HIGHLIGHTED TOPIC | Molecular Adaptations to Exercise, Heat Acclimation, and Thermotolerance

Sexual dimorphism in skeletal muscle protein turnover

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Skeletal muscle is the major constituent (>50%) of lean body mass; it is essential for the body’s locomotor function, posture, and thermoregulation, and is the major site of insulin-stimulated glucose uptake. In individual myocytes, force development is directly proportional to the physiological cross-sectional area. Accordingly, changes in muscle mass are accompanied by almost perfectly proportional increments in strength in healthy and well-conditioned muscle (39, 109). In the general population, however, there is often a dissociation between muscle mass and muscle strength because of neuromuscular adaptations to habitual activities and factors that influence muscle quality (e.g., muscle architecture, innervation, deposition of noncontractile material such as fat and connective tissue) (72, 84, 111).

REGULATION OF MUSCLE MASS

Although skeletal muscle mass is highly heritable—reported heritability estimates range from ≥85% in children, adolescents, and young adults (66, 108) to 50-85% in middle-aged and older adults (4, 85, 96)—it is a highly dynamic organ and can rapidly increase or decrease in size. In healthy people at rest, muscle protein turns over (is renewed/remodeled) at a rate of approximately 1–2% per day. When muscle mass is constant, the rate of muscle protein synthesis (MPS) over the course of a day matches the rate of muscle protein breakdown (MPB). An imbalance between MPS and MPB results in either a net gain (MPS > MPB) or net loss (MPB > MPS) of muscle mass. In healthy people, contractile/physical activity and food intake are the major regulators of MPS and MPB (92) as outlined in Fig. 1 (top). During basal, postabsorptive conditions (overnight fast), the rate of MPB exceeds the rate of MPS, resulting in net loss of muscle protein. Meal intake compensates for these losses because hyperaminoacidemia/protein ingestion and insulin stimulate MPS and inhibit MPB, resulting in net muscle protein accretion. The net muscle protein anabolic response to a meal is largely determined by the amount of protein ingested because amino acids stimulate MPS in a dose-dependent manner up to ~20 g of protein (“ceiling”), whereas the concentration of insulin necessary to achieve maximal suppression of MPB (~15–30 μU/mL) already occurs after consuming a small amount of carbohydrate or protein (11, 43, 82). Exercise (both resistance and endurance) stimulates both MPS and, to a lesser extent, also MPB; consequently, net muscle protein balance after a bout of exercise in the postabsorptive state remains negative, whereas the net protein balance becomes positive after exercise in the postprandial state and exceeds the net balance after meal intake alone (83). Conversely, muscle disuse suppresses MPS (117) and initially (within the first few days) upregulates but then downregulates markers of proteolysis (25, 107). Accordingly, resistance exercise training can rapidly increase muscle size [by approximately 5–10% within 3–6 mo (2, 70)] whereas muscle disuse (as a result of casting or joint immobilization or even just reduced ambulation) rapidly results in loss of muscle mass [approximately 2–5% per wk (117)]. Inadequate protein intake...
An imbalance between MPS and MPB results in either a net gain (MPS > MPB) or net loss (MPB > MPS) of muscle mass. During basal, postabsorptive conditions (overnight fast), the rate of MPB exceeds the rate of MPS, resulting in net loss of muscle protein. Meal intake compensates for these losses because protein/amine acids and insulin stimulate MPS and inhibit MPB, resulting in net muscle protein accretion. Exercise (both resistance and endurance) stimulates MPS, and the net protein balance after exercise in the postprandial state exceeds the net balance after meal intake alone. Conversely, muscle disuse suppresses MPS and initially (within the first few days) upregulates but then downregulates markers of proteolysis and blunts the anabolic response to hyperaminoacidemia/protein ingestion. There are no established sex differences in the regulation of muscle protein turnover in young and middle-aged adults. Bottom: aging is associated with an increase in the basal rate of MPS in women (but not in men) and both men and women have a blunted MPS response to amino acids/protein and exercise but the reduction is greater in women than in men. Potential differences in MPB between older men and women have yet to be evaluated.

