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REVIEW ARTICLE

Endocrinological Roles for Testosterone in Resistance Exercise Responses and Adaptations

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Abstract Chronic increases in testosterone levels can significantly increase hypertrophy and strength, as has been demonstrated by pharmacological intervention. However, decreases in basal testosterone levels can have the opposite result, as has been seen in hypogonadal populations. Because of these profound effects on hypertrophy and strength, testosterone has often been studied in conjunction with resistance exercise to examine whether the endocrine system plays a role in adaptations to the stimulus. Whereas some studies have demonstrated a chronic increase in basal testosterone, others have failed to find an adaptation to regular resistance exercise. However, improvements in strength and hypertrophy appear to be possible regardless of the presence of this adaptation. Testosterone has also been shown to acutely rise immediately following an acute resistance exercise bout. While this substantial mobilization of testosterone is brief, its effects are seen for several hours through the upregulation of the androgen receptor. The role of this acute response at present is unknown, but further study of the non-genomic action and possible intracrinological processes is warranted. This response does not seem to be necessary for resistance training adaptations to occur either, but whether this response optimizes such adaptations has not yet been determined.

Key Points

Neither an acute rise in testosterone following resistance exercise nor a long-term basal increase in testosterone is absolutely necessary to experience gains in strength and hypertrophy.

The rise in testosterone associated with an acute bout of resistance exercise is later followed by an upregulation of the muscle androgen receptor, as well as a subsequent drop in testosterone below baseline levels. This combination of responses may be indicative of the movement of testosterone from the blood to the muscle following resistance exercise.

The role of the acute mobilization of testosterone and its incorporation into the muscle has yet to be determined, but may be related to the non-genomic action of androgens and thus may play a role in the optimization of strength and hypertrophy.

1 Introduction

The development of strength and hypertrophy is sought by a wide range of populations, spanning those experiencing a muscle-wasting disease to elite athletes aiming to optimize human performance. One such means to develop strength and hypertrophy is that of resistance exercise, where careful manipulation of the acute program variables can lead to substantial improvements [1]. In addition, strength and hypertrophy can be gained through the use of testosterone supplementation, even in the absence of resistance exercise, in both young [2] and older men [3]. Owing to the

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anabolic nature of the testosterone hormone, its concentration in human blood has often been measured in conjunction with resistance exercise, either during or immediately after individual bouts of resistance exercise, or to assess whether changes in basal levels exist following several weeks or months of regular training. Such studies have been conducted to assess whether this increased exposure to testosterone is playing a role in the adaptations of strength and hypertrophy to resistance exercise. If these adaptations are indeed mediated by testosterone, either through repeated acute exposures during the exercise bout, or through a chronic upregulation of testosterone, then the manipulation of resistance training programs to specifically increase testosterone responses could help to optimize the development of strength and hypertrophy. The purpose of this review is to synthesize the literature that pertains to the potential role that the endocrine system, and testosterone in particular, plays in adaptations to resistance exercise, as well as to identify important areas for future research.

2 Background

To fully understand the practical applications of testosterone to exercise, it is important to first cover the fundamental concepts of testosterone synthesis, secretion and action.

2.1 Testosterone Synthesis

Each step of the synthesis of testosterone is shown in Fig. 1. The precursor to all steroid hormones is cholesterol, the synthesis of which is complex, with an exorbitant energy cost. The substantial physiological effort required for the circulation of this hormone, therefore, suggests that its role must be valuable, otherwise this process would be seemingly wasteful.

Following the conversion of cholesterol to pregnenolone, there are several possible pathways the body can use to produce androstenedione. However, the human body appears to preferentially use the pathway that involves the conversion of pregnenolone to dehydroepiandrosterone (DHEA), which occurs at approximately a fourfold greater rate than the pathway involving progesterone [4]. Androstenedione is then converted to testosterone via 17-hydroxysteroid-3 (17HSD3) in the testis in men, and also via 17-hydroxysteroid-5 (17HSD5) in women in the ovary [5] and mammary gland [6]. Finally, cytochrome P450 family 19 (often called aromatase) converts testosterone to estradiol in the Leydig cells of the testicles in men or in the ovaries in women.

While it is often stated that 90–95% of total androgen production is from the testes in adult men, it has been well documented that tissue such as the prostate can produce approximately 50% of its own androgens, without a significant release of the hormone into the circulation [7]. This process can occur through so-called peripheral conversions, where a precursor, such as DHEA is converted to testosterone at a site other than the testis, owing to the presence of 3β-hydroxysteroid dehydrogenase and 17HSD3 or 17HSD5 (Fig. 1). These peripheral conversions also explain the presence of testosterone in women, albeit in much lower levels, as a result of peripheral conversion in the ovaries [5], the mammary gland [6], and the adrenal cortex [8]. The application of this process to exercise is described later (Sect. 6).

2.2 Testosterone Secretion

The system for the release and control of the testosterone hormone is known as the hypothalamic-pituitary-gonadal axis (HPGA). A recent advancement in the understanding of the HPGA was the discovery of kisspeptins, produced by the KISS1 gene, and their role in the regulation and secretion of gonadotropin-releasing hormone (GnRH). In fact, KISS1 is the initial signal for GnRH secretion, and is now universally recognized as the major central regulator of the HPGA [9]. Kisspeptin neurons are located in the brain in two areas, the arcuate nucleus and the anteroventral periventricular nucleus. The kisspeptin receptors (KISS1R) are located on GnRH neurons in the preoptic area of the anterior hypothalamus, which stimulates GnRH release into the hypothalamic-hypophyseal portal vein, connecting the hypothalamus to the anterior pituitary. At the anterior pituitary, the GnRH receptor (GnRHR) receives the signal to secrete luteinizing hormone into the circulation, which is done in a pulsatile manner. Luteinizing hormone is then the signal for testosterone secretion, which as described above can occur in several tissues, predominantly the testis in men and the ovaries in women. When appropriate levels are reached, androgens as well as estrogens (which are synthesized from testosterone) provide negative feedback to the KISS1R to stop further secretion [10]. On this note, the discovery that kisspeptins are androgen sensitive answers the question of how the negative feedback mechanism for testosterone secretion occurs when GnRH neurons do not express androgen receptors (ARs) [11].

