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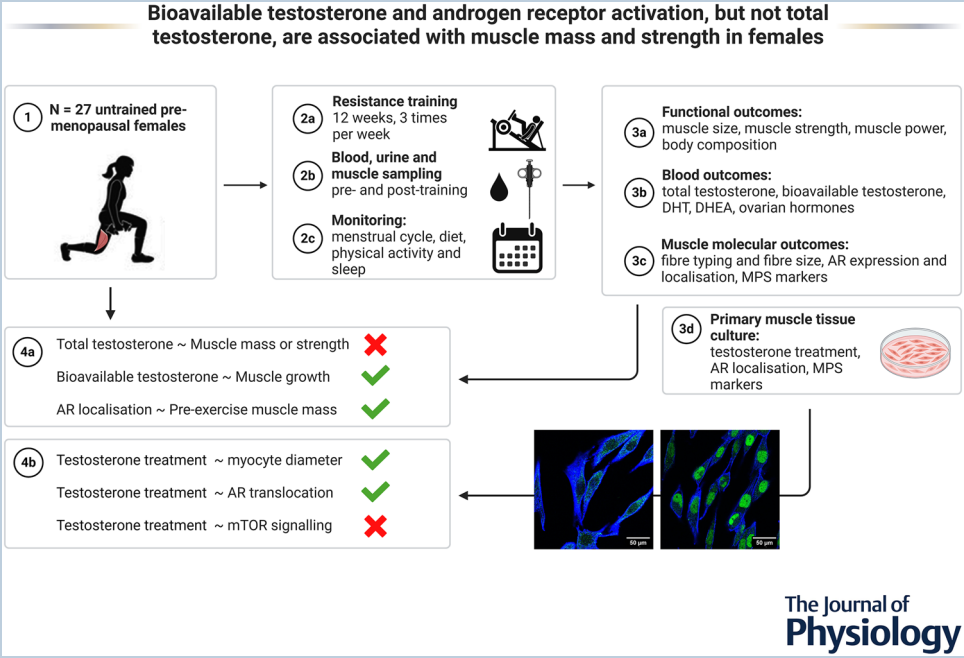

Abstract figure legend Twenty-seven pre-menopausal, untrained females performed a 12-week resistance training programme to explore the association between androgen concentrations, androgen receptor signalling and skeletal muscle size and strength. Total testosterone was not associated with muscle mass or strength, nor with resistance training-induced muscle hypertrophy or strength gains. Bioavailable testosterone and nuclear-localised androgen receptor (AR) were associated with muscle size and strength. *In vitro*, supra-physiological doses of testosterone increased muscle cell size, but not via the Akt/mTOR pathway. Instead, testosterone significantly increased AR protein content and nuclear localisation. Figure created with biorender.com.

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Abstract Testosterone, the major androgen, influences the reproductive and non-reproductive systems in males and females via binding to the androgen receptor (AR). Both circulating endogenous testosterone and muscle AR protein content are positively associated with muscle mass and strength in males, but there is no such evidence in females. Here, we tested whether circulating testosterone levels were associated with muscle mass, function, or the muscle anabolic response to resistance training in pre-menopausal females. Twenty-seven pre-menopausal, untrained females (aged 23.5 ± 4.8 years) underwent a 12-week resistance training programme. Muscle strength, size, power, and plasma and urine androgen hormone levels were measured. Skeletal muscle biopsies were collected before and after the training programme to quantify the effect of resistance training on AR content and nuclear localisation. Primary muscle cell lines were cultured from a subset ($n = 6$) of the participants' biopsies and treated with testosterone to investigate its effect on myotube diameter, markers of muscle protein synthesis and AR cellular localisation. Physiological levels of total testosterone were not associated with muscle mass or strength at baseline or with the changes in muscle mass and strength that occurred in response to resistance training in our cohort of pre-menopausal females. In contrast, bioavailable testosterone and the proportion of nuclear-localised AR were positively associated with skeletal muscle mass and strength in pre-menopausal females. *In vitro*, supra-physiological doses of testosterone increased myocyte diameter, but this did not occur via the Akt/mTOR pathway as previously suggested. Instead, we show a marked increase in AR nuclear localisation with testosterone administration *in vitro*.

