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Upper-body resistance exercise augments vastus lateralis androgen receptor–DNA binding and canonical Wnt/β-catenin signaling compared to lower-body resistance exercise in resistance-trained men without an acute increase in serum testosterone

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ABSTRACT

The purpose of the study was to determine the effect of single bouts of lower-body (LB) and upper- and lower-body (ULB) resistance exercise on serum testosterone concentrations and the effects on muscle testosterone, dihydrotestosterone (DHT), androgen receptor (AR) protein content, and AR–DNA binding. A secondary purpose was to determine the effects on serum *wingless-type MMTV integration site* (Wnt4) levels and skeletal muscle β-catenin content. In a randomized cross-over design, exercise bouts consisted of a LB and ULB protocol, and each bout was separated by 1 week. Blood and muscle samples were obtained before exercise and 3 and 24 h post-exercise; blood samples were also obtained at 0.5, 1, and 2 h post-exercise. Statistical analyses were performed by separate two-way factorial analyses of variance (ANOVA) with repeated measures. No significant differences from baseline were observed in serum total and free testosterone and skeletal muscle testosterone and DHT with either protocol ($p > 0.05$). AR protein was significantly increased at 3 h post-exercise and decreased at 24 h post-exercise for ULB, whereas AR–DNA binding was significantly increased at 3 and 24 h post-exercise ($p < 0.05$). In response to ULB, serum Wnt4 was significantly increased at 0.5, 1, and 2 h post-exercise ($p < 0.05$) and β-catenin was significantly increased at 3 and 24 h post-exercise ($p < 0.05$). It was concluded that, despite a lack of increase in serum testosterone and muscle androgen concentrations from either mode of resistance exercise, ULB resistance exercise increased Wnt4/β-catenin signaling and AR–DNA binding.

1. Introduction

In men, approximately 2% of total testosterone is free testosterone and is the most biologically active [1,2]. Only circulating free/unbound testosterone diffuses across the sarcolemma in skeletal muscle, where a portion is aromatized to dihydrotestosterone (DHT) by 5α-reductase [3]. While DHT displays a greater affinity, both androgens can then bind to a specific nuclear hormone androgen receptor (AR). Once bound, the AR can then translocate into the nucleus, binding to the androgen response element (ARE) on DNA, and result in an up-regulation in the expression of various downstream muscle-specific genes [4]. The AR is a ligand-activated transcription factor composed of a DNA binding domain (DBD) and a ligand binding domain (LBD) [5]. Its N-terminal domain is ligand-independent, whereas the C-terminal domain is ligand-dependent [6].

The ability of androgen ligands to bind the AR, and the subsequent DNA binding with the ARE, is a critical factor controlling the rate of muscle protein synthesis. The ligand binding domain of the AR interacts with a variety of other proteins, such as β-catenin, following androgen binding that coordinately enhance AR transactivation by forming a binary complex on the AREs [7–9]; this interaction has the ability to augment or modulate transcription. β-Catenin serves as an important signaling protein within the Wnt signaling pathway, a molecular transducer involved in up-regulating gene expression. Association of AR with cytosolic β-catenin causes nuclear translocation and activation of Wnt-specific genes, which are implicated in the regulation of muscle hypertrophy [10]. However, it is not known whether the interaction of AR and β-catenin is entirely due to androgenic effects.

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Wnt4 is a member of the *wingless-type mouse mammary virus tumor (MMTV) integration site* (Wnt) family of secreted glycoproteins that functions in a paracrine manner, serves as the ligand of the Frizzled (Fzd) family of cell-surface receptors, and is known to activate intracellular signaling pathways affecting gene expression [11]. Canonical Wnt signaling results in the dephosphorylation and subsequent activation of β-catenin, a multi-functional protein, which is associated with cellular growth through its function as a transcription factor [12]. A specific protein–protein interaction between β-catenin and the AR exists in which the LBD of the AR and the central region spanning the armadillo repeats 1–6 of β-catenin are responsible for the interaction. As a result, β-catenin augments the ligand-dependent activity of the AR [13]. In the presence of androgens, specific regions of β-catenin and AR interact to mediate AR activation and subsequent translocation to the nucleus [14]. However, in the absence of increased androgen content it has been shown that canonical Wnt/β-catenin signaling activated growth-control genes during mechanical overload-induced skeletal muscle hypertrophy [15].

