Androgen Regulation of Anabolic and Catabolic Pathways in Skeletal Muscle

Peter Bond, MSc[a,*]

[a] *PeterBond.nl*

Abstract

Text of abstract. Text of abstract. Text of abstract. Text of abstract. Text of abstract.

Keywords: Androgens, mTOR, FoxO, Akt, β-catenin

1. Introduction

Androgens are well-known for their positive effects on both skeletal muscle size and strength. Skeletal muscle size is determined by the delicate balance between protein synthesis and breakdown. An increase in skeletal muscle size can be achieved by an increase in protein synthesis and/or a decrease in protein breakdown. Androgens influence pathways which coordinate protein synthesis and breakdown and hence regulate skeletal muscle size. In general, androgens increase muscle protein synthesis whereas they decrease breakdown.

In recent years, the molecular mechanisms through which skeletal muscle protein synthesis and breakdown is coordinated have been elucidated. In particular, the protein kinase mammalian/mechanistic target of rapamycin (mTOR) has received a lot of attention as a master regulator of protein synthesis [1, 2]. mTOR forms the catalytic center of the two signaling complexes mTORC1 and mTORC2 [3], of which the first is primarily involved in regulation of protein synthesis. mTORC1 is structurally composed out of the three core subunits mTOR, Raptor and mLST8 [4]. Activation of the complex leads to phosphorylation of its two important sets of substrates which are involved in mRNA translation. One being the eukaryotic initiation factor 4E (eIF4E)-binding proteins 4E-BP1 and 2. 4E-BPs inhibit the formation of the eIF4F complex which facilitates recruitment of the small (40S) ribosomal subunit to the $5'$ end of mRNA [5]. Therefore, 4E-BPs inhibit mRNA translation initiation and phosphorylation by mTORC1 relieves this inhibition. The other important set of substrates of mTORC1 are the ribosomal S6 kinases S6K1 and 2. Phosphorylation of the S6Ks by mTORC1 activates them and resultingly modulate functions of translation initiation factors [6]. Additionally, S6Ks are thought to promote ribosome biogenesis [7].

This paper summarizes the body of literature reporting on androgen-regulation of these pathways which orchestrate skeletal muscle size.

*Corresponding author

Email address: `peter@peterbond.nl` (Peter Bond, MSc)

URL: `PeterBond.nl` (Peter Bond, MSc)

2. Akt/protein kinase B (PKB)

Akt (also known as protein kinase B [PKB]) is a family of serine/threonine-specific protein kinases which play a central role in both anabolic and catabolic pathways [8]. Akt consists of three isoforms, namely: Akt1, Akt2 and Akt3. The first two are expressed in skeletal muscle, whereas the latter is not. Phosphorylation of Akt activates the kinase. Akt can be phosphorylated on two positions, namely at: Ser473 or Ser474 (in Akt1 and Akt2, respectively) [9], and Thr308 or Thr309 (in Akt1 and Akt2, respectively) [10]. Phosphorylation of both residues is necessary for full activation of Akt kinase activity [10, 11]. The threonine residue is phosphorylated by 3-phosphoinositide-dependent protein kinase 1 (PDK1) and the serine residue is likely phosphorylated by mammalian target of rapamycin complex 2 (mTORC2).