**SEXUAL DIMORPHISM IN MUSCLE MASS**

At any given total body weight, young and middle-aged women have less muscle mass and more body fat than age-matched men (65, 78) and are therefore not able to exert the same maximal force as men (38, 40); however, strength relative to muscle cross-sectional area or muscle mass is nearly the same in men and women (67, 73). The difference in body composition between the sexes is evident from infancy but becomes most marked after puberty (when boys experience an accelerated growth spurt) and persists into old age (63, 120) (Fig. 2). The aging-associated loss of muscle mass in women coincides with the onset of menopause, is accelerated during the transition into menopause (3) and then proceeds at a slower rate (~0.25% per year) than in men (~0.40% per year) (37, 62). The hypertrophic response to exercise training and the atrophic response to muscle disuse are similar in young men and women (54, 129), but both are reduced in older women compared with older men (10, 15, 20).

**SEXUAL DIMORPHISM IN MUSCLE PROTEIN TURNOVER**

Young and Middle-Aged Adults

Several groups of investigators, including our own, have compared the rates of muscle protein (mixed or myofibrillar fraction) turnover in young and middle-aged (18–45 yr old) men and women by using the traditional primed, constant amino acid infusion technique to calculate the fractional turnover rate of muscle protein (i.e., by evaluating the rate of
incorporation of the tracer amino acid into muscle protein relative to the enrichment of a validated precursor amino acid pool for protein synthesis with the tracer amino acid; none found differences between the sexes in the rates of MPS and anabolic signaling in muscle during basal postabsorptive conditions (27, 41, 57, 69, 86, 99, 101, 121) or the acute response to nutritional stimuli (99, 101, 121) or resistance-type exercise (27, 74, 121). Measurement of MPB in vivo in people is technically challenging and only two studies to date have compared the rate of MPB in men and women (41, 57). Both found that the basal rate of MPB is not different in young men and women. Two studies compared surrogate markers of proteolytic activity in muscle (e.g., the expression of genes encoding ubiquitin-mediated proteolysis pathway components) and under basal conditions at rest also found no difference between the sexes (121, 122). Taken together, these data are consistent with a stable muscle mass during young and middle-age adulthood (i.e., a state in which MPS and MPB reflect remodeling of muscle but do not result in net growth or loss) and similar hypertrophic responses to resistance exercise training in men and women (1, 54, 93, 118). To our knowledge, the MPS and MPB responses to atrophy-inducing stimuli (reduced activity or protein intake) have not been compared in men and women. It is possible, but unlikely that muscle protein turnover responses during atrophy are different in men and women, because the net atrophic response to muscle disuse is not different in young men and women (129).

In a unique study, Scalzo et al. (95) compared the 4-wk-long cumulative MPS rate during sprint interval training in men and women by using a novel, $^2$H$_2$O-based method. Mixed, cytosolic, and mitochondrial protein fraction synthesis rates were measured over the entire 4-wk period by 1) using gas chromatography/mass spectrometry (GC/MS) analysis of alanine in proteins and 2) using liquid chromatography/mass spectrometry (LC/MS) analysis of dozens of trypptic peptides of positively identified muscle proteins. They reported that the MPS rate in the mixed, cytosolic, and mitochondrial fractions (based on the GC/MS analysis) was almost 50% greater in men than in women and the synthesis rates of individual proteins (analyzed by LC/MS) was ~10% greater in men than women. These results raise the possibility that it requires a substantial amount of time to capture potentially subtle but important differences in muscle protein turnover between men and women that are missed when using the traditional, short-term (several hours) primed, constant amino acid infusion technique to calculate the fractional turnover rate of muscle protein. However, the $^2$H$_2$O method used by Scalzo et al. (95) has some limitations that need to be considered when interpreting the results. First, the method used by Scalzo et al. (95) relies on body water enrichment as the precursor enrichment for MPS, whereas the true precursor enrichment for MPS is that of the tRNA-bound amino acids in muscle. Accordingly, the reported synthesis rates reflect the fraction of protein synthesized de novo (by incorporating hydrogen from body water into the precursor amino acid alanine, which is then incorporated into muscle protein), not the total rate of protein synthesis (which includes protein synthesis from newly synthesized amino acids as well as “recycled” amino acids). Moreover, there is sexual dimorphism in alanine metabolism (41, 52, 94) that could affect the contribution of de novo synthesized alanine to the total plasma alanine pool or the plasma-to-tRNA enrichment ratio and hence the enrichment of alanine in the direct precursor pool for protein synthesis in muscle relative to the body water enrichment. It is therefore possible that the differences in protein turnover reported by Scalzo et al. (95) are attributable to differences in the alanine and body water precursor enrichment equilibrium (due to differences in alanine metabolism between men and women) rather than to actual differences in muscle protein synthesis (i.e., the rate of incorporation of alanine into muscle protein) between men and women. Lastly, Scalzo et al. (95) do not provide baseline data, at rest or data from nonexercising control groups; therefore, it is unclear whether the reported differences represent a differential response to exercise training or reflect baseline differences in muscle protein turnover between men and women in their cohort.