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Key points

- Total circulating testosterone was not related to muscle mass or strength before or after resistance training in pre-menopausal females.
- Bioavailable testosterone was positively related to exercise-induced muscle hypertrophy in pre-menopausal females.
- *In vivo* nuclear localisation of the androgen receptor was positively related to muscle mass in pre-menopausal females at baseline, but not to resistance training-induced hypertrophy.
- Testosterone treatment induced androgen receptor nuclear translocation but did not induce mTOR signalling in primary skeletal myocytes cultured from pre-menopausal female muscle.

Introduction

The maintenance of skeletal muscle mass and function is an essential component of health and ageing (McLeod et al., 2016; Tieland et al., 2018). Muscle mass and function are also performance-determining factors in many sporting disciplines that rely on speed, power or strength, including sprinting (Barbieri et al., 2017) and weightlifting (Zaras et al., 2020). Skeletal muscle dynamically reacts and adapts to external stimuli such

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as mechanical loading and unloading, or internal stimuli such as the hormonal milieu (Schiaffino et al., 2013). One such hormone is testosterone, an androgen (i.e. 'male making') sex hormone with anabolic properties. Females typically exhibit testosterone concentrations that are 10-fold lower than male concentrations (0.5–2.5 and 10−30 nmol l^{-1}, respectively) (Burger, 2002). In males and females, the majority of testosterone circulates bound to carrier proteins, sex hormone binding globulin (SHBG; approximately 45%) or albumin (approximately 50%) (Dunn et al., 1981). Only a small fraction of testosterone (approximately 3−5%) is 'free' and unbound. When bound to SHBG, testosterone is not biologically active (Krakowsky & Grober, 2015). In contrast, testosterone is weakly bound to albumin and can easily dissociate. Therefore, albumin-bound and free testosterone are considered 'bioavailable' and can enter target cells and bind to the androgen receptor (AR) (Burger, 2002). The AR is ubiquitously expressed, and as such, testosterone plays a role in many tissues throughout the body, including skeletal muscle.

The AR exists either in the cytosol of cells bound to chaperone proteins (Berns et al., 1986; de Launoit et al., 1991; Olea et al., 1990), or in the sarcolemma of myocytes linked to a G-protein coupled receptor (Dent et al., 2012). AR exerts its effects via two known mechanisms: non-genomic and genomic signalling. Non-genomic signalling refers to the process by which sarcolemma-bound ARs activate the protein kinase B/mammalian target of rapamycin (Akt/mTOR) or mitogen-activated protein kinase (MAPK) pathways to increase protein synthesis. This has been shown in rat L6 (White et al., 2013; Wu et al., 2010) and mouse C2C12 (Basualto-Alarcón et al., 2013) myocytes *in vitro*, but it is unknown whether testosterone signals through these pathways in humans. Genomic signalling is a process by which cytosolic AR becomes phosphorylated, dissociates from its chaperone protein and translocates to the nucleus of the cell (nAR). nAR act as a transcription factor that increases the expression of over 1000 target genes containing an androgen response element (ARE) in their promoter (Jin et al., 2013; Leung & Sadar, 2017).

Testosterone and its bioactive metabolite dihydrotestosterone (DHT) exhibit anabolic properties. The administration of supraphysiological levels of exogenous testosterone promotes a positive muscle protein turnover and increased muscle mass and function in young (Bhasin et al., 2001) or old males (Storer et al., 2017) and in pre- (Hirschberg et al., 2020) or post-menopausal females (Huang et al., 2014). Conversely, when testosterone concentrations are pharmacologically suppressed, the protein balance switches in favour of protein degradation (Ferrando et al., 1998; Sheffield-Moore et al., 1999) and leads to reduced muscle mass and strength in males (Mauras et al., 1998; Overkamp et al., 2023). Existing evidence of a relationship between endogenous testosterone and muscle outcomes in the literature is, however, conflicting. Some studies found positive associations between endogenous total testosterone and muscle mass or strength in large male cohorts across the lifespan ($n = 252$ (Mouser et al., 2016), $n = 3875$ (Ye et al., 2021)). Other, smaller studies in young males refute the existence of such an association ($n = 49$ (Morton et al., 2018), $n = 23$ (Mitchell et al., 2013), $n = 67$ (Mobley et al., 2018), $n = 49$ (Morton et al., 2016)) and instead propose that increased skeletal muscle AR protein content (Morton et al., 2018), or nAR (Hatt et al., 2024), but not total or bioavailable circulating testosterone (Morton et al., 2016, 2018), is associated with increased muscle hypertrophy and function. Evidence from murine (Yoshioka et al., 2007) and human (Hatt et al., 2024; Pataky et al., 2023) models, however, suggest that there are sex-specific differences in androgen action on the skeletal muscle transcriptome, and that evidence from male cohorts can therefore not be generalised to female cohorts.