Single bouts of resistance exercise involving high intensity and volume have been shown to increase circulating testosterone [16–18]; however, this response is inconsistent and also dependent on other programmatic variables as mode, intensity, exercise selection, and order [19]. Resistance exercise-induced fluctuations in serum testosterone concentrations have been shown to alter skeletal muscle AR content [20–22]. It is generally considered that resistance exercise-induced elevations in serum testosterone will lead to up-regulations in muscle AR content in order to increase testosterone/AR interactions. However, resistance exercise-induced increases in serum testosterone are not always associated with increases in skeletal muscle AR content [16,17,23] and AR activation. Therefore, in the absence of acute increases in serum testosterone following resistance exercise, it is possible that Wnt/β-catenin signaling may instead be involved in mediating AR activation and DNA binding.

Testosterone/AR interactions seem to have a profound effect on skeletal muscle adaptation, ultimately affecting muscle hypertrophy. Nonetheless, research studies have shown inconsistent results regarding resistance exercise-induced increases in testosterone and AR content. In the event that increases in testosterone do not accompany resistance exercise, the effects on AR–DNA binding are presently unknown. In addition, the response of Wnt/β-catenin signaling to resistance exercise in humans, along with the role it may play with AR–DNA binding is not known. Therefore, the purpose of the study was to determine the effect of single bouts of lower-body (LB) and upper- and lower-body (ULB) resistance exercise on serum testosterone concentrations and the effects on muscle testosterone, DHT, AR protein content, and AR–DNA binding. A secondary purpose was to determine the effects on serum Wnt4 concentrations and skeletal muscle β-catenin in order to determine the impact Wnt4/β-catenin signaling may have on AR–DNA binding.

2. Materials and methods

2.1. Experimental approach

In a randomized, cross-over design, participants performed a resistance exercise session on two separate occasions involving either a LB or ULB protocol. Blood and muscle samples were obtained before exercise and 3 and 24 h post-exercise; blood samples were also obtained immediately after and at 0.5, 1, and 2 h post-exercise.

Table 1
Participant demographics.

Sample size (*n*)	8
Age (year)	23.62 (±4.86)
Height (cm)	179.07 (±5.34)
Total body mass (kg)	96.69 (±15.27)
Fat mass (kg)	17.20 (±10.38)
Fat-free mass (kg)	68.25 (±5.36)

Data are expressed as mean (±SD). No significant differences were observed for any of the demographic variables at the onset of the study ($p > 0.05$).

2.2. Participants

Eight apparently healthy, resistance-trained [regular, consistent resistance training (i.e. thrice weekly) for at least 1 year prior to the onset of the study], men between the ages of 18–30 volunteered to serve as participants in this study. Based on self-report from each participant, the average resistance training age for all eight participants was 8.57 (±5.09) years. Ten participants were initially recruited for the study, completed informed consent forms, and participated in an initial familiarization session. Of the 10 participants, nine completed the research study, as one participant became ill due to circumstances unrelated to the study, and was unable to continue. Of the nine who completed the study, eight subjects were utilized in data analysis as one participant's data was considered as a statistical outlier because his pre-exercise levels of free and total testosterone for both resistance exercise sessions were greater than two standard deviations above the group mean. Enrollment was open to men of all ethnicities. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM), and who had not consumed any nutritional supplements (excluding multi-vitamins) 1 month prior to the study were allowed to participate. All eligible subjects signed university-approved informed consent documents and approval was granted by the Institutional Review Board for Human Subjects. Additionally, all experimental procedures involved in the study conformed to the ethical consideration of the Declaration of Helsinki. Table 1 shows the sample size, along with the baseline means (±SD) for height, weight, age, and body composition for the eight participants.

2.3. Procedures

2.3.1. Familiarization, body composition, and muscle strength assessments

Total body mass (kg) was determined on a standard dual beam balance scale (Detecto Bridgeview, IL, USA). Fat mass and fat-free mass were determined using DEXA (Hologic Discovery Series W, Waltham, MA, USA). Quality control calibration procedures were performed on a spine phantom prior to each testing session. Total body water was determined by bioelectric impedance spectroscopy (ImpediMed Ltd., Australia) [24–26]. Based on previous studies in our laboratory, the accuracy of the DEXA for body composition assessment is ±3.7% as assessed by direct comparison with hydrodensitometry and scale weight.

In order to determine muscular strength, participants performed one-repetition maximum (1-RM) tests on the bench press, overhead shoulder press (Nebula, Versailles, OH, USA), seated row, and knee extension (Cybex, Medway, MA, USA), respectively, based on the protocol from our previous studies [24–26]. Participants warmed up by completing 5–10 repetitions at approximately 50% of the estimated 1-RM. The participants rested for 2 min, and then completed three to five repetitions at approximately 70% of the