**Older Adults**

Few studies have evaluated potential differences in muscle protein turnover between older men and older women, and all of them so far have focused solely on MPS; potential differences in MPB between older men and women have been evaluated only by assessing surrogate markers of proteolytic activity (e.g., muscle MAFbx, MuRF1, and FOXO3A mRNA expression), which do not reliably reflect actual rates of MPB (43).

In a series of studies, we have demonstrated that aging affects MPS differently in men and women. In carefully matched young and older men and women, we found that the basal rate of MPS is greater in older women than older men, and older women, compared with older men, have a blunted anabolic response to mixed meal ingestion, combined intravenous infusion of amino acids and insulin, and exercise training (100–102). In addition, we found no difference in the basal rate of MPS between healthy young and older men but a greater rate of MPS in older compared with young women (101, 103). Older men and women both exhibited a blunted anabolic response to nutritional stimuli compared with young men and women (101). These findings are consistent with a slower rate of muscle loss in older women compared with older men (37, 62) [because the greater basal rate of MPS compensates for the
greater anabolic deficit in women than in men (100, 101) and the blunted hypertrophic response to exercise training in older women compared with older men (10, 15). The greater basal rate of MPS in older women in our study was associated with a greater capacity for protein synthesis [i.e., more ribosomal RNA (77)] and a more active translational process at the elongation stage of protein synthesis (40% reduced phosphorylation of muscle eEF2Thr56) (100). In addition, we and others found an age-related upregulation of stimulatory muscle growth regulatory genes in women (91, 103) but not in men (29, 59). The reduced anabolic response to feeding in older women, on the other hand, was associated with reduced stimulation of translation initiation (phosphorylation of muscle eIF4EThr205 and eIF4E-BP1Thr74/76) (100). In addition, it has been reported that older women, but not older men, have an impaired hyperemic response to exercise (64, 87), which could limit amino acid supply to muscle and thereby blunt the anabolic response to exercise in older women.

Bukhari and colleagues (18) studied only older women and, consistent with the results from our studies, reported basal myofibrillar protein synthesis rates that are at least 50% higher (~0.07%/h) than those reported by the same group of investigators and others for older men (0.025–0.043%/h) (24, 61, 80, 126, 127) and young men and women (0.026–0.041%/h) (5, 7, 24, 42, 125). Moreover, the anabolic response to ingestion of 20 g of whey protein (~25%) in their study was much less than that reported for older men (~65%) in studies that used the same experimental protocol (19, 127). Hansen et al. (49), also studied older women only, and they too reported a much higher basal myofibrillar protein synthesis (~0.08% per h) than typically observed in older men and no increase in response to exercise in older women.

Henderson and colleagues (53) evaluated basal rates of MPS in young and older men and women and found a greater rate of MPS in women than in men but no sex × age interaction. However, that study included young men and women who were healthy, but only older men with hypogonadism and older women with low serum dehydroepiandrosterone concentration, which may have confounded the results. Hypoandrogenemia is associated with a reduced lean body mass (58), and treatment with testosterone increases the muscle protein synthesis rate (14, 45, 103, 113).

Markofski and colleagues (69) performed a post hoc analysis of historic basal MPS rate measurements in young and older men and women made over a 10-yr period and found no difference between young and older subjects and men and women. However, there are reasons to believe that this study design made it impossible to pick up the ~30% greater MPS rate in older women (0.058%/h) compared with older men and young men and women (all ~0.045%/h) we (99–103) and others (18, 49) have reported. First, the average MPS values for individual years ranged from ~0.055%/h in 2009 to ~0.075%/h in 2005, and the variance of the MPS rate measurements during the early years was nearly double that in the later years. This suggests that either there were considerable differences among the various cohorts (possibly due to differences in their sex and age distribution) or technical reasons that resulted in less precise measurements early on. Second, the use of oral sex steroid preparations, which can affect MPS (see sex hormone effects on muscle protein turnover), was not an exclusion criterion.