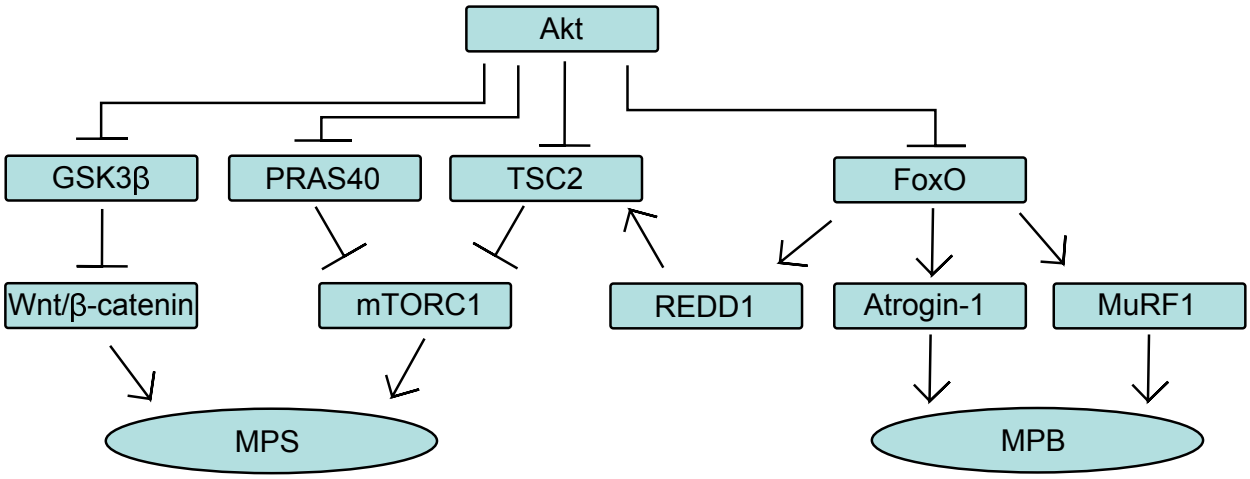

Figure 1: Caption.

When Akt is activated, it phosphorylates several molecules involved in both anabolic and catabolic conditions. Its targets include glycogen synthase kinase 3β (GSK3β) [12], proline-rich Akt substrate of 40 kDa (PRAS40) [13], tuberous sclerosis complex 2 (TSC2) [14] and forkhead box class O (FoxO) family member proteins [15].

GSK3β is a negative regulator of the Wnt/β-catenin signaling pathway as it forms a complex with other proteins and phosphorylates β-catenin leading to degradation of the molecule [16]. Akt phosphorylates GSK3β, which inactivates the enzyme and thereby stimulates Wnt/β-catenin signaling through removal of its inhibiton. β-catenin seems to play an important role in skeletal muscle hypertrophy by functioning as a transcription factor [17] and inhibition of GSK3β stimulates hypertrophy in C_2C_{12} myotubes [18].

PRAS40 is associated with mTORC1 by binding to the substrate binding site of raptor, one of the proteins forming the complex. PRAS40 inhibits mTORC1 and inhibition can be relieved by phosphorylation of the protein [19]. Its two main phosphorylation sites are at Ser183 and Thr246. The latter being phosphorylated by Akt, whereas the first is phosphorylated by mTORC1 itself. After phosphorylation, PRAS40 dissociates from mTORC1 and thus relieving inhibition of the complex.

TSC2 can form a complex with TSC1 and TBC1D7 which inhibits mTORC1 by inhibiting Rheb [20]. In its active GTP-bound state, Rheb activates mTORC1 by unknown mechanisms,

although interaction with the mTOR kinase domain appears to be involved [21]. TSC2 hydrolyzes Rheb-GTP to the inactive Rheb-GDP by virtue of its GTPase activating protein (GAP) domain, and therefore inhibits mTORC1 [22]. Akt phosphorylates TSC2 at multiple sites (Ser939, Ser981, Ser1130, Ser1132 and Thr1462) in order to inhibit the GAP activity of the TSC complex, possibly by moving TSC2 away from the lysosomal surface where mTORC1 is activated by Rheb [23], and therefore stimulates mTORC1 activation through increased Rheb-GTP.

FoxO proteins are key regulators of protein breakdown by modulating ubiquitin-proteasome, as well as autophagy-lysosomal proteolytic pathways [24]. Especially the first seems important in muscle protein breakdown and two E3 ubiquitin ligases, muscle atrophy F-box (MAFbx/atrogin-1) and muscle ring finger 1 (MuRF1) [25, 26], appear to be the two main downstream effectors of FoxO signaling concerning protein breakdown. FoxO proteins are phosphorylated, and thereby inhibited, by Akt [27].