2.3 Factors Influencing Testosterone Levels

Before a simple testosterone level can be interpreted, it is essential to first consider the context that the sample was taken in. There are a multitude of factors that can impact a single testosterone level, including feeding, time of day, and exercise. With an established framework for a testosterone level, one can begin to use the information in a

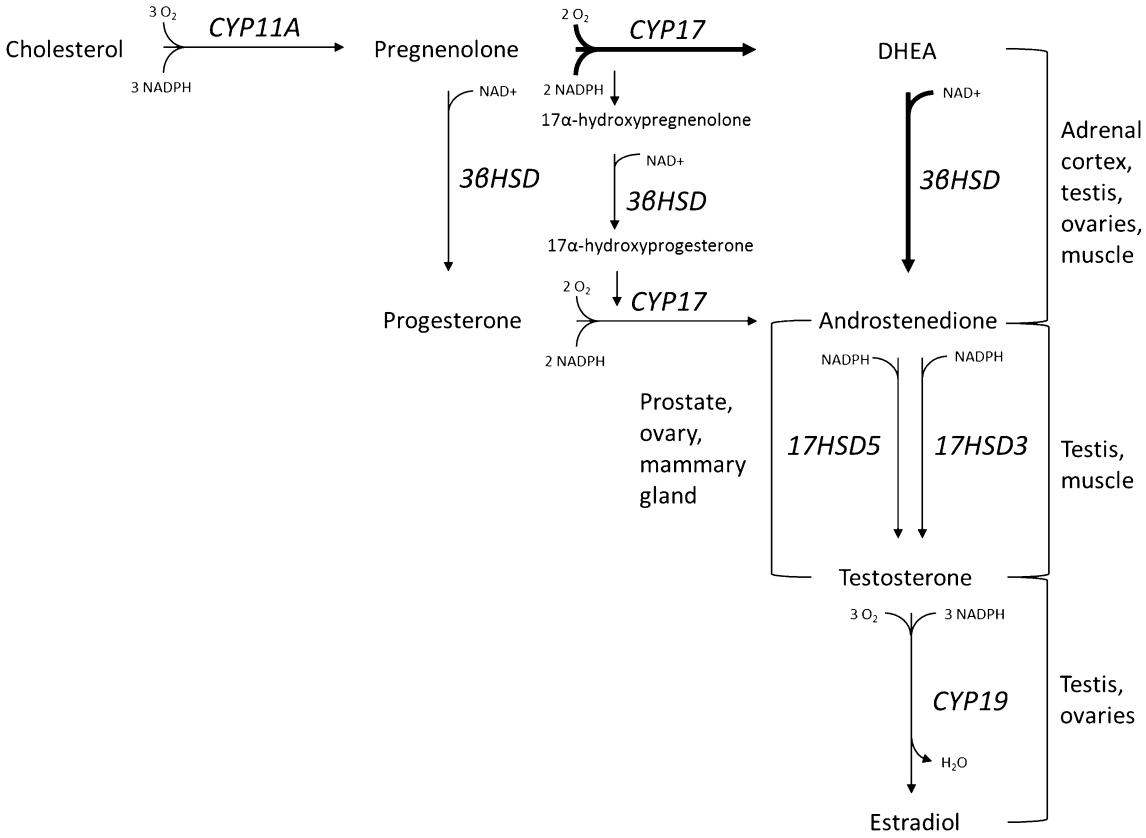

Fig. 1 Testosterone synthesis. Cholesterol is the precursor to all steroid hormones. After conversion to pregnenolone, several pathway permutations are possible, but all lead to the conversion to androstenedione. The pathway via dehydroepiandrosterone (DHEA) is approximately fourfold more common in humans, and is highlighted by *thicker arrows*. The final step in the synthesis is the conversion of androstenedione to testosterone in the testis and prostate in men, and in the ovary and mammary gland in women. Following its production, testosterone can then be converted to estradiol. *CYP* cytochrome P450 family, *NAD+* nicotinamide adenine dinucleotide phosphate, *NADPH* nicotinamide adenine dinucleotide, *3βHSD* 3β-hydroxysteroid dehydrogenase

manner that could contribute to the optimization of resistance training adaptations.