Our knowledge of the association between endogenous testosterone and muscle mass in females is even more limited (Alexander, Pollock, et al., 2022) and the available evidence stems from cross-sectional cohorts from which causal relationships cannot be drawn. We and others showed that, cross-sectionally, there is no association between endogenous total testosterone or nAR and muscle mass and strength in pre- (Alexander et al., 2021; Hatt et al., 2024) or post-menopausal females (Carmina et al., 2009; Gower & Nyman, 2000; Kogure et al., 2015; Pöllänen et al., 2011; Rariy et al., 2011; van Geel et al., 2009). Instead, the free androgen index (FAI), which is indicative of the amount of bioavailable testosterone, is weakly associated with muscle mass in pre-menopausal females (Alexander et al., 2021; Carmina et al., 2009). These findings, however, come solely from cross-sectional observations with a lack of control for confounding factors. In addition, no studies to date have examined the relationship between endogenous androgens and muscle mass and function in females longitudinally, or following an intervention designed to promote muscle strength and hypertrophy and have, therefore, not captured an individual's adaptability or responsiveness to resistance training. Further, no studies have examined androgen signalling pathways or the role of the AR in models of female skeletal muscle.

For these reasons, the aim of the current study was to investigate possible determinants of lower limb muscle mass, strength and power, including basal total testosterone and bioavailable testosterone concentrations, AR content and markers of AR activation in the *vastus lateralis* of untrained, pre-menopausal females. A secondary aim was to investigate the possible determinants of resistance-training-induced changes in lower limb muscle mass, strength and power, including

the area under the curve of total and bioavailable testosterone and average AR protein content. An additional exploratory aim was to investigate the effect of testosterone treatment on AR and mTOR signalling in primary myocytes cultured from pre-menopausal female donors.

We hypothesize that bioavailable, but not total, testosterone will be associated with muscle mass and strength before and after a 12-week resistance training intervention in pre-menopausal females.

Methods

Ethical approval

This research was granted ethical approval by the Deakin University Human Research Ethics Committee (DUHREC 2018-388). All participants provided written, informed consent before taking part in the study, which conformed to the standards set by the *Declaration of Helsinki* (World Medical Organisation, 2018), except for registration in a database.

Participants and exclusion criteria

Thirty-five healthy females aged 18−40 years were recruited from the general population. Four participants were not able to continue the training programme due to COVID-19-related interruptions in 2020, two participants withdrew for health-related reasons and two participants withdrew for personal reasons. Therefore, 27 females completed the training programme. Participants were not resistance-trained (defined as having performed structured resistance training at least twice per week in the previous 6 months), pregnant or breastfeeding, did not smoke and displayed no contraindications to exercise according to the Exercise and Sports Science Australia adult pre-exercise screening system (Exercise & Sports Science Australia, 2019). Participants were excluded if they had a history of anabolic hormone use, used medications or supplements that could affect the anabolic response to training, or if their daily protein intake was outside the Australian dietary guidelines of 15−25% total macronutrient intake, measured through a mobile phone application for 4 days including one weekend day (Easy Diet Diary;Xyris Software, 2019). The health, fitness and anabolic status of young, healthy females are not expected to change over a 12-week period as a passage of time. Therefore, each participant acted as her own control in a pre–post study design.

