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Review article

## Evidence for a non-genomic action of testosterone in skeletal muscle which may improve athletic performance: Implications for the female athlete

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### Abstract

This review will focus on the proposed second mode of testosterone action (now termed non-genomic) that appears to occur independently of the traditional transcriptional mechanism in mammalian skeletal muscle cells which may enhance skeletal muscle contractile properties. This mechanism of testosterone action differs from the traditional pathway, originating at the cell membrane, having a rapid onset of action, requiring second messengers to execute its effects and is insensitive to inhibitors of traditional androgen receptor action, transcription and protein synthesis. Importantly, unlike the traditional action of testosterone in skeletal muscle, this non-genomic pathway is shown to have a direct acute effect on calcium-dependent components important for the contractile process. The changes within the contractile apparatus may enhance the ability of the muscle to produce explosive power during athletic performance. Rapid increases in Inositol triphosphate mass and calcium release from the sarcoplasmic reticulum have been reported in rodent skeletal muscle cells, and a rapid androgen (dihydrotestosterone)-induced increase in peak force production has been recorded in intact rodent skeletal muscle fibre bundles while showing increases in the activity of the Ras/MAP/ERK mediated pathway. Because the non-genomic action of testosterone is enhanced during increases in exposure to testosterone and is acute in its action, implications for athletic performance are likely greater in females than males due to natural fluctuations in circulating testosterone levels during the female menstrual cycle, reproductive pathology, and changes induced by hormonal contraceptive methods. Research should be undertaken in humans to confirm a pathway for non-genomic testosterone action in human skeletal muscle. Specifically, relationships between testosterone fluctuations and physiological changes within skeletal muscle cells and whole muscle exercise performance need to be examined.

**Key words:** Calcium, fatigue, female, rapid, power, androgen.

### Introduction

During exercise, the endocrine system secretes hormones into the bloodstream to regulate metabolism (McKeever, 2002; Mastorakos et al., 2005) and maintain the integrity of the body's internal environment. Therefore the control of hormone secretion must be complex and sensitive to adapt quickly to changing biological stresses within the body during exercise. Testosterone is an anabolic steroid hormone that is found in the bloodstream in 3 forms; strongly bound to sex hormone binding globulin (SHBG) (~70%), weakly bound to albumin (~30%) and unbound (~0.5-3%). Traditionally, the physiological function of testosterone in skeletal muscle tissue is the maintenance and increase of skeletal muscle mass (hypertrophy)

through genomic (long-term, transcriptional) mechanisms and the subsequent indirect increase in muscle strength (Cardinale and Stone, 2006; Griggs et al., 1989). However, steroid hormones including testosterone are now shown to elicit rapid actions (within seconds to minutes) in a number of cell types (Benten et al., 1997; 1999a; Ceballos et al., 1999; Estrada et al., 2003; Furukawa and Kurokawa, 2008; Hamdi and Mutungi, 2010; Jones et al., 2004; Waldkirch et al., 2008) through non-genomic mechanisms (short-term, non-transcriptional) (Benten et al., 1999b). The complex interaction of acute and long term steroid adaptation has however, yet to be described. While testosterone's significant genomic action in skeletal muscle is well described, (Bhasin et al., 2005; Sinha-Hikim et al., 2002; 2003; Urban et al., 1995) little attention has been directed towards such non-genomic actions of testosterone in skeletal muscle. This article will discuss a mode of non-genomic action in skeletal muscle and proposed implications for sporting performance specifically for female athletes.

### What are non-genomic steroid actions?

Non-genomic actions of steroid hormones are those steroid mediated actions in which gene transcription is not directly implicated (shown through insensitivity to inhibitors of transcription and protein synthesis), involves second messenger participation and are rapid in action (within seconds to minutes). As described further in this review, non-genomic actions differentiate themselves from genomic mechanisms in the first instance by the binding of the steroid to an androgen receptor located on the cell-membrane or by linking with a plasma membrane receptor associated with a Pertussis toxin (PTX)-sensitive G protein (Vicencio et al., 2006) rather than binding with the traditional androgen receptor in the cytoplasm of the cell before being translocated into the nucleus. Unlike the genomic effects of steroid hormones, non-genomic effects require the constant presence of the hormone. Once the hormone subsides from the tissue, so too will the non-genomic effects.

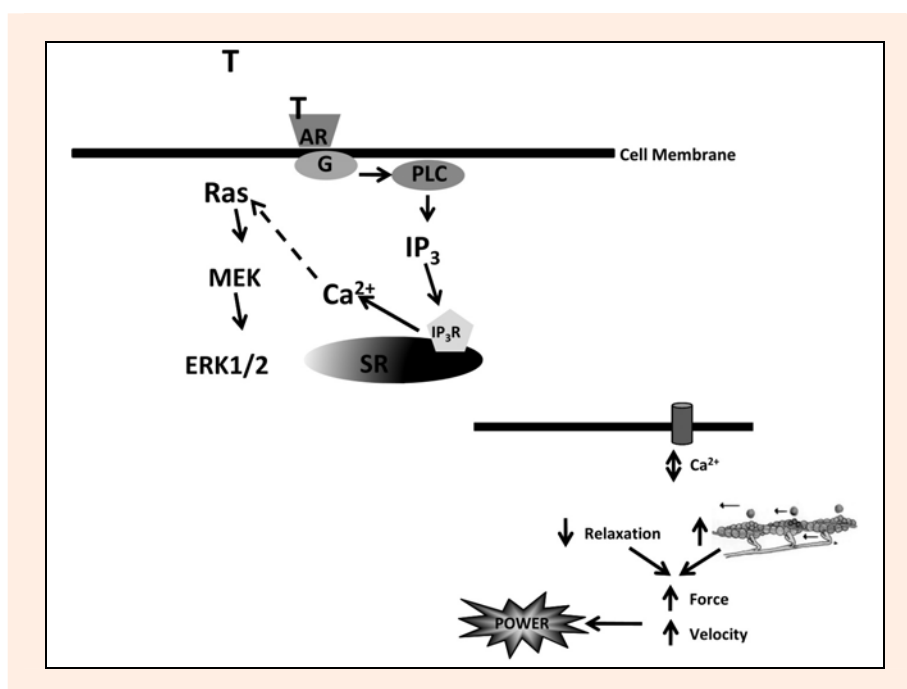
Evidence for a non-traditional testosterone action has been documented regularly in tissues other than skeletal muscle. In the early 1990s, a rapid effect of testosterone on calcium mobility in T-cells, that was initiated at the cell membrane was reported (Benten et al., 1999b), suggesting there was an alternative rapid biological response to testosterone. Similarly, cardiac myocytes from adult rats exposed to testosterone rapidly (1-7 minutes) induced an increase in levels of intracellular calcium