With regard to feeding, studies have demonstrated an acute effect of diet on testosterone levels. For example, fat-rich meals [12], carbohydrate [13], and mixed meals [14] all reduce testosterone in the post-prandial phase (the time period immediately following food intake) to below that of fasting. In fact, the latter study clearly demonstrated the care that must be taken when interpreting a simple circulating testosterone level. Kraemer et al. [14] demonstrated that when a mixed macronutrient supplement (56% carbohydrate, 16% protein, and 28% fat) is consumed, the areas under the concentration-time curve for testosterone levels encompassing measurements from immediately prior to resistance exercise, and for every 10 min following for up to 60 min, were significantly different. In this study, no changes in peak testosterone levels following the workout were seen, but the area under the concentration-time curve was significantly lower (fed 137.25 ± 30.43 nmol L^{-1} vs. fasted 146.37 ± 40.83 nmol L^{-1}) in the fed state. This lower area under the concentration-time curve was driven by a drop in testosterone below baseline following the supplement at 20, 30, 40, 50, and 60 min post-exercise, which did not occur in the fasted state. This reduction in circulating testosterone might have led one to speculate that food intake can negatively affect the contribution of testosterone to resistance training adaptations. However, this drop in testosterone coincided with an increase in AR content in the muscle, suggesting that perhaps the testosterone had moved from the circulation to the muscle where it can exert its positive effects on protein metabolism (Fig. 2). What is certain, however, is that measuring testosterone levels in the fed state could lead to mistakes in interpreting a testosterone value. In addition to feeding, the time of day can affect the circulating testosterone level, with a peak in the early morning, and a substantial nadir in the evening [15]. When considering these factors, it is essential that for a basal level to be determined, the sample should be taken between 7 and 10 a.m., following a normal night's sleep and in a fasted and rested state [16].

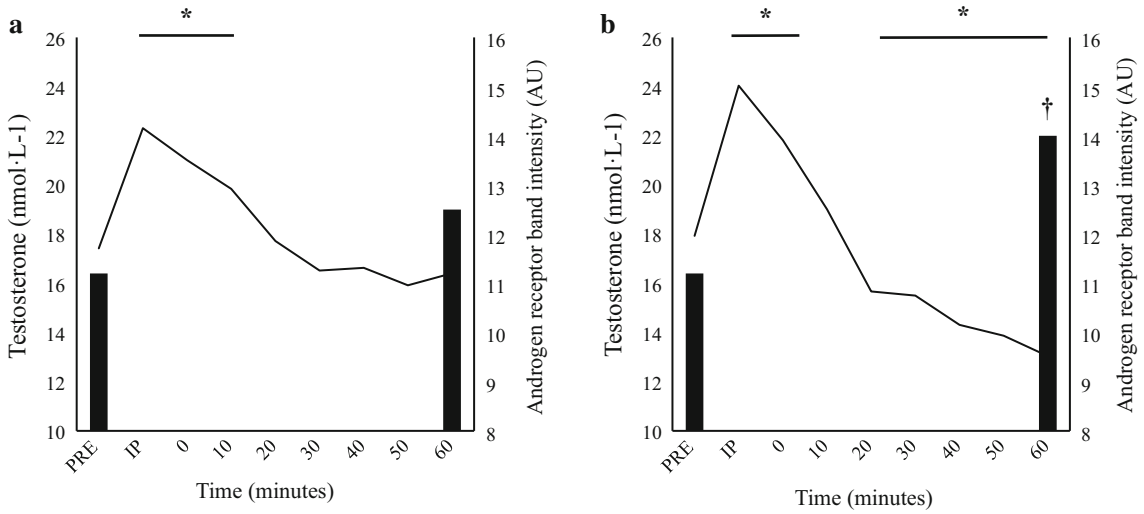

Fig. 2 Testosterone level and androgen receptor content in the fed and fasted state [14]. In the fasted state (**a**), there is an increase in testosterone followed by a return to baseline within 10 min following resistance exercise. There are also no changes in vastus lateralis androgen receptor content 60 min following resistance exercise. In the fed state (**b**), after an initial increase in the testosterone level followed by a return to baseline, testosterone drops below baseline for the remainder of the 60 min following resistance exercise. This is accompanied by a concomitant increase in vastus lateralis androgen receptor content 60 min following resistance exercise. *AU* arbitrary units, *IP* immediately post-resistance exercise, *PRE* before resistance exercise, $^{*}p \leq 0.05$ significantly different from corresponding PRE testosterone; $^{\dagger}p \leq 0.05$ significantly different from corresponding PRE androgen receptor content

3 Basal Levels of Testosterone

An alteration in basal levels of testosterone could potentially have a drastic impact on the body. Despite flaws in the design of early studies evaluating the effectiveness of androgens leading to even the American College of Sports Medicine declaring them ineffective from 1976 to 1984 [17, 18], the positive effects of supraphysiological levels of testosterone on lean mass and strength are no longer questioned [19]. It has also been demonstrated that after suppression of basal testosterone levels, lean mass and strength increase in a dose-response manner over the course of 20 weeks in young men, with exogenous testosterone doses ranging from 25 to 600 mg/week. These doses altered basal levels on a range from below normal (for the 25-mg/week group) to nearly fourfold above normal (for the 600-mg/week group), demonstrating the benefits of testosterone supplementation to populations across an entire spectrum of basal levels [2].

In addition to the clear support for the benefits of increasing basal testosterone levels, the drawbacks of the removal of testosterone are equally powerful in the opposite direction. Following the use of goserelin, a GnRH analog (which blocks testosterone secretion), strength and lean mass gains were attenuated in young men when compared with placebo following 8 weeks of strength training [20]. The processes by which these strength and lean mass attenuations occur have been described more recently by the same author group, where it appears that when testosterone is blocked, satellite cells are unable to differentiate to myonuclei, which prevents the muscle from being able to grow [21].