Assessment of confounding factors

Participants completed a chronotype questionnaire (Horne & Östberg, 1976) to assess the time of day at which they are most alert. Participant chronotype was later tested as a potential covariate in statistical analysis in case it was significantly associated with both the independent and dependent variables of interest. To monitor sleep quantity and energy expenditure, participants wore an activity monitor (Actical Z MiniMitter, Phillips Respironics Inc., Bend, OR, USA) on their non-dominant wrist for 7 days, accompanied by a sleep diary that incorporates several validated sleep rating systems (Jay et al., 2006; Samn & Perelli, 1982). Sleep quantity (total hours) and total daily energy expenditure (metabolic equivalent; METs) measurements were repeated for 24 h on weeks 3, 6 and 9 of the trial to ensure participants' sleep and energy expenditure remained consistent, as any changes to either of these variables could potentially affect the outcome of this study. Sleep quantity and total daily energy expenditure (METs) were tested as covariates in later statistical analysis and were added to the models if they were significantly associated with both the independent and dependent variables of interest.

Protein intake, daily physical activity and sleep quantity were measured at baseline and every 3 weeks throughout the training programme. Sleep and protein intake did not change significantly during the programme, suggesting the participants maintained their habitual diet and sleep patterns throughout the entire 12 weeks (data not shown). Participants decreased their total energy expenditure by 14% during week 6 ($P < 0.001$) and by 10.5% during week 9 ($P = 0.041$) when compared to baseline (data not shown). Despite this change in total energy expenditure, Akaike information criterion (AIC) tests revealed that total energy expenditure was not a significant confounder of the linear models and was, therefore, not included in subsequent analyses.

Menstrual phase standardisation and hormonal contraception use

The pre–post design of this study allowed each participant to act as her own control, and therefore we did not exclude participants based on hormonal contraceptive (HC) use. This study included both normally menstruating females and females using HC. Some research suggests that muscle strength may be greater in the late follicular phase (days 7−14) compared to other phases (Knowles et al., 2019). More recent research, however, suggests that there is no difference in muscle strength between menstrual cycle phases (Colenso-Semple et al., 2023). Despite this, we aimed to minimise any potential confounding effect of the menstrual cycle on muscle performance by avoiding the late follicular phase (days 7−14) of the menstrual cycle during pre- and post-training testing in normally menstruating participants. The data collection period lasted 12 weeks, 3 full cycles of a typical menstrual cycle lasting 28 days, allowing each normally menstruating

participant to undergo pre- and post-testing during the same phase of their cycle. Menstrual phases were verified through menstrual diaries and hormonal analysis, in line with published guidelines for the inclusion of females in exercise physiology cohorts (Elliott-Sale et al., 2021; Knowles et al., 2019). Two separate researchers verified the menstrual phase of each participant, and consensus was reached in each case. HC use and menstrual phase were tested as covariates in all subsequent statistical analysis.

Familiarisation to the training programme

Prior to beginning the training programme, participants attended three one-to-one familiarisation sessions at Deakin University over the course of 1−2 weeks, delivered by an exercise physiologist (Phillips et al., 2004). During these sessions, the participants were coached through all training exercises with little-to-no weight (rate of perceived exertion (RPE) <3/10 – 'moderate') (Borg, 1982a, 1982b) to ensure all participants used the safe and correct technique for all movements. These sessions also aimed to minimise any potential learning effects that may have occurred due to the novelty of the exercises for some participants.

Strength and power testing

Peak muscle power was assessed using a portable force plate (AMTI, Watertown, MA, USA). The sampling frequency was 1000 Hz. No filters were used in the force and power output. Participants performed a countermovement jump (CMJ), without an arm swing. Participants began in a standing position with a straight torso, knees fully extended, hands on their hips and feet shoulder-width apart, according to recommendations (Petrigna et al., 2019). Participants were given identical instructions to 'jump as high as you possibly can'. Participants performed a squatting movement until they reached ∼90° knee flexion before jumping as high as possible with their legs straight, keeping their hands on their hips throughout the entire movement. Four attempts were made, separated by 3 min rest and the highest values were recorded.