Clearly, Akt plays a central role in both muscle hypertrophy and atrophy due to its regulation of both anabolic and catabolic pathways. Several studies suggest Akt also plays a role in androgen-modulation of these pathways [28, 29, 30, 31], whereas others do not [32, 33, 34].

Author(s)	Androgen (dose[s] and duration[s])	Model	Findings
White et al. (2013) [28]	Nandrolone (6 mg/kg bw/wk for 28 d)	Orchidectomized C57BL/6 mice gastrocnemius muscle	p-Akt (Ser473) ↑
White et al. (2013) [28]	Testosterone (5, 50 and 500 nM for 24 h)	C_2C_{12} myoblasts	p-Akt (Ser473) ↔ (5, 50 nM) p-Akt (Ser473) ↑ (500 nM)
White et al. (2009) [29]	Nandrolone (6 mg/kg bw/wk for 5 and 14 d)	Orchidectomized C57BL/6 mice tibialis anterior muscle	p-Akt (Ser 473) ↑ (all time points)
White et al. (2009) [29]	Nandrolone (6 mg/kg bw/wk for 5 and 14 d)	Orchidectomized bupivacaine-injured C57BL/6 mice tibialis anterior muscle	p-Akt (Ser 473) ↑ (all time points)
Basualto-alarcón et al. (2013) [30]	Testosterone (100 nM for 1, 5, 15, 30 and 60 m)	Cultured rat hindlimb muscles myotubes	p-Akt (Ser473) ↔ (1, 5, 30 and 60 m) p-Akt (Ser473) ↑ (15 m)
Wu et al. (2013) [34]	Testosterone (100 nM for 20 m, 2 h or 20 h)	L6AR myoblasts	p-Akt (Thr308) ↔ (all time points)
Ma et al. (2014) [33]	Testosterone (single 20 mg 15-d release pellet for 1, 4, 7 and 14 d)	Burn-injured male Wister rats tibialis anterior muscle	p-Akt (Thr308) ↔ (all time points)
Hourdé et al. (2009) [32]	Nandrolone (3 mg/kg bw/d for 3 mo)	Orchidectomized male Wister rats soleus muscle	p-Akt (Ser473) ↔
Hourdé et al. (2009) [32]	Dihydrotestosterone (single 150 mg 90-d release pellet for 3 mo)	Orchidectomized male Wister rats soleus muscle	p-Akt (Ser473) ↔
Jones et al. (2010) [31]	Testosterone (0.1, 1, 10 nM, 100 nM and 1000 nM for 24 h)	C_2C_{12} myoblasts treated with 100 nM dexamethason	p-Akt (Ser473) ↑ (all concentrations)
Jones et al. (2010) [31]	Testosterone (25 mg/kg bw/d for 8 d)	Sprague Dawley rats receiving 600 μg/kg bw/d dexamethason	p-Akt (Ser473) ↑

Table 1: Caption.

Adult male rats receiving near-physiological doses of either nandrolone or dihydrotestosterone (DHT) for 3 months after orchidectomy, did not show differences among total Akt content, nor p-Akt (Ser473), compared to orchidectomized rats treated with vehicle or sham operated rats [32]. Notably, protein measurements were taken from a biopt of the soleus muscle and orchidectomy did not result in significant atrophy of soleus muscle and muscle fibers. However, lean mass significantly differed from sham operated rats in all three treatment groups. In contrast with these results, adult male orchidectomized mice did show a significant reduction of soleus muscle mass 30 d post-intervention [35]. This effect was attenuated by testosterone administration at a physiological rate. Unfortunately, the researchers did not assess Akt status. The contrasting results might be due to the different animal models used (rats vs. mice) or because of the time at which the measurements were taken (3 mo vs. 30 d post-intervention).