Although testosterone levels were artificially reduced in the aforementioned study, these findings may be of concern for individuals who acquire hypogonadism. Such populations include individuals with a disease of the pituitary, which can alter testosterone secretion, as well as older populations because cross-sectional studies have demonstrated testosterone levels decrease with normal aging [22]. In addition, low-testosterone levels are associated with decreased lean mass in otherwise healthy hypogonadal men when compared with controls [23]. Moreover, a wide range of symptoms have been reported in association with hypogonadism, such as infertility, reduced libido, reduced muscle mass and strength, reduced bone mass, loss of body hair, and breast discomfort [16]. Therefore, it appears changes in basal testosterone levels can have a powerful impact on the health of the individual, and long-term resistance exercise has been shown to alter these levels.

3.1 Basal Testosterone Responses to Resistance Exercise

Basal levels of testosterone have been found to demonstrate significant increases after just 5 weeks of resistance training [24]. Beyond initial increases in previously untrained individuals, testosterone levels have been shown even in elite weightlifters to continue to increase over the

course of 2 years of training [25]. However, other studies have failed to show any chronic increases, leading to the speculation that perhaps varied strength training programs using higher volumes may be needed to alter resting levels of testosterone [26]. When increases in testosterone levels have been found, while they did not match the elevations seen with pharmacological intervention [2], positive changes in basal testosterone levels are associated with increases in lean mass and strength; therefore, it is reasonable to surmise that such adaptations from resistance training would be positive for human performance. Although the exact role of this adaptation is unknown, it has been speculated it may lead to optimized strength development [25].

Such chronic adaptation in the HPGA could be particularly valuable to older men, as it has been shown that the incidence of hypogonadal testosterone levels increases from well below 10% in men under 40 years of age, to about 20% in men over 60 years of age, 30% in men over 70 years of age, and 50% in men over 80 years of age [22]. Therefore, Ahtiainen et al. [27] studied the effects of 12 months of resistance training on many aspects of testosterone synthesis and action in younger (28 ± 3 years) and older men (70 ± 2 years), including testosterone production and metabolic clearance, luteinizing hormone level, AR content, and testosterone response to gonadotropin stimulation. Despite evidence of reduced testosterone metabolism and urinary excretion of testosterone metabolites in older men prior to the intervention, resistance training had no effect on any of the variables. However, most importantly, despite a reduction in testicular steroidogenesis, the older men were still able to gain similar strength to younger men as measured by leg press 1 repetition maximum (RM).

4 Acute Testosterone Level

Although the positive effects of an increase in basal testosterone levels have been well documented, the effects of the acute changes in testosterone levels during and immediately following resistance exercise are unlikely to be as potent owing to the much shorter exposure time. However, considering the powerful anabolic effects of testosterone that have been previously described, an examination of the impact of an elevation in its level, albeit brief, seems warranted.

4.1 Acute Testosterone Response to Resistance Exercise

The acute rise in testosterone levels immediately following resistance exercise was initially reported by Fahey et al. [28]. In this study, college-aged male and female individuals as well as high school-aged male individuals performed a weightlifting session, with testosterone levels measured before and immediately after the workout. While Fahey et al. [28] were the first to demonstrate the acute rise in testosterone levels associated with resistance exercise in the college-aged male group, they failed to see the response in the college-aged female individuals or high school-aged male individuals. However, importantly, the college-aged male individuals performed an entirely different workout that included five sets of 5RM barbell deadlifts, as opposed to the college-aged female individuals and high school-aged male individuals, who performed three sets of 5RM bench press, seated press, and leg press on resistance machines. The authors also noted that female individuals lacked 'aggressiveness' towards the workout, while the high school-aged male individuals (age 16.0 ± 0.8 years) had lower motivation, which may have led to less vigorous weight training sessions, ultimately contributing to the lack of response. This suggestion from the authors appears to have been validated, at least with regard to the high school-aged male individuals, by a later study by Kraemer et al. [29], who were able to demonstrate the acute rise in testosterone levels in elite junior weightlifters (age 17.3 ± 1.4 years), but only in the subjects with a minimum of 2 years of training experience. Despite the limitations of the early study by Fahey et al. [28], the authors did conduct a landmark study as the first to demonstrate this response, which has since been followed up by hundreds of studies assessing the acute hormonal responses to resistance training using a wide variety of exercises, loads, and rest periods, as well as having been compared in many different populations, including men and women, younger and older individuals, and trained and untrained persons.

A review of the impact of manipulation of the acute program variables (e.g., exercise selection, exercise order, intensity, volume, and rest period) on the acute testosteronemia (AT) response has been previously published by Vingren et al. [30]. In short, it appears that a volume threshold must be met to induce the AT response. In conjunction with adequate volume, the addition of a metabolic demand, as evidenced by elevated blood lactate, can further enhance the AT response, which can be achieved through the reduction of between-set rest periods. Other changes can be made by altering the speeds of the resistance exercise movement, such as consciously increasing the duration of the eccentric phase. For example, Goto et al. [31] compared the testosterone responses with four different timing patterns of leg extensions, including slow concentric (5 s concentric, 1 s eccentric), slow eccentric (1 s concentric, 5 s eccentric), balanced contractions (3 s concentric, 3 s eccentric), and normal velocity (1 s concentric, 1 s eccentric). While there were no differences in

free testosterone responses, slower contraction speeds were able to raise free testosterone to the same degree despite a lower metabolic stress, evidenced by higher pH and lower lactate levels [31]. This combination of results demonstrates just how sensitive the AT response to resistance training can be, as it can be manipulated by subtle changes in program design.