released from intracellular stores through Inositol trisphosphate ( $IP_3$ ) receptors. This calcium response was not linked with the intracellular androgen receptor, but instead through activation of a plasma membrane receptor associated with a (PTX)-sensitive G protein (Vicencio et al., 2006). Similarly again, testosterone rapidly stimulated increases in calcium concentration in rat osteoblasts (within 5 s via enhanced calcium influx) and increased calcium mobilisation from the endoplasmic reticulum, as well as increasing  $IP_3$  formation within 10 s (Lieberherr and Grosse, 1994).  $IP_3$  is used for signal transduction in biological cells via the release of calcium from the endoplasmic reticulum via the  $IP_3$  receptor ( $IP_3R$ ). Testosterone appears to exert these rapid actions through an unidentified G-protein (guanine nucleotide-binding proteins communicate signals from hormones extracellular that then regulate changes intracellular) located on the cell membrane (Benten et al., 1999a; Vicencio et al., 2006).

Rapid actions of testosterone in skeletal muscle have been less researched compared with other tissues such as cardiac muscle and bone (Benten et al., 1997; 1999a) as outlined above. However, the few papers investigating non-genomic testosterone action in skeletal muscle suggest testosterone is capable of producing similar rapid (within 2 min) effects in skeletal muscle cells (Estrada 2000; 2003; Hamdi and Mutungi, 2010). To date, non-genomic testosterone action in skeletal muscle has only been investigated in isolated rodent cells and fibre bundles, with most evidence originating from two studies by Estrada et al (Estrada et al., 2000; 2003). These two studies isolated myotubes and achieved a response to 100 nM of testosterone in 70% of cells. Supraphysiological levels of testosterone (100 nM) at the single-cell level in primary myotubes induced a relatively fast (<2 min) tran-

sient increase in intracellular calcium, which was frequently accompanied by oscillations and a transient increase in the mass of  $IP_3$  to threefold the basal levels within 45 s. Both unbound and albumin-bound testosterone initiated this non-genomic action. These results are similar to those previously identified in other tissue cells (cardiac myocytes, T-Cells, osteoblasts). These results provided support for a G-protein-linked receptor at the plasma membrane, and an  $IP_3$ /calcium, Ras/MEK/ERK mediated pathway. ERK1/2 increased in response to testosterone in a transient and dose-dependent (50-100nM) manner, while G-protein inhibitors blocked the fast rise in calcium and  $IP_3$  and thus the fast effect of testosterone. Therefore, the signal transduction mechanisms of non-genomic actions within skeletal muscle are likely regulated by second messengers such as intracellular calcium and  $IP_3$  (Estrada et al., 2000; Estrada et al., 2003). These non-genomic signal pathways have been previously identified in the action of testosterone, aldosterone, (Estrada et al., 2000) and 17- $\beta$ -estradiol (Morley and Whitfield et al., 1992) and are outlined in Figure 1.

The only study of rapid steroid action in intact skeletal muscle fibres was recently published by Hamdi and Mutangi (2010). This study used intact isolated skeletal muscle fibres from the extensor digitorum longus (predominantly fast-twitch) and soleus (predominantly slow twitch) muscles of adult male and female mice. Like the previous studies, this study used formerly published methods to investigate the effects of steroid hormones on maximum isometric force. Physiological concentrations of dihydrotestosterone (DHT) ( $630 \text{ pg}\cdot\text{ml}^{-1}$ ), significantly increased force produced in both twitch and titanic contractions in fast twitch fibres. A significant 24% increase in maximum isometric tension in fast twitch fibres



**Figure 1. Possible non-genomic pathway/action of testosterone in skeletal muscle.**

Testosterone (T) attaches to a traditional androgen receptor (AR) located at the cell membrane, coupled with a PTX-sensitive G-protein that activates phospholipase C (PLC). This in turn increases  $IP_3$  levels, which are liberated and diffuse to receptors ( $IP_3R$ ) on the sarcoplasmic reticulum (SR). This in turn increases intracellular calcium levels through the release of calcium from the SR. A possible calcium-induced activation of the Ras/ERK phosphorylation cascade may occur, resulting in transcription of DNA, which is then expressed as proteins.

in male mice and a 30% increase in female mice, though not statistically different from each other, suggests it may be appropriate to investigate potential gender differences in non-genomic steroid action. Testosterone however, had no effect on either twitch or tetanic contractions in either fast or slow twitch muscle in intact rodent muscle fibre bundles (Hamdi and Mutungi, 2010). In parallel with the increases in maximum isometric force seen with exposure to DHT, physiological concentrations of DHT increased phosphorylation of ERK1/2 by 2-3 fold in both fibre types adding support for a non-genomic androgen RAS/MAP/ERK mediated pathway. Testosterone increased ERK1/2 phosphorylation in slow twitch fibres only.

While the aforementioned animal studies provide evidence for a non-genomic mechanism, the ability to deduce human effects from rodent studies is unclear. It is therefore important that human research be undertaken to provide evidence for a non-genomic steroid action in human skeletal muscle. Based on the current understanding of non-genomic testosterone action, we suggest that testosterone may be able to produce an increase in intracellular calcium levels and calcium mobility within the human skeletal muscle cell. This may increase the sensitivity of the contractile elements to calcium, which could increase the speed of myosin head binding and/or the force at which the myosin head pulls, such that more force is produced per contraction. Combined, these effects would likely result in greater whole muscle power production (refer to Figure 1). Although current research provides a substantiated basis for this theory (Estrada et al., 2000; 2003; Hamdi and Mutungi, 2010), it is vital that research in human skeletal muscle is undertaken to provide evidence to support both the molecular and physiological undertones of this theory. Until further research is undertaken, implications for athletic performance will remain speculative.