A different experimental setup examing testosterone's effect on severe burn injury in male Wister rats also examined the role of Akt [33]. Measurements were taken at 1 d, 4 d, 7 d and 14 d post-treatment. Testosterone treatment had no effect on total Akt protein content nor p-Akt (Thr308) in the tibialis anterior (TA) at any of the time points. Remarkably, testosterone administration did increase p-FoxO3a (Residue???) compared to vehicle. Moreover, testosterone administration significantly attenuated the burn-induced increase in atrogin-1 mRNA at all time points, but not that of MuRF1. These results suggest androgens can suppress atrogin-1 expression via its effect on FoxO3a in an Akt-independent manner. A line of rat L6 myoblasts stably expressing human androgen receptor (AR) under a retroviral transgene also showed no change in p-Akt (Thr308) after short-term (20 m, 2 h and 20 h) incubation with testosterone or DHT, despite an increase of protein content and cell diameter [34].

In contrast with these results which do not implicate Akt in androgen-modulation of anabolic and catabolic pathways, White et al. reported an increased ratio of p-Akt (Ser473) to total Akt (p-Akt/Akt) after nandrolone decanoate (6 mg/kg bw/wk) administration for 42 d to orchidectomized C57BL/6 mice [28]. Gastrocnemius muscles were used for analysis and the ratio was 4.5 fold higher ($p = 0.004$) compared to castrated mice treated with vehicle and 2 fold higher ($p = 0.012$) compared to sham operated mice. Additionally, the researchers examined the effects of incremental concentrations (5, 50 and 500 nM) of testosterone for 24 h in C_2C_{12} myoblasts. Interestingly, incubation with 5 and 50 nM testosterone did not affect p-Akt, whereas 500 nM led to a significant increase. Moreover, the two Akt-targets GSK3β and FoxO3a showed an increased phosphorylation state at 500 nM, as well as 50 nM. An increase in FoxO3a and GSK3β phosphorylation was also observed in the nandrolone treated mice. However, PRAS40 phosphorylation was not increased in the C_2C_{12} myoblasts, whereas it was in the nandrolone treated mice.

White et al. had also examined the effect of nandrolone decanoate (6 mg kg bw/wk) administration for 5 and 14 d to orchidectomized C57BL/6 mice with bupivacaine-induced muscle injury [29]. The researchers randomized mice to four groups: 1) uninjured; 2) nandrolone only (uninjured + nandrolone); 3) bupivacaine (injured); and 4) bupivacaine + nandrolone (injured + nandrolone). Nandrolone treatment led to a significant increase of p-Akt (Ser473) in both uninjured and injured mice at 14 d after injury. The p-Akt/Akt ratio was nearly 8-fold higher in the injured + nandrolone group compared to the injured group. Moreover, the p-Akt/Akt ratio was also slightly higher in the injured + nandrolone group compared to the injured group at 5 d after injury, as well as in the nandrolone + uninjured group compared to the uninjured group. As would be expected from the increase in p-Akt, the p-GSK3β (Ser9)/GSK3β ratio was also increased in the injured + nandrolone group compared to the injured group as well as the nandrolone + uninjured group

compared to the uninjured group at both 5 and 14 d.