As well as observing the AT response to changes in resistance training programming, other studies assessed the variation in the AT response among different groups of individuals. These studies demonstrated that the AT response appears to follow the same pattern as basal testosterone levels. For example, women exhibit lower basal levels of testosterone than men, and also do not appear to exhibit a significant AT in resistance training programs even when they are identical to those performed by men, who themselves did exhibit the AT response [32, 33]. Another such example is the reduction of AT response with age, as shown by Kraemer et al. [34], who compared the AT response in younger men (aged 29.8 ± 5.3 years) with older men (aged 62 ± 3.2 years), and demonstrated a significantly higher magnitude of increase in testosterone levels over the course of the 30-min post-resistance exercise in the younger men. Finally, as mentioned before, previously untrained high school-aged boys do not appear to demonstrate a discernible AT response, when compared with college-aged men that do [28], although the AT response does appear in highly trained, high school-aged boys [29].

While the AT response has been well demonstrated, along with the impact of changes in resistance training programming and the variation of the response in different populations, the question of the importance of this response and its long-term effects on hypertrophy have not been determined. While an increase in the circulation of an anabolic hormone would appear to be beneficial, as it has been shown to be with the use of pharmacological intervention [19], the long-term impact of the AT response associated with resistance training has not been well documented. For comparison, the testosterone levels that have been observed as part of the AT response are substantially smaller than those seen in individuals who have received testosterone enanthate supplementation [2]. In this study, men who received doses of 300 mg of testosterone enanthate in addition to a GnRH agonist to suppress endogenous testosterone production, exhibited a basal testosterone level increase from 22.6 to 46.6 nmol L^{-1}, an ~100% increase. Even though such a dose is markedly lower than those used by bodybuilders, who have been reported to use over 1000 mg/week [35], such a dose was still sufficient to increase leg press strength and power even without undertaking any resistance training over the course of 20 weeks. By contrast, those who have observed the AT response, have demonstrated increases from ~18 nmol L^{-1} before exercise to ~30 nmol L^{-1} during the exercise, an ~60% increase [36]. Furthermore, the level changes observed following resistance training do typically return to baseline in 15–30 min, whereas those who have received pharmacological intervention experience an elevated basal level, for as much as 7 days following injection [2].

While the level changes associated with AT vs. testosterone injections are approximately half, the time of exposure to the increased level is obviously nowhere near as substantial. However, although the AT response is back to baseline within 30 min following resistance exercise, there is evidence to suggest that the physiological responses to the elevation in testosterone may continue for several hours, as evidenced by an upregulation of the AR. This was an essential area of discovery as we know that the hormone itself does not have an effect on protein synthesis until it has interacted with its receptor. Several studies together characterized the changes in expression of the muscle AR, which appears to stabilize immediately following an AT-inducing workout [37], then downregulates at the 1-h timepoint [38], before showing upregulation [37] for at least 3 h. When mapped along with the AT response, it appears that after the stimulation of circulating testosterone, the presence of testosterone in the blood begins to dissipate and the expression of the receptor in the muscle increases, suggesting that the hormone has moved from the circulation to the muscle, where it can interact with its receptor and induce its protein synthesis response. Interestingly, each of these aforementioned studies were conducted with their participants in the fasted state, which is known to be a state in which net protein balance remains negative [39]. Only one study has measured the AR response in the fed state, which in contradiction to prior research that detected a downregulation of the AR at the 1-h timepoint in the fasted state [38] found a significant increase in AR content at the same timepoint following consumption of a mixed meal (56% carbohydrate, 16% protein, 28% fat). Although speculative, there appears to be a link between increased protein synthesis in the fed state, at a time that also shows an upregulation in muscle AR content along with a movement of testosterone from the circulation to the muscle (Fig. 2).

In light of the suggestion that an AT response to resistance training could upregulate the AR, enhance testosterone uptake to the muscle, and therefore potentially exert anabolic effects, some studies have attempted to discern whether resistance training programs that maximize the AT response lead to greater hypertrophy than programs with a lower AT response. In addition to the aforementioned paradigm regarding testosterone, other anabolic hormones such as growth hormone(s) (22 kD and aggregates and

splice variants) and insulin-like growth factor-I have been shown to be elevated following resistance training. The responses of these anabolic hormones together and whether or not this response could potentiate hypertrophy have been dubbed by some as the "the hormone hypothesis" [40].

With regard to this so-called "hormone hypothesis", it appears that an acute rise in testosterone is not necessary for hypertrophy to occur, particularly in untrained individuals. It has been demonstrated that low-load resistance training, such as 40 or 70% of 10RM [32], or low-volume resistance training, such as six or fewer total sets, even when performed with higher loads (e.g., 80–88% 1RM) fails to stimulate an AT response [41]. Despite this, light load (30% 1RM), low-volume resistance training (three total sets) at just 30% 1RM, which is very unlikely to stimulate an AT response, has been shown to result in significant hypertrophy following 10 weeks of resistance exercise three times per week in a previously untrained population [42]. However, this is not always the case, as other studies have failed to find significant increases in hypertrophy following light-load resistance training (20–28RM) in beginners [43].

A possible explanation for a lack of differences in untrained populations could be a lack of divergence in cell signaling responses in these individuals. When an exercise stimulus is novel, as is the case for those without prior resistance training experience, there appears to be a generalized response. For example, despite the substantial differences in strength and hypertrophy adaptations as a result of chronic resistance and aerobic exercise, mammalian target of rapamycin (mTOR), an important signaling molecule in the development of hypertrophy, was not different during the initial 60-min recovery period following acute bouts of aerobic vs. resistance exercise in untrained individuals [44]. If these drastically different exercise protocols (resistance vs. aerobic) are unable to induce distinctions in muscle signaling, it is not surprising that manipulations of program design within resistance training programs have failed to measure detectable changes in hypertrophy following short-term interventions. However, when similar comparisons are made in trained individuals, a differential response in mTOR has been noted, with mTOR being preferentially activated following resistance exercise but no differences seen following aerobic exercise [45]. These studies together provide evidence for the importance of distinguishing between trained and untrained individuals, and how over time, the exercise stimulus may need to progress to continue to achieve adaptations.