### Testosterone and athletic performance

From testosterone supplementation studies in males (3 mg·kg<sup>-1</sup>·wk<sup>-1</sup> for 12 wk) (Griggs et al., 1989) and (600 mg·wk<sup>-1</sup> for 10 wk), (Bhasin et al., 1996) the traditional (transcriptional) action of testosterone demonstrates the ability to increase protein synthesis (27%), (Griggs et al., 1989) fat free mass (6.1 ± 0.6 kg), (Bhasin et al., 1996) muscle mass (20%), (Griggs et al., 1989) muscle size (triceps brachii 501 ± 104 mm<sup>2</sup>, quadriceps 1174 ± mm<sup>2</sup>) (Bhasin et al., 1996) and strength (bench press 22 ± 2 kg, squat 38 ± 4 kg) (Bhasin et al., 1996). Subsequently testosterone was officially listed as a banned substance in athletic events. While chronic testosterone supplementation can increase strength; (Bhasin et al., 1996) power or counter-movement jump height has also been shown to positively correlate with acute natural levels (0.62 ± 0.06 ng·ml<sup>-1</sup> and 6.49 ± 0.37 ng·ml<sup>-1</sup>) of testosterone (r = 0.061, p < 0.001) in female and male elite athletes, respectively (Cardinale and Stone, 2006). Natural basal testosterone levels and counter-movement jump height are higher in explosive athletes, such as sprinters, and lowest in endurance athletes such as cross-country skiers (Bosco, 1998). Given testosterone's dual mechanisms (i.e., genomic and

non-genomic action), natural testosterone levels may prove to be more important in the ability to produce acute explosive power; a variable that is a determinant of performance in sprint, jumping and throwing events (Bourdin et al., 2010; Hori et al., 2008; Sleivert and Taingahue, 2004; Van Ingen Schenau et al., 1990) than previously thought.

### Fatigue hypothesis: How the non-genomic action of testosterone may counteract fatigue

Calcium is an important metabolite in muscle contraction, with both the concentration surrounding the myofilaments and the sensitivity of the myofilaments to calcium important for the production of force by the individual cross-bridges (maximum calcium activated force). Intensive and repetitive contraction of skeletal muscle causes a decline in peak performance (i.e. fatigue) characterized by reduced force production, decreased shortening velocity, and delayed relaxation of the muscle following contraction (Bigland-Ritchie et al., 1979; Edman and Mattiazzi, 1981; Haan et al., 1989; Jones et al., 1979; Milner-Brown and Miller, 1986; Westerblad and Lännergren, 1991; Cheng and Rice, 2010). One principal theory of skeletal muscle fatigue is characteristic changes in calcium regulation and sensitivity that occur during the decline in performance (Kabbara and Allen, 1999).

Previous research provides evidence that during fatiguing muscle contraction there is a decline in calcium transport; a decline in sarcoplasmic reticulum calcium release (Kabbara and Allen, 1999; Ward et al., 1998; Westerblad and Allen, 1991) and/or a reduction in the sensitivity of the contractile apparatus to calcium (Godt and Nosek, 1989; Westerblad and Allen, 1993). Both scenarios would result in impaired excitation-contraction coupling such that less force is generated for each individual membrane excitation.

A possible protective effect of testosterone against skeletal muscle fatigue was suggested when Bosco et al. (2000) investigated neuromuscular activity and hormonal profile following an acute resistance exercise session in male and female sprint athletes. Full squat power decreased by 10% at the end of the session in males only. EMG/power ratio calculated in the half squat test were decreased in both males and females, but only reached significance in males (p < 0.05). Levels of circulating testosterone, cortisol and luteinising hormone were significantly lower post exercise in males only, while a negative correlation (r = -0.61) was found between change in testosterone concentration and EMG/power ratio in half squat performance in both groups. Bosco et al. (2000) suggested adequate testosterone levels may compensate for or offer protection against the effect of fatigue in fast-twitch muscle fibres by ensuring a better neuromuscular efficiency (Bosco et al., 2000).

We therefore propose, based on results of prior non-genomic testosterone research, that acute elevations in testosterone concentration (such as those during the female menstrual cycle) may be able to reduce or compensate the effects of fatigue in fast-twitch fibres. Due to non-genomic rapid increases in intracellular calcium levels and increased mobilisation of calcium from the

sarcoplasmic reticulum, testosterone may reduce or protect against impaired excitation-contraction coupling during repeated high-intensity muscle contraction. However, gender differences in hormonal changes, notably testosterone following squat exercise reported by Bosco et al., (Bosco et al., 2000) suggest future research should investigate if there are indeed gender specific responses in non-genomic testosterone action.

### **Specific importance of testosterone for female athletes**

Testosterone levels have often been difficult to accurately measure in females due to the combined challenge of both the naturally low levels of circulating testosterone coupled with low sensitivity and precision of assays. Previous studies have however shown that circulating testosterone fluctuates throughout the menstrual cycle (Judd and Yen, 1973; Sinha-Hikim et al., 1998). Serum total and free testosterone levels in the luteal and follicular phases are not significantly different from each other, but an approximate 30-45% pre-ovulatory increase in both total and free testosterone roughly three days prior to the luteinising hormone peak was recorded in two separate studies investigating testosterone levels across a full menstrual cycle (Judd and Yen, 1973; Sinha-Hikim et al., 1998). However, even though a clear peak in both total and free testosterone was shown in these two studies, no studies have yet to specifically investigate changes in muscle strength, explosive power or fatigue when testosterone levels are peaked, which could prove an important measure as previous studies have shown strong positive correlations with acute natural testosterone concentration and acute power performance such as counter-movement jump height.

