

# Phytoecdysteroids: A Novel, Non-Androgenic Alternative for Muscle Health and Performance

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## Introduction

Skeletal muscle is the most abundant tissue in the adult human body, comprising more than 40% of total body mass in normal, healthy individuals [1], and is required for movement and locomotion. Muscle mass (volume) is an important determinant of muscle function, such that physiological cross-sectional area is correlated to peak isometric force [2,3]. The maintenance of skeletal muscle mass exists as a delicate balance between the rates of muscle protein synthesis and muscle protein degradation. Rates of muscle protein synthesis and degradation are finely tuned according to activity level, nutrient availability, and health status. For example, rates of muscle protein synthesis are positively influenced by exercise and nutrition, and negatively regulated by inactivity (e.g. disuse), aging (i.e. sarcopenia), and muscle wasting-related diseases (e.g. cancer) [4]. The PI3K/Akt signaling pathway controls both catabolic and anabolic mechanisms in skeletal muscle. Activating this pathway through exercise and nutritional interventions has a positive effect on skeletal muscle. Phytoecdysteroids (PEs) are natural steroid analogs synthesized by many plant species that possess biological, pharmacological, and medicinal properties with no known side effects in mammals [5], and are believed to elicit anabolic effects on skeletal muscle, in a non-androgenic manner, via activation of the PI3K/Akt signaling pathway.

Activated Akt has at least two anti-catabolic effects: inhibition of glycogen synthase kinase (GSK)-3 $\beta$  [6] and inhibition of the ubiquitin proteasome system [7]. Eukaryotic initiation factor-2B (eIF-2B), a key regulatory factor of protein translation initiation [8], is a suppressed by activated GSK-3 $\beta$  [9], thus inhibiting protein synthesis [10]. Further, activated Akt can suppress an aspect of skeletal muscle atrophy regulated by the ubiquitin proteasome system [7]. Proteasomal degradation requires ligation of ubiquitin to proteins by E3 ubiquitin ligases. Polyubiquitinated proteins are then marked for degradation by the proteasome. Forkhead box O1/O3 (FOXO1/3) regulates the transcription of the E3 ligases muscle ring finger 1 (MuRF-1) and muscle atrophy F box (MAFbx, aka Atrogin-1) [11]. Activated Akt phosphorylates FOXO proteins, resulting in their export from the nucleus; thus suppressing transcription of the E3 ligases and muscle protein degradation [7].

During skeletal muscle hypertrophy, activated Akt stimulates mammalian target of rapamycin (mTOR) [12]. The mammalian target of rapamycin complex (mTORC1) functions as an intracellular signaling hub that stimulates muscle protein synthesis and other essential cellular processes [13]. Resistance exercise training, growth factors (insulin and insulin-like growth factor), and nutritional interventions, such as supplementation of essential amino acids, are all well-known anabolic stimuli whose signaling mechanisms converge upon mTORC1 to stimulate muscle protein synthesis [13]. In contrast, the highly anabolic nature of testosterone is independent of mTORC1 in stimulating hypertrophy and protein synthesis in skeletal muscle [14]. This review will focus on the potential of PEs to modulate these pathways and the implications of PEs for skeletal muscle health.

## Anabolic Effects of Phytoecdysteroids

Ecdysteroids are a class of steroid hormones originally discovered in insects that control molting and other metamorphic processes, but since have been identified to regulate many biochemical and physiological processes in both invertebrates and vertebrates [15]. Analogs of ecdysteroids, called phytoecdysteroids, are found in high abundance in a variety of plant species, such as *Ajuga*, *Serratula*, *Silene*, and *Leuzea* [5] and the commonly consumed spinach (*Spinacia oleracea*) [16]. While their function in plants is still conjectural, it is believed that plants synthesize PEs to provide protection against plant-eating insects [17]. A vast amount of research (most of which full text versions are unavailable in English) suggests that PEs possess a broad spectrum of biological, pharmacological, and medicinal properties in mammals, with no known adverse side effects [5]. PEs elicit anabolic, hepatoprotective, immunoprotective, antioxidant, and hypoglycemic effects. Furthermore, PEs are considered adaptogenic by enhancing physical performance, promoting vitality, and enhancing resistance to stress [5]. In general, PEs are characterized as polyhydroxylated basic carbon ring structures of 27-29 carbon atoms [15]. To date, approximately 250 variants of PEs have been identified, but the most widely investigated and physiologically significant PE appears to be 20-hydroxyecdysone (20E).

The anabolic effects of PEs, primarily via increases in protein synthesis, have been thoroughly described in various tissues of mammals [15]; however, less is known about the anabolic effects of PEs on skeletal muscle tissue. Folklore describes Siberians consuming hardy plants (now identified as containing high levels of PEs) to enhance stamina and ward off fatigue. Early research in this area demonstrated that a plant extract enriched in PEs increases skeletal muscle mass in young adult and growing rats [18]. More recently, 20E has been reported to increase strength in young rats and stimulate protein synthesis in muscle cells *in vitro* [19]. Additionally, longer treatments (five to seven days) of 20E increases muscle mass in young mice [20] and rats [21], respectively. Most intriguing is that 20E, unlike testosterone, elicits anabolic growth effects on tissues without androgenic side effects, e.g. increased prostate or seminal vesicle mass [22,23]. It is believed

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that 20E works via a G protein-coupled receptor to activate the PI3K/Akt signaling pathway, resulting in mTORC1-mediated protein synthesis [24].