While it appears that AT is not necessary for hypertrophy, particularly in untrained individuals, the context for which AT has been suggested to be beneficial for hypertrophic adaptations in resistance training is in trained populations, where such a response may optimize adaptations [1]. However, there is a dearth of studies comparing resistance training programs with and without AT in trained populations [40]. Although such a direct comparison does not exist in the literature at present, it is interesting to note that both the AT and hypertrophic responses to resistance exercise appear to have a similar threshold with regard to the load used. As described previously by Vingren et al. [30], once a load and volume threshold are met, the AT response is demonstrated. For example, Linnamo et al. [32] showed that five sets of ten repetitions at 10RM induced an AT response. However, when the load was reduced to 40 and 70% 10RM, the AT response was lost. Although there is variation between exercises, typically 10RM is approximately 75% 1RM, which is similar to the optimal threshold for hypertrophy that has been previously suggested [46]. Thus, this evidence does point to a link between optimal hypertrophy and the AT response.

In an attempt to assess the impact of the rise in anabolic hormones, including testosterone, associated with resistance training, a recent study observed the changes in strength and hypertrophy to two strength training programs that included the same five exercises, with one program using high repetitions (20–25 repetitions, ~30–50% 1RM) and another using moderate repetitions (8–12 repetitions, ~75–90% 1RM) [47]. The authors found no significant differences between the groups in fat-free mass, or type I or II muscle fiber cross-sectional area, along with no significant correlations between the acute rise in any purported anabolic hormone and the change in strength or hypertrophy. However, it is important to note that there were no differences in the AT response between the two programs, making it impossible to gauge the impact of the AT response. The lack of difference between the programs in AT response, despite the differences in load, could have been because of the low volume. Each workout consisted of three sets of each of the five exercises performed, whereas prior studies that have demonstrated a substantial AT response from resistance exercise have used as many as eight exercises [36].

Other studies have attempted to discount the role of AT by comparing the effects of resistance training programs that do and do not stimulate AT and balance their effects on muscle protein synthesis (MPS). The measurement of MPS is achieved by assessing the incorporation of tracer amino acids into the muscle, which are infused via the antecubital vein. In a between-subjects design, an identical resistance training program was conducted on men (who would demonstrate an AT response) and women (who were unable to produce an AT response) and their respective post-exercise MPS rates were compared [33]. After the failure to uncover any differences in MPS between the two sexes, the authors concluded that the AT response must not

be relevant in stimulating protein synthesis. However, it is important to consider the context for which MPS can be used. Of course, resistance training is beneficial owing to its positive effects on net protein balance, where synthesis exceeds degradation and hypertrophy can occur. In a within-subjects design, particularly if the stimulus is identical, it is fair to assume that protein degradation would be similar, such as in the studies that have compared protein ingestion protocols on MPS and developed protein recommendations. However, protein degradation can be drastically different when comparing populations, as was demonstrated by a cross-sectional study comparing young/old and male/female subjects, which revealed that older women have the highest MPS rates, but they also have the highest degradation rates [48]. Thus, changes in MPS alone, particularly when comparing across sexes do not adequately predict changes in protein balance as they do not take into account protein degradation. It has also been noted that changes in protein synthesis measured following resistance training do not always occur in parallel with chronic upregulation of causative myogenic signals [49] and are not necessarily predictive of long-term hypertrophic response to regular resistance training [50], as evidenced by a lack of correlation between changes in MPS and hypertrophy following 16 weeks of resistance exercise [51]. As a result, this study is also unable to adequately discount a positive effect of the AT response on long-term changes in protein balance.

In addition to MPS, the previous study also measured differences in anabolic signaling in men vs. women [33]. Despite the lack of changes in MPS, the study did demonstrate increased phosphorylation of Akt (protein kinase B) and mTOR in men in conjunction with greater AT response and increased AR content. The link between these signaling responses and the role of testosterone could be explained through the recently identified non-genomic actions of testosterone, which suggests that there are alternative mechanisms of testosterone action to the classic free hormone hypothesis, as will be described in the following section.

5 Testosterone Action

Once testosterone is in circulation it can be loosely bound to albumin (~20–30%), tightly bound to sex hormone-binding globulin (~50–70%), bound to other proteins (~4%), or unbound, known as 'free' (~1–3%) [52]. Traditionally, free testosterone has been thought of as the only form of testosterone that is biologically available, often referred to as the "free hormone hypothesis" [53]. Owing to the hydrophobic nature of testosterone, the binding proteins were believed to act as a mode of transport for the hormone, which would not readily dissolve in the blood. It was also suggested that binding proteins were used as a way to keep testosterone in an inactive state and thus serve as a means of regulating the amount of active hormone available for diffusion.

