health Initiative study (http://www.whi.nih.gov/) was halted prematurely due to a higher incidence of stroke in women on HRT (estrogen and progesterone). On average, 44 of 10,000 women receiving HRT experienced stroke compared with 32 of 10,000 women not on HRT. Retrospective analyses have provided insights that were not evident at the time the decision to halt the trial was made. Women on HRT were in their 60s and 70s, rather than menopausal, and the incidence of preexisting heart disease had not been determined in subjects. Thus, the potential detrimental effects of HRT for perimenopausal and postmenopausal women are still unclear. Women in the Women’s Health Initiative receiving HRT experienced 733 fractures compared with 896 fractures among women taking a placebo. Overall, a 24% reduction in all fractures and a 33% reduction in hip fracture were observed. Hip bone density increased 3.7% in 3 yr in women on HRT, confirming the importance of estrogen for bone health (7).

A summary of estrogen effects on bone in women is shown in Table 4.

Testosterone Effects on Skeletal Muscle in Men

The profound anabolic effect of testosterone on muscle becomes evident at puberty, when boys gain ∼35% more muscle mass than girls. Testosterone stimulates myoblasts and increases the number of satellite cells, which promotes protein synthesis. Once men are in their 20s, testosterone levels begin to decline, and this decline is continuous throughout their lifetime (Fig. 5). If serum levels of testosterone fall below 320 ng/dl, men are considered hypogonadal, a common state after the age of 70 yr. While testosterone values decline with natural aging, there are a number of factors that diminish testosterone levels at all ages, including obesity, inactivity, trauma, diet, disease, and drugs.

Skeletal muscle has many androgen receptors, and when receptors are stimulated, muscle protein synthesis occurs. Androgen receptors are also responsive to IGF-1 and growth hormone, providing additional stimulation to increase muscle size. To illustrate the potent effect of testosterone, one investigator nearly obliterated endogenous testosterone production in normal young men with GnRH. These young men became hypogonadal (testosterone values of 31 ng/dl) with GnRH and remained hypogonadal for 10 wk. Before and after GnRH
administration, the quantities of lean body mass and fat mass were measured. Muscle mass significantly decreased by ~1 kg and fat mass increased proportionately such that body weight was not different at the end of the study. This study (3) illustrates the role of testosterone in the maintenance of normal body composition in men. In another study, GnRH was used to block endogenous testosterone production for 8 wk. Various doses of testosterone were given back for the 8 wk that GnRH was being given (3). Muscle mass and strength decreased in men on the low doses of testosterone but increased once testosterone levels reached the minimum of 320 pg/dl. Although none of the events were serious, the young men in this study experienced 55 adverse events, primarily prostate-specific antigen (PSA) above 4 μg/ml, hematocrits >54%, and leg edema.

A more important question is what happens when testosterone is given to older men who are already hypogonadal? Several investigators have addressed this question. Ferrando et al. (12) administered 100 mg of testosterone to six healthy men (average age: 67 yr) who were hypogonadal (defined in this study as ≤480 ng/dl) to bring testosterone levels to within normal. Following 4 wk of testosterone administration, knee extension and flexion strength was significantly increased, and the fractional synthetic rate of quadriceps muscle protein synthesis was significantly elevated. Bhasin et al. (4) administered graded doses of testosterone for 20 wk to 60 older men (60–75 yr) who were made hypogonadal following GnRH administration. The primary outcome measures were fat-free mass and maximum leg press strength. Muscle mass and strength increased in a dose-dependent manner (r = 0.77); the higher the dose of testosterone, the greater the increase in muscle size and strength. Decreases in fat mass also occurred and were inversely correlated with testosterone dose. The highest dose of testosterone increased muscle strength by nearly 50%, which has clear functional implications for the older man at risk for loss of independence. Unfortunately, there were 147 adverse events in this study, 12 of which were serious. Serious adverse events included hematocrit >54%, leg edema with shortness of breath, urinary retention, prostate cancer, and hematuria with elevated PSA. Additional side effects of testosterone administration included a dramatic drop in HDL-cholesterol, which may have long-term cardiovascular consequences, and a general overall increase in PSA values. In a recent evaluation on the safety of testosterone, Bhasin et al. concluded that “an androgen receptor modulator with anabolic properties that are free of dose-limiting adverse effects of testosterone” is needed. With so many concerned

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Table 5. Summary of testosterone effects on skeletal muscle in men

<table>
<thead>
<tr>
<th>Testosterone effects on skeletal muscle in young men:</th>
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<tbody>
<tr>
<td>● Testosterone directly stimulates muscle protein synthesis</td>
</tr>
<tr>
<td>● A huge increase in muscle mass occurs with puberty secondary to the increase in testosterone</td>
</tr>
<tr>
<td>● Hypogonadal young men have atrophied muscle. Testosterone administration increases muscle mass and strength</td>
</tr>
</tbody>
</table>

Testosterone effects on skeletal muscle in older men:

| ● Testosterone naturally decreases with age. Loss of testosterone is associated with the age-related decline in muscle mass |
| ● Testosterone given to hypogonadal older men increases muscle mass and strength |
| ● Adverse events are a major concern when testosterone is given to older men (e.g., prostate cancer, edema, high prostate-specific antigens) |

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Table 6. Summary of testosterone and estrogen effects on bone in men

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<thead>
<tr>
<th>Testosterone and estrogen effects on bone in men:</th>
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<tbody>
<tr>
<td>● Estrogen is related to bone mass in aging men, independent of testosterone</td>
</tr>
<tr>
<td>● As levels of testosterone and estrogen decrease with age, bone loss occurs</td>
</tr>
<tr>
<td>● Aging men with low testosterone and estrogen levels combined are at the greatest risk for hip fracture</td>
</tr>
</tbody>
</table>
hormones and the maintenance of bone with aging in men (27). The authors suggested that perhaps testosterone is aromatized to estrogen and that estrogen is responsible for the maintenance of bone mass with advancing age. It is recognized that estrogen and testosterone use different cellular pathways to inhibit osteoclastic activity and bone resorption, and perhaps hormone balance and pathway activation shift as hormone levels are altered with age. More recently, data from the Framingham study were analyzed for 793 men who had had serum estrogen and testosterone measures taken in the early 80s. These men were followed until 1999, and the incidence of hip fracture was calculated for those with low estrogen and testosterone. The findings indicated there were no significant increased risks for hip fracture among men with low testosterone. Men with the lowest levels of estrogen had the highest incidence rates of hip fracture. In subsequent analyses, men with low estrogen and testosterone combined had the greatest risk for hip fracture (1). Bone health in men has been minimally examined and provides an ample opportunity for future inquiry.

In summary, falling testosterone levels with age are associated with the loss of lean muscle and bone mass. Testosterone supplementation is probably not warranted for older men due to a high incidence of detrimental effects. Inactivity is likely a major factor contributing to lower testosterone values at all ages. Exercise increases testosterone levels in young men, but it is not clear if exercise has a similar effect in older men. A summary of testosterone and estrogen effects on bone in men is shown in Table 6.

Considering how little is understood about processes so fundamental, there is a need for more physiological, biochemical, endocrine, molecular, genomic, and medical knowledge on age-related changes in men and women. Skeletal muscle and bone are vitally important, and strategies to maintain the well-being of these tissues throughout the course of an average lifetime are needed.

GRANTS
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REFERENCES