### Implications for Use as an Anti-Sarcopenic Therapy

The involuntary loss of skeletal muscle mass and strength that occurs as we age is termed sarcopenia. Sarcopenia is a major health burden because it is associated with functional disability, loss of independence, and premature death [25-27]. Traditional pharmacological treatments for sarcopenia, such as hormone replacement therapy, are not entirely effective or have negative side effects. With aging, the rate of skeletal muscle protein synthesis declines [28], predominately due to decreased activation of the PI3K/Akt/mTORC1 signaling pathway [29]. Furthermore, the ability of anabolic stimuli (e.g. essential amino acid supplementation and resistance exercise training) to activate the PI3K/Akt/mTORC1 signaling pathway and muscle protein synthesis is blunted in aged skeletal muscle, a condition known as "anabolic resistance". Given that PEs may activate skeletal muscle protein synthesis through a non-androgenic, G protein-coupled receptor pathway, the therapeutic potential for PEs to treat anabolic resistance and to be used of as an anti-sarcopenic therapy is encouraging.

### Potential Aid for Post-Damage Muscle Regeneration

Another potential therapeutic effect of PEs is their influence on systemic and cellular processes that influence muscle injury and subsequent regeneration. Evidence suggests that post-injury supplementation with PEs has a beneficial effect on skeletal muscle regeneration by increasing the size of the regenerating fibers [21]. Of course, any agent with known effects on the muscle hypertrophy-promoting Akt pathway, as many PEs have, are good candidates for therapeutic treatment of muscle injury, but additional properties of phytoecdysteroids, such as their proposed anti-inflammatory and anti-fibrotic properties, might also have beneficial effects on muscle regeneration.

The negative effects of heightened inflammation on skeletal muscle are well documented [30], and it has been noted that attenuating excessive inflammation, especially through the use of natural supplements, can be beneficial to muscle regeneration [31]. Several PEs have been shown to have potent anti-inflammatory properties in experiments utilizing known pro-inflammatory factors [32,33]. Skeletal muscle exhibits enhanced proteolysis in response to the pro-inflammatory agent 12-O-tetradecanoylphorbol-13-acetate (TPA) [34]. TPA likely upregulates the ubiquitin proteasome pathway through NFκB. NFκB is a molecular regulator that has been implicated in slowing of the muscle regeneration process [35]. However, Sun and Yasukawa have proven that several PEs have inhibitory effects on TPA-induced inflammation [32]. Likewise, carrageenan is a known pro-inflammatory agent which negatively affects muscle strength [36]. Ochieng et al. have noted a powerful inhibitory effect of isolated PEs on carrageenan-induced inflammation [33], but others have suggested that some PEs do not have anti-inflammatory properties [37,38]. While these results are not definitive proof that PEs can modulate skeletal muscle damage due to inflammation, further research is certainly warranted to explore the potential therapeutic potential of PEs to modulate skeletal muscle inflammation and enhance regeneration.

Skeletal muscle fibrosis is often a negative consequence of the regeneration response following injury [39]. Dysregulation of TGF-β1 and SMAD signaling [40], as well as aberrant matrix metalloproteinase activity [41], contribute to fibrosis and prevent optimal functional

regeneration of skeletal muscle. Preventing the formation of this excessive connective tissue helps to better restore function to the injured muscles [42]. Unfortunately, these effects have yet to be studied in a model of skeletal muscle damage utilizing PEs as a potential treatment. Evidence from other tissues, such as skin [43] and kidney [44], implicates PEs as potential regulators of fibrosis through their actions on matrix metalloproteinases and TGF-β1. The logical next step in understanding the effects of PEs on muscle damage is to explore their role in aiding regeneration through these same mechanisms by which they benefit other tissues.

### Implications for Athletic Performance Enhancement

Synthetic forms of anabolic-androgenic steroids have been extensively studied for possible enhancement of athletic performance [45]. However, several adverse side effects have been documented with their usage [46]. As an alternative, anecdotal evidence indicates that various sport coaches may have experimented with deriving these effects from PEs. While there are several structural similarities between PEs and mammalian steroid hormones, they differ in the mechanism by which they induce anabolic signaling transduction [5]. As previously mentioned, in humans PEs possess anabolic, hepatoprotective, immunoprotective, and hypoglycemic effects. Limited data in humans is available as to whether the anabolic effects would be significant enough to translate into meaningful changes in actual athletic performance. Comparisons between PE and anabolic steroid effectiveness have occurred primarily in animal models. One investigation reported that both PEs and methandrostenolone stimulate biosynthesis of myofibrillar proteins to a similar extent in mice [47]. Studies in human models are limited, but Wilborn et al. [48] examined PE supplementation on changes in muscle strength, muscular endurance, anaerobic capacity, and body composition in resistance-trained males. This study reported no significant effect of PE supplementation on any of the measured variables. There are currently many commercially available nutritional supplements on the market that contain PEs and PEs are also present in several food sources such as spinach, quinoa, and suma root. However, the World Anti-Doping Agency currently has PEs listed on the banned substance list for their use as performance enhancement, therefore use by athletes under this governing body is not allowed. While the actual effectiveness of PEs on human athletic performance is still questionable, further research is needed to determine the performance enhancement of PEs in human models.

### Conclusion

An abundance of data exists on the anabolic effects of PEs in rodent models with limited adverse side effects. The non-androgenic nature of PEs on tissues makes investigation of PEs on skeletal muscle and their potential use for attenuating sarcopenia, treating post-damage muscle regeneration, and enhancing athletic performance intriguing. However, data from supplementation of PEs in human models are still limited. While the potential use of PEs for enhancing skeletal muscle health and performance is promising, conclusions are tentative.

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