### **Influence of oral contraceptives**

Combined hormonal contraceptives prevent ovulation as their primary mechanism of action (Rivera and Jacobson et al., 1999). This elimination of ovulation also eliminates the natural peaking in testosterone prior to the luteinizing hormone surge. Oral estrogens ingested in common varieties of the oral contraceptive pill, can have significant consequences on circulating free testosterone levels (Edwards and O'Neal, 2009; Raj et al., 1983; Rickenlund et al., 2004; Thorneycroft et al., 1999; Van der Vange et al., 1990; Wiegratz et al., 1995; 2003a). Oral estrogens can increase the levels of sex hormone binding globulin (Campagnoli et al., 1993; Thorneycroft et al., 1999; Wiegratz et al., 1995; 2003a), which binds to testosterone making it biologically unavailable thus reducing the ratio of circulating free testosterone to total testosterone.

Progestin's can also influence circulating testosterone levels in females, (Gordon et al., 1970) Medroxyprogesterone, a synthetic version of the naturally synthesized human progesterone (often used in oral contraceptives) decreases the production rate of testosterone, likely due to the inhibition of pituitary secretion of luteinizing hormone and may increase the rate of removal of testosterone from the circulation (Gordon et al., 1970) (Palatsi et al., 1984; Wiegratz et al., 2003b). Along with the elimination of the peak in testosterone during the cycle, and reductions in total testosterone concentration, oral contraceptives may,

in our opinion, affect genomic and non-genomic actions of testosterone, reducing optimal hormonal physiology for elite female athletic performance.

The potential impact of the menstrual cycle on anaerobic performance has received less attention than the impact on aerobic variables. There is a lack of consensus on whether sex hormone fluctuations have an influence on anaerobic performance, with some studies concluding performance is unaffected by menstrual cycle phase, (Doolittle and Engebretsen, 1972; Giacomoni et al., 2000; Lebrun, 1993; 1994; Lebrun et al., 1995) and others reporting differences in anaerobic performance variables with menstrual cycle phase (Davies et al., 1991; Masterson, 1999; Wearing et al., 1972). The strength of the research looking at anaerobic performance and sex hormones is faltered by a lack of experimental controls, including variation in the determination of cycle phase (hormonal assay vs. body temperature), unclear subject selection criteria, and differing measures of performance (sprinting vs. jumping vs. swimming and acute vs. repeated efforts). There is yet no published research on the effect of menstrual cycle hormones on anaerobic/power variables in elite female athlete populations.

Studies in which oral contraceptive users were compared with eumenorrheic females have reported a trend towards lower strength across an oral contraceptive cycle compared with a natural cycle. As well, a complete reduction in the natural fluctuation of testosterone and strength often seen with a natural cycle has been shown in women using an oral contraceptive (Phillips et al., 1996; Sarwar et al., 1996). There is little research that has specifically looked at the effect of the menstrual cycle or oral contraceptives on explosive power in female athletes. However, one study examined team sport performance variables during an oral contraceptive cycle (Rechichi and Dawson, 2009), with the only significant difference found in drop jump height in the late withdrawal phase (end of the sugar pills), where a reduced drop jump height coincided with increased serum estrogen levels (Rechichi and Dawson, 2009). It is possible that changes may have occurred in performance variables in females with a natural menstrual cycle due to the fluctuations in circulating testosterone; however, although testosterone is known to reach a peak during the menstrual cycle, no study has specifically investigated athletic performance at this time. Furthermore, there are no studies available that have conducted performance tests every day of the menstrual cycle to ensure all hormonal fluctuations are investigated. In spite of this, due to testosterone's correlation with explosive power and evidence of a non-genomic testosterone action in skeletal muscle, individual female athletes whose events require strength or power may benefit from using non-hormonal based contraceptive methods.

### **Polycystic ovarian syndrome and other menstrual disorders: A physiological advantage?**

Previously, oligomenorrhea in exercising women was seen as a symptom of menstrual disorders, secondary in response to metabolic perturbations due to extreme energy deficit (often seen in endurance runners and associated with leanness) (Rosetta et al., 1998; Sanborn et al.,



1982; Torstveit and Sundgot-Borgen, 2005). However, with the potential for oligomenorrhea to be associated with hyperandrogenism [particularly increased levels of testosterone outside (or within the higher range) of the physiological limits for normally menstruating women] many athletic women suffering from oligomenorrhea may not, as expected, present with symptoms of energy deficit. Therefore, our opinion is that the perception that female athletes who present with oligomenorrhea are most likely to be experiencing extreme energy deficit, may be unfounded.

Although research into the prevalence of hyperandrogenism and polycystic ovarian syndrome in athletic populations is scarce, the available data suggest that the most common diagnosis of menstrual disturbance in Olympic athletes (Hagmar and Berglund et al., 2009) and women in sports where muscle mass is advantageous or non-detrimental to performance (Lebrun, 1994; Masterson, 1999) is polycystic ovarian syndrome. It may be that women with polycystic ovarian syndrome or hyperandrogenism are inherently attracted to and succeed in athletic activities. This suggests that oligomenorrhea is also likely to be a polycystic ovarian syndrome or metabolic syndrome symptom in athletic women, as opposed to an exercise-induced trait *per se*. We suggest that polycystic ovarian syndrome may be a competitive advantage due to the proposed dual mechanism of testosterone (long-term effects on muscle strength and size, and a rapid acute effect on contractile efficiency).

Further research is needed to confirm whether acute testosterone fluctuations seen during a natural menstrual cycle or heightened levels seen in conditions such as polycystic ovarian syndrome are significant enough to result in a greater efficiency of the skeletal muscle to produce force/power. Polycystic ovarian syndrome or hyperandrogenism could lead to a physiological advantage for female athletes, especially those competing in athletic events that require rapid and/or forceful movements.

### Non-genomic actions of estrogen

While this review focuses on the specific role of non-genomic testosterone action in skeletal muscle and its effects within the contractile compartment of skeletal musculature it is within the scope of this article to briefly discuss the potential effects of a subsidiary non-genomic action of another prominent sex hormone that fluctuates throughout the female menstrual cycle, estrogen.