5.1 The Free Hormone Hypothesis

The idea of binding proteins inactivating circulating hormones centers on the classical action of testosterone (Fig. 3, left side), where the bound complex is unable to cross the cell membrane. In contrast, free testosterone is hydrophobic and can readily be diffused through the membrane into the cell cytoplasm where it can reach the intracellular AR [54]. Before testosterone reaches the AR, the AR is associated with a large complex of chaperones, including heat shock proteins, which keep the AR inactive yet still ready for binding [55]. The AR binds both dihydrotestosterone and testosterone with high affinity, although testosterone binds with a twofold lower affinity as well as a fivefold higher dissociation rate than dihydrotestosterone [56]. Upon binding, heat shock proteins are then dissociated, accompanied by a conformational change of the complex. This change is associated with an increased affinity for the androgen response element on the DNA sequence. Once the complex has been translocated to the nucleus, the receptor then dimerizes and binds to DNA sequences known as the androgen response element, where it can now influence transcription. This occurs by an interaction between the AR and many different classes of transcription factors, including general, sequence specific, co-activators, co-repressors, and chromatin factors. The end result of this process is a promotion of the expression of target genes [57]. Therefore, the effects of testosterone do not occur until the increased gene expression produces the protein. Such effects on protein synthesis are not observed for at least half an hour [58] and up to hours or days [59]. This process has also been referred to as the "slow action" of testosterone due to the amount of time required to notice a measurable response as a result of the genomic nature [58].

5.2 Non-Genomic Action

The rate at which the effects of testosterone are seen is an important difference in the two mechanisms of response (as illustrated in Fig. 3). Whereas the genomic response requires an interaction with nuclear DNA before changes can be observed, another mechanism of testosterone action (Fig. 3, right side) can exert its effects via intracellular signaling molecules (such as protein kinase A, protein kinase C, phospholipase C, phosphoinositide-3 kinase, and mitogen-activated protein kinase) and can show a

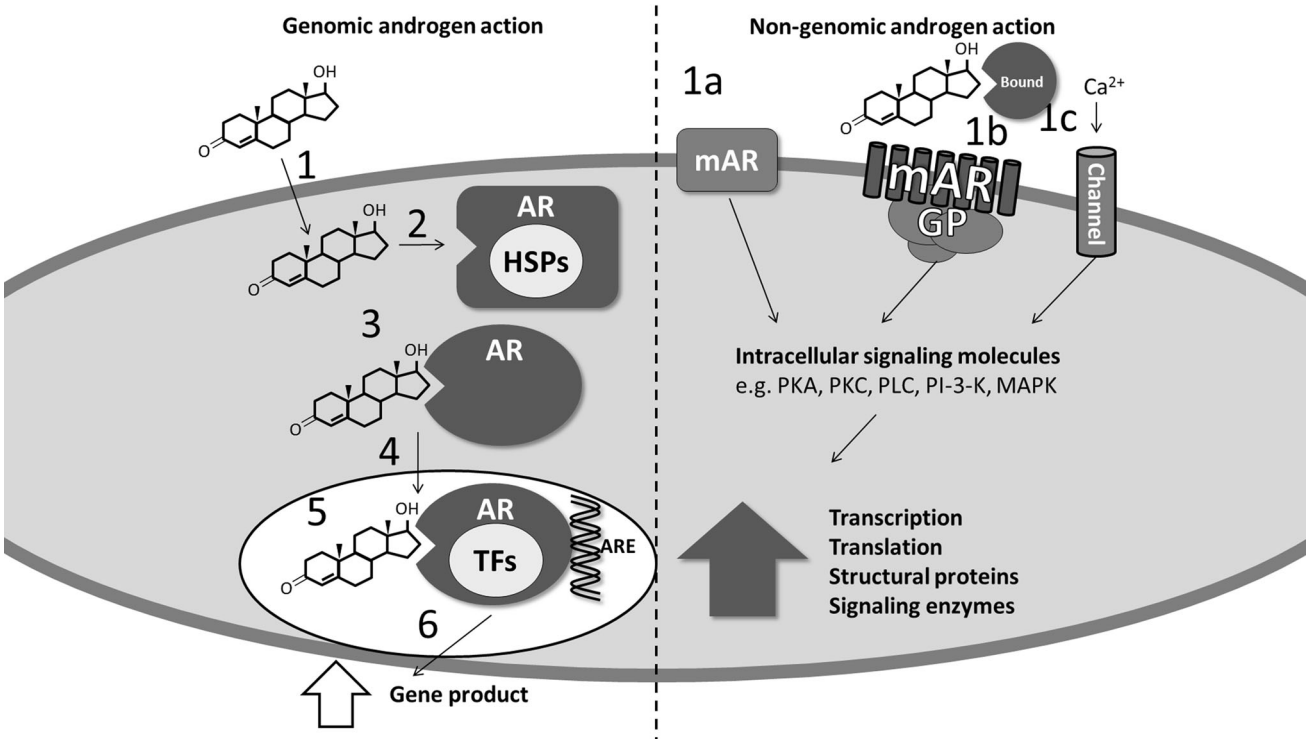

Fig. 3 Genomic and non-genomic androgen action. *Left side*: *1* Hydrophobic testosterone readily diffuses across the cell membrane. *2* The androgen receptor is inactive before testosterone binds owing its association with heat shock proteins. *3* Testosterone binding occurs after heat shock protein dissociation and results in a conformational change of the complex. The receptor also dimerizes, facilitating future binding to the androgen response element (ARE). *4* The complex translocates to the cell nucleus. *5* With the help of transcription factors, the complex binds to the ARE on the DNA sequence near the target gene. *6* Following an increase in transcription, there is an increase in the product of the gene. *Right side*: The bound testosterone molecule can induce changes within the cell without passing the cell membrane, by a putative membrane receptor (*1a*), a G-protein receptor (*1b*), or via a calcium channel (*1c*). Following the stimulation of a variety of possible signal transduction systems, bound testosterone is ultimately able to increase transcription, translation, and the synthesis of structural proteins and signaling enzymes, all of which can contribute to hypertrophy. *AR* androgen receptor, *GP* G-protein, *HSPs* heat shock proteins, *mAR* membrane androgen receptor, *PI-3-K* phosphoinositide 3-kinase, *PKA* protein kinase A, *PKC* protein kinase C, *PLC* phospholipase C, *MAPK* mitogen-activated protein kinase, *TFs* transcription factors