Similar results to that of White et al. of testosterone administration on p-Akt (Ser473) where found in a rat model of dexamethasone-induced atrophy [31]. Rats treated with dexamethasone (600 μg/kg bw/d) for 8 d showed a strong decrease in p-Akt. Administration of testosterone propionate (25 mg/kg bw/d) maintained phosphorylated status of Akt in the levator ani. Moreover, the authors measured several downstream effectors of Akt and found decreases in p-GSK3β, p-p70S6K and p-FoxO3A after dexamethason administration, of which the down-regulation of the first two was blocked by testosterone and attenuated for the latter. The two ubiquitin ligases MuRF-1 and Atrogin-1 also showed a strong increase in mRNA expression after dexamethasone administration in the levator ani, gastrocnemius, extensor digitorum longus and soleus muscles. Testosterone completely inhibited this up-regulation in the levator ani and attenuated it in the gastrocnemius, extensor digitorum longus and soleus muscles. Remarkably, while the decrease in levator ani size was blocked by testosterone, its administration did not attenuate the decrease in gastrocnemius, extensor digitorum longus and soleus size. This might be due to timing of the administration of testosterone. In a rat model of denervation-induced atrophy, nandrolone regulated almost entirely different genes when administered directly after deneveration compared to 4 weeks after denervation [36]. Administration of nandrolone for 7 d slows down denervation atrophy when begun 4 weeks after nerve transaction, whereas it does not when administration is started at the day of transection. The same might apply to a model dexamethasone-induced atrophy.

In addition, similar effects were found by Jones et al. in C_2C_{12} myoblasts expressing the AR incubated with 100 nM dexamethasone, in which they examined increasing concentrations of testosterone (0.1, 1, 10, 100 and 1000 nM) incubation for 24 h [31]. Notably, testosterone already exhibited marked effects at 0.1 nM on Atrogin-1 mRNA expression, with a near-maximal effect at 1 nM. Moreover, the dexamethasone-induced decrease in p-Akt was already completely blocked at 10 nM of testosterone.

In order to examine the rapid effects exerted by androgens, Basualto-Alarcón et al. incubated cultured rat skeletal muscle myotubes with 100 nM testosterone [30]. Measurements of total Akt and p-Akt (Ser473) were taken 1, 5, 15, 30 and 60 m after incubation. At 15 m, p-Akt was significantly increased. Measurements of α-actin mRNA and protein were taken 6 and 12 h after testosterone incubation and both were significantly increased, thus indicating an increase in contractile protein synthesis. Indeed, the cross-sectional area (CSA) was significantly increased after 12 h. An interesting finding was that, prior to the increase in p-Akt, extracellular signal-regulated kinases 1/2 (ERK1/2) phosphorylation (Thr202/Tyr204) was significantly increased at 5 m. Nevertheless, ERK1/2 inhibition had no effect on α-actin protein levels, although it did seem to attenuate the increase in p-S6K1/S6K1 ratio. However, inhibition of phosphoinositide 3-kinase (PI3K), Akt or mTOR did block the effect on α-actin. As such, it appears likely that androgens exert rapid effects by activation of the PI3K/Akt/mTOR pathway. PI3K is a family of membrane-localized serine/threonine kinases which phosphorylate phosphatidylinositol 4,5-bisphosphate (PIP2) to produce phosphatidylinositol (3,4,5)-trisphosphate (PIP3). Interaction of PIP3 with the pleckstrin-homology (PH) domain of Akt aids in recruitment of the kinase to the cell membrane where it can be phosphorylated (and therefore activated) [37]. Due to the rapid effect observed, as well as the localization of PI3K, it appears highly likely that the increase in p-Akt after 15 m of incubation with testosterone is mediated by a cell membrane receptor. Indeed, multiple lines of evidence implicate a cell membrane-localized receptor in the rapid effects of androgens [38],