While ageing, muscle wasting and pathology research support a role for estrogens in skeletal muscle, particularly in females it is likely that the prevention of strength loss through estrogen hormone replacement therapy is likely a genomic or transcriptional mechanism rather than a rapid non-transcriptional mechanism. As this review is also focusing on the effects of testosterone on the contractile apparatus it seems pertinent to discuss those findings in rodent studies that indicate estrogen can influence the force generating capacity of skeletal muscle not by maintaining size of individual fibres but by maintaining the integrity and ability of individual fibres to generate force. This idea is supported by Wattanaperm-

pool et al. (1999) who measured cross sectional area (CSA) and the peak isometric tension of isolated rat soleus muscle fibres 10 and 14 wk post ovariectomy. While CSA was not reduced following ovariectomy, CSA was significantly increased compared with non-ovariectomy controls at 14 wk, peak tension was significantly lower in ovariectomised rats compared with sham-operate controls in both 10-wk (~19%) and 14 wk (~20%) post ovariectomy. These results show that fibres from ovariectomised rats were not weaker due to their smaller size but rather there was a deficit in the contractile apparatus likely due to the absence of estrogen that resulted in a reduced ability to produce force.

Similarly to testosterone, non-genomic estrogen action has been regularly identified in tissues other than skeletal muscle (Morley et al., 1992; Rubio-Gayosso and Sierra-Ramirez et al., 2000; Watson et al., 2008; Younglai et al., 2005). The most regularly reported non-genomic effect of androgen exposure is a rapid (within seconds) enhancement in intracellular calcium concentration (Ceballos et al., 1999; Morley et al., 1992; Vicencio et al., 2006; Watson et al., 2008). Estrogen is not exempt from this effect and it was originally demonstrated in chicken granulosa cells subjected to 17- $\beta$  estradiol, that there was an immediate (less than 5 s) 4-8 fold increase in calcium concentration in all of the 76 cells exposed [67]. Estrogen receptors have also been found to interact with a G-protein on the cell membrane of osteoblasts leading to a rapid increase in intracellular  $\text{Ca}^{2+}$  concentration due distinctly to increased  $\text{Ca}^{2+}$  mobilisation from the endoplasmic reticulum and the formation of  $\text{IP}_3$  demonstrating a similar mechanistic pathway as that shown during the mobilisation of intracellular  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum in skeletal muscle cells by rapid testosterone action. At this time no research has specifically investigated a non-genomic action of estrogen in skeletal muscles at either the molecular or whole muscle level.

Again, like testosterone, estrogen has demonstrated possible positive effects in skeletal muscle, in particular producing positive effects within the contractile machinery and even though these effects have been seen be in the presence (hormone replacement therapy) or absence (ageing/ovariectomy) of constant exposure or non-exposure to estrogen (17- $\beta$  estradiol), the non-genomic actions of estrogen in tissues other than skeletal muscle are similar to those demonstrated by testosterone in skeletal muscle cells. Therefore research is required to investigate a presence of a non-genomic action of estrogen in skeletal muscles and determine its ability to modulate force-generating processes. It may therefore be likely that both testosterone and estrogen are able to enhance myosin and actin binding processes due to modulations in calcium mobilisation, resulting in a greater force and/or velocity of contraction during acute changes in their concentrations.

### Conclusion

At this time, there is small evidence of non-genomic testosterone action in human skeletal muscle and the specific mode of action, as well as testosterone's practical importance to athletic competition are yet to be identified. Some

evidence points towards direct non-genomic activation of calcium-mediated events in skeletal muscle cells, which may modulate significant physiological responses such as the acute modulation of force in individual fibres and acute prevention or protection against calcium-mediated fatigue. These responses are likely complex and mediated by interplay between testosterone and second messengers IP<sub>3</sub> and calcium, which ultimately may result in simultaneous non-genomic and genomic modulations of skeletal muscle events. However, more research is needed to elucidate the potential membrane receptor involved, as well as the second-messenger pathway, the resulting action at the single-cell level and the transference of testosterone to whole muscle significance. Due to fluctuations in testosterone during the female menstrual cycle, research should also aim to identify if there are genuine gender differences in the response or mechanism of non-genomic action of testosterone in skeletal muscle. There may be a physiological advantage for female athletic performance during particular phases of the menstrual cycle. As well, polycystic ovarian syndrome and similar hyperandrogenism disorders may be a physiological advantage for specific athletes. Research to date suggests the main physiological function of non-genomic testosterone action in skeletal muscle is the enhancement of force production particularly in fast twitch fibres; however the actions are likely multifaceted and further research is needed to provide evidence of a whole body performance effect in humans.