Participants' repetition maximum (RM) was assessed for leg press, as well as all the exercises included in the training programme. Leg press was included in the strength testing but not in the training programme and represents the major measure of muscle strength in this study. Using an exercise that was not included in the training programme minimised any learning effect, as participants did not train in the movement. Lower body 1RM was calculated from 5RM tests using the equation (Abadie & Wentworth, 2000): $estimated1RM = 4.67 + (1.14 \times weightlifted)$. Upper body 1RM was calculated from 10RM tests using the equation (Abadie & Wentworth, 2000): $estimated1RM = 1.43 + (1.20 \times weightlifted)$. Participants were given identical instructions to 'lift as much weight as you possibly can for five repetitions'. Participants were asked to provide an RPE after each attempt, and 3 min separated each attempt. Participants continued until they reached muscle failure or volitional failure, with a maximum of four attempts.

For both the strength and power tests, verbal encouragement was standardised in wording (e.g. 'push', 'explode' and 'keep going') and enthusiasm for all participants (Engel et al., 2019).

Plasma and urine collection

A resting plasma sample was collected from participants in the fasted state before and after the training programme, as well as before exercise in weeks 2, 4, 6, 8 and 10. At 07.00 h, 10 ml of venous blood was taken from the antecubital vein in vacutainer tubes containing 7.2 mg K_2-EDTA (Becton Dickinson, Franklin Lakes, NJ, USA). Blood was centrifuged immediately for 10 min at 1500 *g*, 4°C and plasma was stored at −80°C until further use. A first-void urine sample was collected at the same time points and the urine was stored at −20°C until further use.

Body composition analysis

Participants' body composition was assessed before and after the 12-week training programme via bioelectrical impedance analysis (BIA; Tanita, Kewdale, WA, USA) and dual-energy X-ray absorptiometry (DXA; Lunar Prodigy Advance, GE Healthcare, Madison, WI, USA). At the time of measurement, participants had abstained from vigorous exercise, caffeine and alcohol for the previous 48 h, minimising the chances of water-retention or dehydration that may occur, as per standard recommendations (Walter-Kroker et al., 2011).

Assessment of thigh muscle cross-sectional area

The cross-sectional area (CSA) of the thigh muscle groups (quadriceps and hamstrings) at 50% of femur length was assessed via peripheral quantitative computed tomography (pQCT) (XCT 3000, Stratec Medizintechnik GmBH, Pforzheim, Germany).

Collection of muscle tissue

Participants abstained from caffeine, alcohol and vigorous activity for 48 h prior to the collection of muscle biopsies. The night before, participants consumed a low-protein, standardised meal of pasta and tomato-based sauce as

Table 1. The resistance training programme prescribed to participants (n = 27)

Exercise	Sets	Reps	Intensity (%RM)	Rest between sets (s)	Tempo (s)	Volume load (reps × sets × intensity)
Gym-based resistance training programme						
Squat	3	8	80	90	2, 0, 2, 0	19.2
Leg extension	3	8	80	90	2, 0, 2, 0	19.2
Hamstring curl	3	8	80	90	2, 0, 2, 0	19.2
Seated shoulder press	3	9	70	90	2, 0, 2, 0	18.9
Seated row	3	10	60	90	2, 0, 2, 0	18.0
Seated biceps curl	3	9	70	90	2, 0, 2, 0	18.9
Home-based resistance training programme						
Squats	3	15*	Approx. 45% RM	90	2, 0, 2, 0	20.3
Forward lunges	3	15 leg^{-1}*	Approx. 45% RM	90	2, 0, 2, 0	20.3
Hamstring sliders	3	10*	Body weight	90	2, 0, 2, 0	N/A
Seated shoulder press	3	9*	70% RM	90	2, 0, 2, 0	18.9
Bent over row	3	9*	70% RM	90	2, 0, 2, 0	18.9
Seated biceps curl	3	9*	70% RM	90	2, 0, 2, 0	18.9

The gym-based programme was followed by all participants when access to the gym was possible. A sub-cohort of participants (n = 10) performed a portion of their training programmes using the home-based resistance training programme when access to the training facility was not possible. *Last set was prescribed as many repetitions as possible (AMRAP).

previously described (Lamon et al., 2021). Portion size and water consumption were *ad libitum*. Participants recorded the portion size and water consumption from the pre-training trial and replicated this for the post-training trial.