and the G-protein coupled receptor (GPCR) GPRC6A has been shown to mediate a rapid signaling response to testosterone, including involvement of PI3K and ERK [39]. Nevertheless, when the AR antagonist bicalutamide was added, the increase in CSA was blocked, despite an increase in α-actin protein level. This indicates crosstalk between the intracellular AR and the PI3K pathway activated by testosterone. Strikingly, the intracellular AR has been shown to interact with the p85α regulatory subunit of PI3K in androgen-sensitive epithelial cells, enhancing its activity [40]. However, the addition of bicalutamide in that experiment blocked, rather than attenuated, the androgen-induced Akt (Ser473) phosphorylation. Since p-Akt inhibition blocked the increase in α-actin protein level in the experiment of Basualto-Alarcón et al., but bicalutamide did not, there appears to be a discrepancy in the data. This might be due to the differences in cell lines and AR ligands used. Nevertheless, AR-PI3K crosstalk might (partly) underlie the absence of an increase in CSA with the addition of bicalutamide in the experiment of Basualto-Alarcón et al., despite an increase in α-actin. Additionally, activation of the PI3K/Akt pathway can, in turn, regulate AR activity due to phosphorylation by Akt [41]. It is, however, unclear what the effect would be on AR transcriptional activity in skeletal muscle, since no study has evaluated this yet. Highlighting its dependency on cellular context is that the passage number of cells can already influence the effect it has on AR transcriptional activity. Low passage and high passage LNCaP cells have been shown to have opposite effects on Akt-phosphorylated AR transcriptional activity [42].

Concluding, the effect of androgens on p-Akt in skeletal muscle is dependent on several factors.

References

[1] S. C. Bodine, T. N. Stitt, M. Gonzalez, W. O. Kline, G. L. Stover, R. Bauerlein, E. Zlotchenko, A. Scrimgeour, J. C. Lawrence, D. J. Glass, et al., Akt/mtor pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo, Nature cell biology 3 (11) (2001) 1014–1019.

[2] O. A. Adegoke, A. Abdullahi, P. Tavajohi-Fini, mtorc1 and the regulation of skeletal muscle anabolism and mass, Applied Physiology, Nutrition, and Metabolism 37 (3) (2012) 395–406.

[3] K. Huang, D. C. Fingar, Growing knowledge of the mtor signaling network, in: Seminars in cell & developmental biology, Vol. 36, Elsevier, 2014, pp. 79–90.

[4] C. C. Dibble, B. D. Manning, Signal integration by mtorc1 coordinates nutrient input with biosynthetic output, Nature cell biology 15 (6) (2013) 555–564.

[5] N. Sonenberg, A. G. Hinnebusch, Regulation of translation initiation in eukaryotes: mechanisms and biological targets, Cell 136 (4) (2009) 731–745.

[6] X. M. Ma, J. Blenis, Molecular mechanisms of mtor-mediated translational control, Nature reviews Molecular cell biology 10 (5) (2009) 307–318.

[7] M. Brian, E. Bilgen, C. F. Diane, Regulation and function of ribosomal protein s6 kinase (s6k) within mtor signalling networks, Biochemical Journal 441 (1) (2012) 1–21.

[8] M. Wu, M. Falasca, E. R. Blough, Akt/protein kinase b in skeletal muscle physiology and pathology, Journal of cellular physiology 226 (1) (2011) 29–36.

[9] D. D. Sarbassov, D. A. Guertin, S. M. Ali, D. M. Sabatini, Phosphorylation and regulation of akt/pkb by the rictor-mtor complex, Science 307 (5712) (2005) 1098–1101.

[10] D. R. Alessi, S. R. James, C. P. Downes, A. B. Holmes, P. R. Gaffney, C. B. Reese, P. Cohen, Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase bα, Current Biology 7 (4) (1997) 261–269.

[11] J. Feng, J. Park, P. Cron, D. Hess, B. A. Hemmings, Identification of a pkb/akt hydrophobic motif ser-473 kinase as dna-dependent protein kinase, Journal of Biological Chemistry 279 (39) (2004) 41189–41196.

[12] D. A. Cross, D. R. Alessi, P. Cohen, M. Andjelkovich, B. A. Hemmings, Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase b, Nature 378 (6559) (1995) 785–789.