In addition, several investigators have compared the basal rates of MPS between young and older men only and reported no difference or a lower basal MPS rate in older compared with young men (e.g., 24, 28, 61, 116, 119); only one other study, to our knowledge, has compared MPS rates in young and older women only and found no difference (23).

In summary, the composite of the results obtained in older men and women suggest that aging is associated with an increase in the basal rate of MPS in women (but not in men), and that both older men and older women (compared with young men and women) have a blunted MPS response to amino acids/protein and exercise but the reduction is greater in women than in men (Fig. 1, bottom).

**SEX HORMONE EFFECTS ON MUSCLE PROTEIN TURNOVER**

Considering the well-known anabolic effect of testosterone, which is mediated by its stimulatory effect on MPS (14, 45, 103, 113) and possibly also an inhibitory effect on MPB (34), it may seem surprising that there are no differences in muscle protein turnover between young men and women (see sexual dimorphism in muscle protein turnover, young and middle-aged adults). This is most likely due to the fact that during young and middle-age adulthood when muscle mass is constant, MPS and MPB simply reflect turnover over a stable muscle mass (i.e., remodeling of muscle). In fact, the rapid growth of lean body and muscle mass during adolescence and early adulthood in boys is most certainly mediated by the surge in testosterone secretion (36, 55), whereas growth of lean body and muscle mass in girls simply proceeds in parallel with overall skeletal growth (i.e., constant relative to height); the surge in ovarian hormone production during puberty in girls stimulates growth of fat mass but does not suppress lean tissue and muscle growth (120). Together, the continued growth in lean body and muscle mass in adolescent boys and the increase in fat mass in adolescent girls result in the well-established sexual dimorphism in body composition in adulthood. On the other hand, it is intriguing that differences in muscle protein turnover between men and women become apparent in older age (see sexual dimorphism in muscle protein turnover, older adults) because the loss of testosterone with aging in men, although significant, is very small (51, 116) compared with the change in female sex steroid availability at the onset of menopause. This would suggest that the female sex steroids, estrogens and progesterone, are important regulators of muscle protein turnover. Indeed, studies conducted in rodents suggest that female sex steroids suppress MPS (110). Interpretation of these results, however, is complicated by the ovariectomy-induced changes in physical activity and body weight in rats (35, 44). The results from studies that evaluated the effect of female sex steroids on muscle protein turnover in people are conflicting. Several studies found that the basal rate of MPS is greater in older, postmenopausal compared with young, premenopausal women (101, 103); lower in women who received estrogen replacement therapy after surgically induced menopause than age-matched postmenopausal women (49); and lower in women who use certain types of oral, hormonal contraceptives (i.e., Lindyette) compared with those who do not (47). These results are consistent with a suppressive effect of female sex steroids on MPS. On the other hand, numerous other studies found no difference in the rate of MPS between...
women who use certain other oral steroidal contraceptives (i.e., Cilest) and those who do not (47), and no difference between women who were studied during the follicular and luteal phases of their menstrual cycles (74), which would suggest that female sex steroids are not important regulators of muscle protein turnover. It is possible that menstrual cycle and oral contraceptive use-induced changes in female sex steroid availability during young and middle-age adulthood are often too subtle compared with the prevailing hormone milieu, which makes it difficult to evaluate their effect until cessation of ovarian function with menopause. Interpretation of the results from these studies is also difficult because of their cross-sectional design, the difference in progestin potency in various oral contraceptive formulations, and potential confounding influences [e.g., differences in free testosterone, IGF-1, and insulin concentrations between hormone-treated and untreated women (47, 49)], which may have been induced by the oral hormone treatments (97, 104) that were used in those studies. To avoid these confounding influences, we recently evaluated the effects of systemically delivered estradiol and micronized progesterone and found that transdermally delivered estradiol has no effect on MPS or the expression of several genes in muscle that encode proteins involved in the regulation of muscle mass, whereas vaginally delivered micronized progesterone has potent stimulatory effects on MPS and MYOD1 mRNA expression (103). Congruent with the results from our study, other investigators found that estradiol has no effect on basal muscle growth stimulatory factor expression in ovariectomized female and orchidectomized male mice and rats (30, 31, 106, 112, 114), whereas combined (estradiol plus progesterin) hormone replacement stimulates growth regulatory gene expression in muscle of postmenopausal women (26, 90). In addition, the results from studies conducted in rodents demonstrate that 1) both testosterone and estrogen can counteract the disuse-induced loss of muscle atrophy (32, 105, 124, 128), 2) estrogen is necessary for the recovery of muscle loss in ovariectomized rats (16, 71, 98) and augments muscle myoD expression during recovery (114), and 3) hormone replacement therapy stimulates myogenic gene expression after muscle-damaging eccentric exercise in postmenopausal women (26). Testosterone, on the other hand, does not affect recovery from disuse (50). In summary, these results suggest that during adulthood testosterone and progesterone are probably important for muscle maintenance, whereas estradiol could be a critical mediator for muscle growth following/during periods of muscle atrophy (Table 1); this helps explain the blunted anabolic response to exercise training in older women compared with younger women and old men.