measureable biological response within seconds [59]. In this mechanism, testosterone interacts with other transcription factors without direct binding to DNA [54, 60], hence the name 'non-genomic'. Evidence of these non-genomic responses has been presented in all classes of steroids across many laboratories [59]. Such research into the non-genomic response has unveiled a complex network of signaling cascades beyond the scope of this review. For a detailed review of these effects, see Michels and Hoppe [58] and Norman et al. [59]. However, the main concept (highlighted in Fig. 3) is that these networks appear to lead to activation of many important processes for hypertrophy, such as increases in transcription, translation, structural proteins, and signaling enzymes.

Interestingly, although the non-genomic role of testosterone might be seen as an alternative to the free hormone hypothesis, a seminal paper by Mendel [53] for the proposal of the free hormone hypothesis actually predicted that it may not hold for all hormones and all tissues, and particularly questioned its validity for steroid hormones [11]. However, it was only recently that alternatives to the free hormone hypothesis were studied. As previously mentioned, the reason for bound testosterone initially being considered inactive is owing to the attached protein preventing the complex from crossing the membrane and blocking any interaction with the intracellular receptor. Therefore, these non-genomic signaling cascades in response to testosterone center on the potential role for the bound hormone through a putative membrane receptor.

In a pivotal article with regard to the non-genomic role of androgens, Estrada et al. [61] demonstrated that testosterone conjugated with bovine serum albumin (and therefore unable to pass through the cell membrane) led to an increase in extracellular signal-related kinase 1 and 2 phosphorylation comparable to that of free testosterone in skeletal muscles. Further experiments identified that such

an increase was not inhibited by an AR antagonist, clearly indicating an alternate AR present in the cell membrane. Additionally, extracellular signal-related kinase 1 and 2 phosphorylation was blocked by a G-protein antagonist (pertussis toxin), suggesting that this pathway is mediated by a G-protein mechanism. Estrada [61] went on to state that calcium was likely the second messenger responsible for signal transduction, although at the time was unable to provide a mechanism as to how this signal went on to stimulate the Ras/MEK/extracellular signal-regulated kinase pathway, but did allude to the possibility that the process was mediated by calmodulin or protein kinase C. Although study of the non-genomic role of testosterone is still in its infancy, there is substantial evidence for its existence and it should now begin to be considered in studies that pertain to the role of testosterone in resistance exercise physiology, particularly as there is evidence the upregulation of these pathways is associated with AT [33].

6 Intracrinology

Another possible means of testosterone playing a role in resistance training adaptation is via the process of intracrinology. In this process first put forward by Labrie [62], testosterone may act on skeletal muscle in an intracrine manner, in which precursors to testosterone such as DHEA, progesterone, and androstenedione may be converted to testosterone in the muscle itself without ever entering circulation. The significance of the role of intracrinology is clearly evidenced by the fact that when testicular synthesis is removed, the circulating testosterone level drops 95–97%, but function only reduces to approximately 40% [63]. These processes have also been supported by the presence of 17HSD3 and 3β-hydroxysteroid dehydrogenase in human skeletal muscle, which perform the aforementioned peripheral conversions of testosterone precursors to testosterone and led to the study of their responses following resistance training [64]. Although this study found no evidence of intracrinology in the AT response, this particular study was undertaken in the fasted state. However, protein synthesis is much higher in the fed state than the fasted state [65] and the process of intracrinology may have required a supply of energy as well as amino acids to fully function, and therefore this study may have failed to activate intracrinological processes. In addition, the presence of testosterone in muscle was measured at a time (70 min post-resistance exercise) that previous studies have demonstrated that the AT is downregulated [38], or unchanged from baseline [14]. With these factors in mind, the role of intracrinology following resistance exercise cannot be ruled out and may warrant further investigation.

7 Conclusion

Changes in basal levels of testosterone have proven to have a dramatic impact on human performance, illustrated by reduced strength and hypertrophy in hypogonadal populations, and enhanced strength and hypertrophy following testosterone supplementation. Although some studies have shown an increase in basal testosterone levels following long-term resistance exercise, not all studies have demonstrated such an adaptation. However, even when this adaptation was not found, such as in older men, resistance exercise was still capable of stimulating strength and hypertrophy gains [27].

Similarly, just as a basal increase in testosterone is not necessary for strength and hypertrophy adaptation, nor is an acute rise in testosterone following a resistance exercise bout absolutely necessary. However, the mobilization of a highly energetically expensive molecule, in conjunction with the upregulation of its receptor in the muscle following resistance exercise, suggests that the acute rise in testosterone may play a role in adaptation. The nature of this role could be found through future investigations of the non-genomic actions of androgens or the intracrine actions of testosterone. At present, there is a dearth of literature investigating the prolonged effects of resistance training programs which induce an acute testosterone response compared with programs that do not. Thus, the long-term implications of the AT response are unclear.

Compliance with Ethical Standards

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Conflict of Interest David Hooper, William Kraemer, Brian Focht, Jeff Volek, William DuPont, Lydia Caldwell, and Carl Maresh declare they have no conflicts of interest relevant to the content of this review.

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