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### References

- Benten, W.P., Lieberherr, M., Sekeris, C.E. and Wunderlich, F. (1997) Testosterone induces Ca<sup>2+</sup> influx via non-genomic surface receptors in activated T cells. *FEBS letters* **407**(2), 211-214.
- Benten, W.P., Lieberherr, M., Giese, G., Wrehlke, C., Stamm, O., Sekeris, C.E., Mossmann, H. and Wunderlich, F. (1999a) Functional testosterone receptors in plasma membranes of T cells. *The FASEB Journal* **13**(1), 123-133.
- Benten, W.P., Lieberherr, M., Stamm, O., Wrehlke, C., Guo, Z. and Wunderlich, F. (1999b) Testosterone signaling through internalizable surface receptors in androgen receptor-free macrophages. *Molecular Biology of the Cell* **10**(10), 3113-3123.
- Bhasin, S., Storer, T.W., Berman, N., Callegari, C., Clevenger, B., Phillips, J., Bunnell, T.J., Tricker, R., Shirazi, A. and Casaburi, R. (1996) The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *The New England Journal of Medicine* **335**(1), 1-7.
- Bhasin, S., Woodhouse, L., Casaburi, R., Singh, A.B., Mac, R.P., Lee, M., Yarasheski, K.E., Sinha-Hikim, I., Dzekov, C., Dzekov, J., Magliano, L. and Storer, T.W. (2005) Older Men Are as Responsive as Young Men to the Anabolic Effects of Graded Doses of Testosterone on the Skeletal Muscle. *Journal of Clinical Endocrinology & Metabolism* **90**(2), 678-688.
- Bigland-Ritchie, B., Jones, D.A. and Woods, J.J. (1979) Excitation frequency and muscle fatigue: electrical responses during human voluntary and stimulated contractions. *Experimental Neurology* **64**(2), 414-427.
- Bosco, C., Colli, R., Bonomi, R., Von Duvillard, S.P. and Viru, A. (2000) Monitoring strength training: neuromuscular and hormonal profile. *Medicine & Science in Sports Exercise* **32**(1), 202-208.
- Bosco, C.A.V., A. (1998) Testosterone and cortisol levels in blood of male sprinters, soccer players and cross-country skiers. *Biology of Sport* **15**, 3-8.
- Bourdin, M., Rambaud, O., Dorel, S., Lacour, J.R., Moyon, B. and Rahmani, A. (2010) Throwing performance is associated with muscular power. *International Journal of Sports Medicine* **31**(7), 505-510.
- Campagnoli, C., Biglia, N., Altare, F., Lanza, M.G., Lesca, L., Cantamessa, C., Peris, C., Fiorucci, G.C. and Sismondi, P. (1993) Differential effects of oral conjugated estrogens and transdermal estradiol on insulin-like growth factor I, growth hormone and sex hormone binding globulin serum levels. *Gynecological Endocrinology* **7**(4), 251-258.
- Cardinale, M. and Stone, M.H. (2006) Is testosterone influencing explosive performance? *Journal of Strength and Conditioning Research* **20**(1), 103-107.
- Ceballos, G., Figueroa, L., Rubio, I., Gallo, G., Garcia, A., Martinez, A., Yanez, R., Perez, J., Morato, T. and Chamorro, G. (1999) Acute and nongenomic effects of testosterone on isolated and perfused rat heart. *Journal of Cardiovascular Pharmacology* **33**(5), 691-697.
- Cheng, A.J. and Rice, C.L. (2010) Fatigue-Induced Reductions of Torque and Shortening Velocity Are Muscle Dependent. *Medicine & Science in Sports & Exercise* **42**(9), 1651-1659.
- Davies, B.N., Elford, J.C. and Jamieson, K.F. (1991) Variations in performance in simple muscle tests at different phases of the menstrual cycle. *Journal of Sports Medicine and Physical Fitness* **31**(4), 532-537.
- Doolittle, T.L. and Engebretsen, J. (1972) Performance variations during the menstrual cycle. *Journal of Sports Medicine and Physical Fitness* **12**(1), 54-58.
- Edman, K.A.P. and Mattiazzi, A.R. (1981) Effects of fatigue and altered pH on isometric force and velocity of shortening at zero load in frog muscle fibres. *Journal of Muscle Research and Cell Motility* **2**(3), 321-334.
- Edwards, D.A. and O'neal, J.L. (2009) Oral contraceptives decrease saliva testosterone but do not affect the rise in testosterone associated with athletic competition. *Hormones and Behavior* **56**(2), 195-198.
- Estrada, M., Liberona, J.L., Miranda, M. and Jaimovich, E. (2000) Aldosterone- and testosterone-mediated intracellular calcium response in skeletal muscle cell cultures. *American Journal of Physiology. Endocrinology & Metabolism* **279**(1), E132-139.
- Estrada, M., Espinosa, A., Muller, M. and Jaimovich, E. (2003) Testosterone stimulates intracellular calcium release and mitogen-activated protein kinases via a G protein-coupled receptor in skeletal muscle cells. *Endocrinology* **144**(8), 3586-3597.
- Furukawa, T. and Kurokawa, J. (2008) Non-genomic regulation of cardiac ion channels by sex hormones. *Cardiovascular & Hematological Disorders Drug Targets* **8**(4), 245-251.
- Giacomoni, M., Bernard, T., Gavarry, O., Altare, S. and Falgairette, G. (2000) Influence of the menstrual cycle phase and menstrual symptoms on maximal anaerobic performance. *Medicine & Science in Sports & Exercise* **32**(2), 486-492.
- Godt, R.E. and Nosek, T.M. (1989) Changes of intracellular milieu with fatigue or hypoxia depress contraction of skinned rabbit skeletal and cardiac muscle. *The Journal of Physiology* **412**(1), 155-180.
- Gordon, G.G., Southren, A.L., Tochimoto, S., Olivo, J., Aaltman, K., Rand, J. and Lemberger, L. (1970) Effect of Medroxyprogesterone Acetate (Provera) on the Metabolism and Biological Activity of Testosterone. *Journal of Clinical Endocrinology & Metabolism* **30**(4), 449-456.
- Griggs, R.C., Kingston, W., Jozefowicz, R.F., Herr, B.E., Forbes, G. and Halliday, D. (1989) Effect of testosterone on muscle mass and muscle protein synthesis. *Journal of Applied Physiology* **66**(1), 498-503.
- Haan, A., Jones, D.A. and Sargeant, A.J. (1989) Changes in velocity of shortening, power output and relaxation rate during fatigue of rat medial gastrocnemius muscle. *Pflügers Archiv European Journal of Physiology* **413**(4), 422-428.
- Hagmar, M., Berglund, B., Brismar, K. and Hirschberg, A.L. (2009) Hyperandrogenism may explain reproductive dysfunction in olympic athletes. *Medicine & Science in Sports & Exercise* **41**(6), 1241-1248.
- Hamdi, M.M. and Mutungi, G. (2010) Dihydrotestosterone activates the MAPK pathway and modulates maximum isometric force