Participants arrived at the testing facility at 07.00 h after an overnight fast from 21.00 h the previous evening. A muscle biopsy of the *vastus lateralis* was performed via a percutaneous needle biopsy technique modified to include suction (Bergstrom, 1962). Briefly, the skin over the *vastus lateralis* was sterilised, and the area was anaesthetised with 1% lidocaine without adrenaline. An incision was made through the skin and muscle fascia. A muscle sample of 150−300 mg in size (Russell et al., 2013) was immediately snap-frozen in liquid N_2-cooled isopentane and stored in liquid N_2 until required.

Training programmes

After all the baseline measures were assessed, the 12-week resistance training programme commenced. Every Monday, Wednesday and Friday, participants arrived at Deakin University between 06.00 and 08.00 h after an overnight fast from 21.00 h the previous evening. Briefly, the gym-based training programme consisted of squats, leg extensions, hamstring curls, shoulder press, biceps curls and seated row exercises. Participants performed three sets of 8−10 repetitions at 60−80% 1RM. Progressive overload (add 5% load) was applied to each exercise when an individual was able to complete two additional repetitions in the last set of an exercise in two consecutive sessions.

Due to the various SARS-CoV-2-related lockdowns experienced throughout Victoria, Australia in 2020 and 2021 (Dunstan, 2021), there was a requirement for a sub-cohort of participants (n = 10) to undertake an average of 4 out of 36 (11%; range 2−6 sessions) of their training sessions at home. The remaining 30−34 sessions were completed in the gym at Deakin University following the same training protocol as participants who completed all their sessions in the gym. For the home-based training, participants were provided with weights and the online training sessions were delivered via videoconferencing at 07.30 h, replicating the time and days of the gym-based training sessions. The home-based programme consisted of squats, lunges, hamstring slides, shoulder press, biceps curls and bent-over rows. Participants performed three sets of 15 repetitions at approximately 40−50% 1RM. The last set of each exercise was performed until failure as evidence suggests that loads below 60% of an individual's 1RM can induce similar levels of skeletal muscle hypertrophy and strength (Nóbrega et al., 2018; Schoenfeld et al., 2017), provided the individual performs the exercise to failure. Table 1 outlines the two different training programmes undertaken by participants. Both programmes were designed by an Exercise and Sports Science Australia-accredited exercise scientist and an Australian Strength and Conditioning Association-accredited strength and conditioning coach.

All participants were given a 25-g protein supplement (Ascent Protein, Denver, CO, USA) either immediately

before or after each training session to optimise the anabolic response to resistance training. The protein supplement was approved by Informed Choice (Informed Choice, 2021), thereby minimising the risk that the supplement contained any substances that are banned by the World Anti-Doping Agency (WADA). Figure 1 provides a visual timeline of the intervention and key outcomes of the study.

Laboratory analysis

Hormone analysis. Testosterone, sex hormone binding globulin (SHBG), dehydroepiandrosterone (DHEA) and 5α-dihydrotestosterone (DHT) were measured by enzyme-linked immunosorbant assay (ELISA; cat. no. IBRE52151, cat. no. IB30176808, cat. no. IBRE52221, cat. no. IBDB5202, Abacus Dx, Parkville, Australia), according to the manufacturer's instructions.

The Free Androgen Index (FAI) in plasma was calculated as: $\frac{\text{Total testosterone (nmol·L}^{-1})}{\text{Sex hormone binding globulin (nmol·L}^{-1})} \times 100$

The full steroid profile in urine (including testosterone, its precursors and metabolites) was measured via gas chromatography–mass spectrometry (GC/MS) in a WADA-accredited laboratory as described previously (Salamin et al., 2022). GC/MS was used to exclude confounding factors having a potential impact on endogenous testosterone production (e.g. alcohol, ketoconazole, aromatase inhibitors), while also ensuring that the participants were not using exogenous testosterone. All hormonal markers from urine were corrected for the specific gravity of urine, using the equation: $\text{Con}_{\text{adj}} = \frac{(1.020-1)}{\text{SG}_{\text{samplemax}}} \times \text{Con}_{\text{measured}}$, where $\text{SG}_{\text{samplemax}} = \text{SG}_{\text{sample}} + 0.002$.