[13] K. S. Kovacina, G. Y. Park, S. S. Bae, A. W. Guzzetta, E. Schaefer, M. J. Birnbaum, R. A. Roth, Identification

of a proline-rich akt substrate as a 14-3-3 binding partner, Journal of Biological Chemistry 278 (12) (2003) 10189–10194.
[14] K. Inoki, Y. Li, T. Zhu, J. Wu, K.-L. Guan, Tsc2 is phosphorylated and inhibited by akt and suppresses mtor signalling, Nature cell biology 4 (9) (2002) 648–657.
[15] H. Tran, A. Brunet, E. C. Griffith, M. E. Greenberg, The many forks in foxo's road, Science Signaling 2003 (172) (2003) re5.
[16] S. Ikeda, S. Kishida, H. Yamamoto, H. Murai, S. Koyama, A. Kikuchi, Axin, a negative regulator of the wnt signaling pathway, forms a complex with gsk-3β and β-catenin and promotes gsk-3β-dependent phosphorylation of β-catenin, The EMBO journal 17 (5) (1998) 1371–1384.
[17] D. D. Armstrong, K. A. Esser, Wnt/β-catenin signaling activates growth-control genes during overload-induced skeletal muscle hypertrophy, American Journal of Physiology-Cell Physiology 289 (4) (2005) C853–C859.
[18] D. R. Vyas, E. E. Spangenburg, T. W. Abraha, T. E. Childs, F. W. Booth, Gsk-3β negatively regulates skeletal myotube hypertrophy, American Journal of Physiology-Cell Physiology 283 (2) (2002) C545–C551.
[19] C. Wiza, E. B. Nascimento, D. M. Ouwens, Role of pras40 in akt and mtor signaling in health and disease, American Journal of Physiology-Endocrinology and Metabolism 302 (12) (2012) E1453–E1460.
[20] C. C. Dibble, W. Elis, S. Menon, W. Qin, J. Klekota, J. M. Asara, P. M. Finan, D. J. Kwiatkowski, L. O. Murphy, B. D. Manning, Tbc1d7 is a third subunit of the tsc1-tsc2 complex upstream of mtorc1, Molecular cell 47 (4) (2012) 535–546.
[21] X. Long, Y. Lin, S. Ortiz-Vega, K. Yonezawa, J. Avruch, Rheb binds and regulates the mtor kinase, Current Biology 15 (8) (2005) 702–713.
[22] Y. Li, M. N. Corradetti, K. Inoki, K.-L. Guan, Tsc2: filling the gap in the mtor signaling pathway, Trends in biochemical sciences 29 (1) (2004) 32–38.
[23] S. Menon, C. C. Dibble, G. Talbott, G. Hoxhaj, A. J. Valvezan, H. Takahashi, L. C. Cantley, B. D. Manning, Spatial control of the tsc complex integrates insulin and nutrient regulation of mtorc1 at the lysosome, Cell 156 (4) (2014) 771–785.
[24] A. M. Sanchez, R. B. Candau, H. Bernardi, Foxo transcription factors: their roles in the maintenance of skeletal muscle homeostasis, Cellular and Molecular Life Sciences 71 (9) (2014) 1657–1671.
[25] S. C. Bodine, E. Latres, S. Baumhueter, V. K.-M. Lai, L. Nunez, B. A. Clarke, W. T. Poueymirou, F. J. Panaro, E. Na, K. Dharmarajan, et al., Identification of ubiquitin ligases required for skeletal muscle atrophy, Science 294 (5547) (2001) 1704–1708.
[26] S. C. Bodine, L. M. Baehr, Skeletal muscle atrophy and the e3 ubiquitin ligases murf1 and mafbx/atrogin-1, American Journal of Physiology-Endocrinology and Metabolism 307 (6) (2014) E469–E484.
[27] X. Zhang, N. Tang, T. J. Hadden, A. K. Rishi, Akt, foxo and regulation of apoptosis, Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 1813 (11) (2011) 1978–1986.
[28] J. P. White, S. Gao, M. J. Puppa, S. Sato, S. L. Welle, J. A. Carson, Testosterone regulation of akt/mtorc1/foxo3a signaling in skeletal muscle, Molecular and cellular endocrinology 365 (2) (2013) 174–186.
[29] J. P. White, K. A. Baltgalvis, S. Sato, L. B. Wilson, J. A. Carson, Effect of nandrolone decanoate administration on recovery from bupivacaine-induced muscle injury, Journal of Applied Physiology 107 (5) (2009) 1420–1430.
[30] C. Basualto-Alarcón, G. Jorquera, F. Altamirano, E. Jaimovich, M. Estrada, Testosterone signals through mtor and androgen receptor to induce muscle hypertrophy., Medicine and science in sports and exercise 45 (9) (2013) 1712–1720.
[31] A. Jones, D.-J. Hwang, R. Narayanan, D. D. Miller, J. T. Dalton, Effects of a novel selective androgen receptor modulator on dexamethasone-induced and hypogonadism-induced muscle atrophy, Endocrinology 151 (8) (2010) 3706–3719.
[32] C. Hourde, C. Jagerschmidt, P. Clément-Lacroix, A. Vignaud, P. Ammann, G. Butler-Browne, A. Ferry, Androgen replacement therapy improves function in male rat muscles independently of hypertrophy and activation of the akt/mtor pathway, Acta physiologica 195 (4) (2009) 471–482.
[33] L. Ma, C. Shen, J. Chai, H. Yin, H. Deng, R. Feng, Extracellular signal–regulated kinase–mammalian target of rapamycin signaling and forkhead-box transcription factor 3a phosphorylation are involved in testosterones effect on severe burn injury in a rat model, Shock 43 (1) (2015) 85–91.
[34] Y. Wu, W. A. Bauman, R. D. Blitzer, C. Cardozo, Testosterone-induced hypertrophy of l6 myoblasts is dependent upon erk and mtor, Biochemical and biophysical research communications 400 (4) (2010) 679–683.
[35] H. De Naeyer, S. Lamon, A. Russell, I. Everaert, A. De Spaey, B. Vanheel, Y. Taes, W. Derave, Androgenic and estrogenic regulation of atrogin-1, murf1 and myostatin expression in different muscle types of male mice, European journal of applied physiology 114 (4) (2014) 751–761.
[36] W. Qin, J. Pan, W. A. Bauman, C. P. Cardozo, Differential alterations in gene expression profiles contribute to