- through the EGF receptor in isolated intact mouse skeletal muscle fibres. *The Journal of Physiology* **588**(Pt 3), 511-525.
- Hori, N., Newton, R.U., Andrews, W.A., Kawamori, N., McGuigan, M.R. and Nosaka, K. (2008) Does Performance of Hang Power Clean Differentiate Performance of Jumping, Sprinting, and Changing of Direction? *The Journal of Strength & Conditioning Research* **22**(2), 412-418
- Jones, D.A., Bigland-Ritchie, B. and Edwards, R.H. (1979) Excitation frequency and muscle fatigue: mechanical responses during voluntary and stimulated contractions. *Experimental Neurology* **64**(2), 401-413.
- Jones, R.D., English, K.M., Jones, T.H. and Channer, K.S. (2004) Testosterone-induced coronary vasodilatation occurs via a non-genomic mechanism: evidence of a direct calcium antagonism action. *Clinical Science* **107**(2), 149-458.
- Judd, H.L. and Yen, S.S.C. (1973) Serum Androstenedione and Testosterone Levels During the Menstrual Cycle. *Journal of Clinical Endocrinology & Metabolism* **36**(3), 475-481.
- Kabbara, A.A. and Allen, D.G. (1999) The role of calcium stores in fatigue of isolated single muscle fibres from the cane toad. *The Journal of Physiology* **519**(1), 169-176.
- Lebrun, C.M. (1993) Effect of the different phases of the menstrual cycle and oral contraceptives on athletic performance. *Sports Medicine* **16**(6), 400-430.
- Lebrun, C.M. (1994) The effect of the phase of the menstrual cycle and the birth control pill on athletic performance. *Clinical Sports Medicine* **13**(2), 419-441.
- Lebrun, C.M., McKenzie, D.C., Prior, J.C. and Taunton, J.E. (1995) Effects of menstrual cycle phase on athletic performance. *Medicine & Science in Sports & Exercise* **27**(3), 437-444.
- Lieberherr, M. and Grosse, B. (1994) Androgens increase intracellular calcium concentration and inositol 1,4,5-trisphosphate and diacylglycerol formation via a pertussis toxin-sensitive G-protein. *The Journal of Biological Chemistry* **269**(10), 7217-7223.
- Masterson, G. (1999) The Impact of Menstrual Phases on Anaerobic Power Performance in Collegiate Women. *The Journal of Strength & Conditioning Research* **13**(4), 325-329.
- Mastorakos, G., Pavlatou, M., Diamanti-Kandarakis, E. and Chrousos, G.P. (2005) Exercise and the stress system. *Hormones* **4**(2), 73-89.
- McKeever, K.H. (2002) The endocrine system and the challenge of exercise. *The Veterinary Clinics of North America. Equine practice* **18**(2), 321-353, vii.
- Milner-Brown, H.S. and Miller, R.G. (1986) Muscle membrane excitation and impulse propagation velocity are reduced during muscle fatigue. *Muscle & Nerve* **9**(4), 367-374.
- Morley, P., Whitfield, J.F., Vanderhyden, B.C., Tsang, B.K. and Schwartz, J.L. (1992) A new, nongenomic estrogen action: the rapid release of intracellular calcium. *Endocrinology* **131**(3), 1305-1312.
- Palatsi, R., Hirvensalo, E., Liukko, P., Malmiharju, T., Mattila, L., Riihilauma, P. and Ylostalo, P. (1984) Serum total and unbound testosterone and sex hormone binding globulin (SHBG) in female acne patients treated with two different oral contraceptives. *Acta Dermato Venereologica* **64**(6), 517-523.
- Phillips, S.K., Sanderson, A.G., Birch, K., Bruce, S.A. and Woledge, R.C. (1996) Changes in maximal voluntary force of human adductor pollicis muscle during the menstrual cycle. *The Journal of Physiology* **496**(Pt 2), 551-557.
- Raj, S.C., Raj, M.H.G., Talbert, L.M., Sloan, C.S. and Hicks, B. (1983) Normalization of Testosterone Levels Using a Low Estrogen-containing Oral Contraceptive in Women with Polycystic Ovary Syndrome. *Obstetrical & Gynecological Survey* **38**(2), 110-111.
- Rechichi, C. and Dawson, B. (2009) Effect of oral contraceptive cycle phase on performance in team sport players. *Journal of Science and Medicine in Sport* **12**(1), 190-195.
- Rickenlund, A., Carlstrom, K., Ekblom, B., Brismar, T.B., Von Schoultz, B. and Hirschberg, A.L. (2004) Effects of oral contraceptives on body composition and physical performance in female athletes. *The Journal of Clinical Endocrinology and Metabolism* **89**(9), 4364-470.
- Rivera, R., Yacobson, I. and Grimes, D. (1999) The mechanism of action of hormonal contraceptives and intrauterine contraceptive devices. *American Journal of Obstetrics and Gynecology* **181**(5), 1263-1269.
- Rosetta, L., Harrison, G.A. and Read, G.F. (1998) Ovarian impairments of female recreational distance runners during a season of training. *Annals of Human Biology* **25**(4), 345-357.
- Rubio-Gayosso, I., Sierra-Ramirez, A., García-Vazquez, A., Martínez-Martínez, A., Muñoz-García, O., Morato, T. and Ceballos-Reyes, G. (2000) 17[beta]-Estradiol Increases Intracellular Calcium Concentration Through a Short-Term and Nongenomic Mechanism in Rat Vascular Endothelium in Culture. *Journal of Cardiovascular Pharmacology* **36**(2), 196-202.
- Sanborn, C.F., Martin, B.J. and Wagner, W.W., Jr. (1982) Is athletic amenorrhea specific to runners? *American Journal of Obstetrics and Gynecology* **143**(8), 859-861.
- Sarwar, R., Niclos, B.B. and Rutherford, O.M. (1996) Changes in muscle strength, relaxation rate and fatiguability during the human menstrual cycle. *The Journal of Physiology* **493** (Pt 1), 67-72.
- Sinha-Hikim, I., Arver, S., Beall, G., Shen, R., Guerrero, M., Sattler, F., Shikuma, C., Nelson, J.C., Landgren, B.-M., Mazer, N.A. and Bhasin, S. (1998) The Use of a Sensitive Equilibrium Dialysis Method for the Measurement of Free Testosterone Levels in Healthy, Cycling Women and in Human Immunodeficiency Virus-Infected Women. *Journal of Clinical Endocrinology & Metabolism* **83**(4), 1312-1318.
- Sinha-Hikim, I., Artaza, J., Woodhouse, L., Gonzalez-Cadavid, N., Singh, A.B., Lee, M.I., Storer, T.W., Casaburi, R., Shen, R. and Bhasin, S. (2002) Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. *American Journal of Physiology - Endocrinology & Metabolism* **283**(1), E154-E164.
- Sinha-Hikim, I., Roth, S.M., Lee, M.I. and Bhasin, S. (2003) Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men. *American Journal of Physiology - Endocrinology & Metabolism* **285**(1), E197-E205.
- Sleivert, G. and Taingahue, M. (2004) The relationship between maximal jump-squat power and sprint acceleration in athletes. *European Journal of Applied Physiology* **91**(1), 46-52.
- Thornycroft, I.H., Stanczyk, F.Z., Bradshaw, K.D., Ballagh, S.A., Nichols, M. and Weber, M.E. (1999) Effect of low-dose oral contraceptives on androgenic markers and acne. *Contraception* **60**(5), 255-262.
- Torstveit, M.K. and Sundgot-Borgen, J. (2005) Participation in leanness sports but not training volume is associated with menstrual dysfunction: a national survey of 1276 elite athletes and controls. *British Journal of Sports Medicine* **39**(3), 141-147.
- Urban, R.J., Bodenbun, Y.H., Gilkison, C., Foxworth, J., Coggan, A.R., Wolfe, R.R. and Ferrando, A. (1995) Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. *American Journal of Physiology - Endocrinology & Metabolism* **269**(5), E820-E826.
- Van Der Vange, N., Blankenstein, M.A., Kloosterboer, H.J., Haspels, A.A. and Thijssen, J.H.H. (1990) Effects of seven low-dose combined oral contraceptives on sex hormone binding globulin, corticosteroid binding globulin, total and free testosterone. *Contraception* **41**(4), 345-352.
- Van Ingen Schenau, G.J., De Konong, J.J. and De Groot, G. (1990) A simulation of speed skating performances based on a power equation. *Medicine & Science in Sports & Exercise* **22**(5), 718-728.
- Vicencio, J.M., Ibarra, C., Estrada, M., Chiong, M., Soto, D., Parra, V., Diaz-Araya, G., Jaimovich, E. and Lavandero, S. (2006) Testosterone induces an intracellular calcium increase by a nongenomic mechanism in cultured rat cardiac myocytes. *Endocrinology* **147**(3), 1386-1395.
- Waldkirch, E., Uckert, S., Schultheiss, D., Geismar, U., Bruns, C., Scheller, F., Jonas, U., Becker, A.J., Stief, C.G. and Hedlund, P. (2008) Non-genomic effects of androgens on isolated human vascular and nonvascular penile erectile tissue. *British Journal of Urology International* **101**(1), 71-5; discussion 75.
- Ward, C.W., Spangenburg, E.E., Diss, L.M. and Williams, J.H. (1998) Effects of varied fatigue protocols on sarcoplasmic reticulum calcium uptake and release rates. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **275**(1), R99-R104.