For validation of menstrual phase, oestradiol (E2) and progesterone (P) were measured via a competitive binding immune-enzymatic assay according to manufacturer's instructions (Beckman Coulter, Lane Cove, Australia). Luteinising hormone (LH) was analysed via a sequential two-step immune-enzymatic assay according to manufacturer's instructions (Beckman Coulter) and follicle stimulating hormone (FSH) was analysed via a microparticle enzyme immunoassay (MEIA) according to manufacturer's instructions (Beckman Coulter).

Protein extraction. Protein was extracted from 20–25 mg of skeletal muscle tissue via manual homogenisation in 15 µl mg^{-1} muscle 1× RIPA lysis buffer 1 (cat. no. J62524-AE, Thermo Fisher Scientific, Scoresby, VIC, Australia) containing 10 µl ml^{-1} phosphatase inhibitor and 1 µl ml^{-1} protease inhibitor cocktail (cat. no. 78440, Thermo Fisher Scientific). The protein concentration of each sample was determined via Pierce Bicinchoninic Acid (BCA) assay (cat. no. 23225, Thermo Fisher Scientific) according to the manufacturer's instructions. Absorbance of samples was read at 562 nm using bovine serum albumin as a standard.

Western blotting. The total protein and phospho-protein levels of the AR as well as markers of skeletal muscle protein synthesis were analysed via western blot. Thirty micrograms of denatured protein from each sample was loaded into a 4−15% gradient Criterion Tris–glycine extended (TGX) Stain Free gel (cat. no. 5678085, Bio-Rad, Gladesville, Australia), separated via electrophoresis at 200 V, 40 min. The separated proteins were transferred to an Immobilon PVDF-FL membrane (cat. no. IPFL00005, Millipore, Billerica, MA, USA) at 100 V, 60 min and

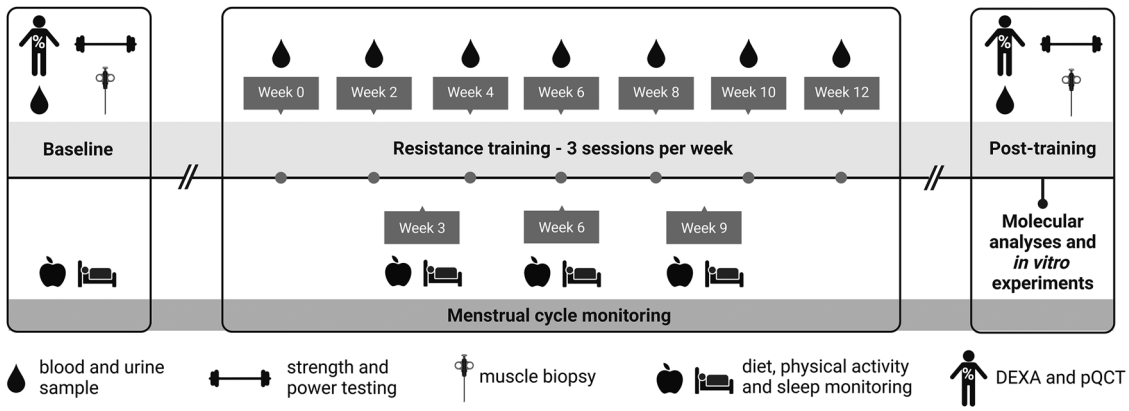

Figure 1. Visual timeline of the intervention and key outcomes
Before and after the 12-week training intervention, participants underwent muscle biopsies, DXA, pQCT scans of the thigh muscles and strength and power testing. Menstrual phase was verified through hormone levels and menstrual diaries before and during the training intervention. Physical activity, sleep and dietary protein intake was recorded before the intervention and after 3, 6 and 9 weeks of training. Plasma and urine samples were collected before the intervention and after 2, 4, 6, 8 and 10 weeks of training. *n = 10 participants completed an average of 11% of their sessions at home via videoconferencing. Figure created with BioRender.com