## NONCONTRACTILE PROTEINS

Little is known about the turnover of noncontractile proteins that provide structural support and are critical for force transfer from the muscle to the skeleton and joint stabilization (i.e., collagenous proteins of the extracellular matrix and tendons and ligaments). Overall, the turnover rate of these proteins is similar to that of contractile proteins (~0.05%/h) and they are very responsive to changes in muscle loading (they increase with exercise and decrease with unloading) (25, 76, 81). However, unlike contractile proteins, they are not responsive to nutritional stimuli (8, 79). Muscle collagen turnover is not different in men and women at rest, and increases to the same extent in response to exercise in young men and women; however, the effect of exercise is blunted in postmenopausal women and may be restored with estrogen replacement therapy (49, 74, 89). Tendon collagen turnover, on the other hand, is ~50% slower in women than in men, and women (unlike men) do not increase tendon collagen synthesis in response to exercise, regardless of age (46, 48, 75, 89). This could have detrimental consequences because it might adversely affect the balance between force-producing (contractile proteins) and force-transmitting proteins (tendons) and could help explain the greater risk of soft tissue injury during physical activity in women than men. The effect of sex hormones on tendon collagen turnover has been assessed by only two studies and the results are equivocal (46, 89).

### SUMMARY, CONCLUSION, AND FUTURE PERSPECTIVES

Women have less muscle mass than men, and the greater muscle mass in men is most likely due to a testosterone-driven growth spurt in adolescence. During young and middle-age adulthood, when muscle mass is stable, there are no differences in muscle protein turnover between men and women, either at rest or after exercise. However, the age-associated decline in muscle mass, which starts around the fourth decade of life, is slower in women than in men and older women appear to be less responsive to the hypertrophic effect of exercise training. The specific mechanisms responsible for this phenomenon are not entirely clear but the relative preservation of muscle mass in older women compared with older men appears to be driven by an age-associated increase in the expression of growth regulatory genes and MPS, whereas the blunted hypertrophic response to exercise training in older women may, at least in part, be due to an impaired hyperemic response and limited substrate supply for MPS. How much of the differences between men and women can be attributed to differences in the sex hormone milieu is unclear because the results from studies evaluating the effect of sex steroids are equivocal and often difficult to interpret because orally delivered synthetic sex steroids can cause changes in circulating anabolic and catabolic hormone concentrations. In addition, women of all ages fail to adapt tendon collagen synthesis to increased loading, which might adversely affect the balance between force-producing (contractile proteins) and force-transmitting (tendons) proteins. Carefully designed and adequately powered prospective stud-

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**Table 1. Effects of sex hormones on regulation of skeletal soft tissue mass**

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<th>Testosterone</th>
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<td>Recovery from disuse</td>
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<td>Tendon collagen synthesis</td>
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<td>Rest</td>
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<td>Response to exercise</td>
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↑ indicates stimulatory effect; ↓ indicates inhibitory effects; - indicates no effect; ? indicates effect not known; multiple symbols indicate equivocal findings.

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ies that focus on the combined study of MPS and MPB in older men and women are needed to fully appreciate the age-associated differences in muscle protein turnover while mechanistic studies (including, for example, exercise and nutritional dose-response studies and sex hormone interventions) will help delineate the sex-specific pathophysiology of sarcopenia.

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AUTHOR CONTRIBUTIONS
B.M. conceived and designed the research; G.I.S. and B.M. drafted manuscript; G.I.S. and B.M. edited and revised manuscript; B.M. approved final version of manuscript.

REFERENCES


69. McCracken AJ, Cameron-Smith D, Poppitt SD. It is not just muscle mass: a review of muscle quality, composition and metabolism during...
Review