- Watson, C.S., Jeng, Y.-J. and Kochukov, M.Y. (2008) Nongenomic actions of estradiol compared with estrone and estriol in pituitary tumor cell signaling and proliferation. *The FASEB Journal* **22**(9), 3328-3336.
- Wattanapernpool, J. and Reiser, P.J. (1999) Differential effects of ovariectomy on calcium activation of cardiac and soleus myofilaments. *American Journal of Physiology - Heart and Circulatory Physiology* **277**(2), H467-H473.
- Wearing, M.P., Yuhasz, M.D., Campbell, R. and Love, E.J. (1972) The effect of the menstrual cycle on tests of physical fitness. *Journal of Sports Medicine and Physical Fitness* **12**(1), 38-41.
- Westerblad, H. and Allen, D.G. (1991) Changes of myoplasmic calcium concentration during fatigue in single mouse muscle fibers. *The Journal of General Physiology* **98**(3), 615-635.
- Westerblad, H. and Lännergren, J. (1991) Slowing of relaxation during fatigue in single mouse muscle fibres. *The Journal of Physiology* **434**(1), 323-336.
- Westerblad, H. and Allen, D.G. (1993) The contribution of  $[Ca^{2+}]_i$  to the slowing of relaxation in fatigued single fibres from mouse skeletal muscle. *The Journal of Physiology* **468**(1), 729-740.
- Wiegatz, I., Jung-Hoffmann, C. and Kuhl, H. (1995) Effect of two oral contraceptives containing ethinylestradiol and gestodene or norgestimate upon androgen parameters and serum binding proteins. *Contraception* **51**(6), 341-346.
- Wiegatz, I., Kutschera, E., Lee, J.H., Moore, C., Mellinger, U., Winkler, U.H. and Kuhl, H. (2003a) Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. *Contraception* **67**(1), 25-32.
- Wiegatz, I., Kutschera, E., Lee, J.H., Moore, C., Mellinger, U., Winkler, U.H. and Kuhl, H. (2003b) Effect of four oral contraceptives on thyroid hormones, adrenal and blood pressure parameters. *Contraception* **67**(5), 361-366.
- Younglai, E.V., Wu, Y.J., Kwan, T.K. and Kwan, C.Y. (2005) Non-genomic action of estradiol and progesterone on cytosolic calcium concentrations in primary cultures of human granulosa-lutein cells. *Human Reproduction* **20**(9), 2383-2390.

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## Key points

- Non-genomic calcium mediated events activated by testosterone have been identified in skeletal muscle cells.
- The non-genomic action originates at the cell membrane, is rapid in onset and is directed by second messengers' calcium and  $IP_3$ .
- A possible action of non-genomic testosterone may be the initiation of a more efficient contraction through the mobilisation of calcium from the SR resulting in greater force production or velocity of contraction in fast twitch fibres.
- Physiologically, females with menstrual disorders that cause hyperandrogenism may have a performance advantage in events that require great force or power production.