time-dependent effects of nandrolone to prevent denervation atrophy, BMC genomics 11 (1) (2010) 596.
[37] B. T. Hennessy, D. L. Smith, P. T. Ram, Y. Lu, G. B. Mills, Exploiting the pi3k/akt pathway for cancer drug discovery, Nature reviews Drug discovery 4 (12) (2005) 988–1004.
[38] C. Foradori, M. Weiser, R. Handa, Non-genomic actions of androgens, Frontiers in neuroendocrinology 29 (2) (2008) 169–181.
[39] M. Pi, A. L. Parrill, L. D. Quarles, Gprc6a mediates the non-genomic effects of steroids, Journal of Biological Chemistry 285 (51) (2010) 39953–39964.
[40] S. Baron, M. Manin, C. Beaudoin, L. Leotoing, Y. Communal, G. Veyssiere, L. Morel, Androgen receptor mediates non-genomic activation of phosphatidylinositol 3-oh kinase in androgen-sensitive epithelial cells, Journal of Biological Chemistry 279 (15) (2004) 14579–14586.
[41] D. Gioeli, B. M. Paschal, Post-translational modification of the androgen receptor, Molecular and cellular endocrinology 352 (1) (2012) 70–78.
[42] H.-K. Lin, Y.-C. Hu, L. Yang, S. Altuwaijri, Y.-T. Chen, H.-Y. Kang, C. Chang, Suppression versus induction of androgen receptor functions by the phosphatidylinositol 3-kinase/akt pathway in prostate cancer lncap cells with different passage numbers, Journal of Biological Chemistry 278 (51) (2003) 50902–50907.