blocked for 1 h in 5% skim milk in Tris-buffered saline plus 0.1% Tween-20 (TBST; cat. no. P1379, Sigma-Aldrich, North Ryde, Australia). Membranes were incubated at 4°C overnight in the primary antibody. The antibodies and conditions used were for Akt (cat. no. 2920, 1:1000, mouse, Cell Signaling Technology, Danvers, MA, USA), p-Akt^{ser473} (cat. no. 4060, 1:1000, rabbit, Cell Signaling Technology), AR (cat. no. 5153, 1:500, rabbit, Cell Signaling Technology), p-AR^{ser213} (cat. no. PA537478, 1:500, rabbit, Thermo Fisher Scientific), p-AR^{ser650} (cat. no. 537479, 1:500, rabbit, Thermo Fisher Scientific), MAPK (cat. no. 4696, 1:1000, mouse, Cell Signaling Technology), p-MAPK$^{thr202/tyr204}$ (cat. no. 9101, 1:500, rabbit, Cell Signaling Technology), mTOR (cat. no. 4517, 1:1000, mouse, Cell Signaling Technology), p-mTOR^{ser2448} (cat. no. 5536, 1:1000, rabbit, Cell Signaling Technology), MuRF-1 (cat. no. MP3401, 1:1000, rabbit, ECM Biosciences), 4E-BP1 (cat. no. 9452, 1:1000, rabbit, Cell Signaling Technology), p-4E-BP1$^{thr37/46}$ (cat. no. 2855, 1:500, rabbit, Cell Signaling Technology), rpS6 (cat. no. 2217, 1:1000, rabbit, Cell Signaling Technology) and p-rpS6$^{ser235/236}$ (cat. no. 4856, 1:1000, rabbit, Cell Signaling Technology). Following a 1 h incubation in the corresponding secondary antibody (cat. no. 5151, 1:10000, anti-rabbit IgG Dylight 800 or cat. no. 5470, 1:10000, anti-mouse IgG DyLight 680; Cell Signaling Technology) the proteins were exposed on an Odyssey CLx Infrared Imaging System and individual protein band optical densities were determined using the Odyssey Infrared Imaging System software (Image Studio V5.2, Li-Cor Biosciences, Lincoln, NE, USA). All blots were normalized against the total protein load using the Bio-Rad Image Lab software (v6.0).

Immunohistochemical staining. Muscle fibre type composition and CSA were assessed via immunohistochemistry (IHC), staining for myosin heavy chain (MHC I and IIx) and laminin. Eight-micrometre cross-sections of the muscle samples were cut on a microtome cryostat and loaded onto glass slides. The muscle sections were blocked in 10% goat serum (cat. no. 16210072, Thermo Fisher Scientific) in 1× phosphate-buffered saline (PBS) for 1 h at room temperature and incubated for 1 h at room temperature in a cocktail containing primary antibodies specific to anti-MHCI (cat. no. BA-F8, 1:20, Developmental Studies Hybridoma Bank; DSHB, University of Iowa, IA, USA), anti-MHCIIx (cat. no. 6H1, 1:20, DSHB) and anti-laminin (cat. no. L9393, 1:100, Sigma-Aldrich) in 10% goat serum/PBS. Following this, sections were incubated for 1 h at room temperature in a secondary antibody cocktail containing goat anti-mouse IgG2b Alexa Fluor 647 (cat. no. A-21242, 1:500, Thermo Fisher Scientific), goat anti-mouse IgM Alexa Fluor 555 (cat. no. A-21426, 1:500, Thermo Fisher Scientific) and goat anti-rabbit IgG Alexa Fluor 405 (cat. no. A-31556, 1:500, Thermo Fisher Scientific) in 10% goat serum/PBS. One image of the entire muscle section was visualised using a Fluoview fv0i confocal microscope (Olympus, Tokyo, Japan) at ×10 magnification and analysed using Semi-automatic Muscle Analysis using Segmentation of Histology (SMASH) software (MATLAB application, MathWorks, Natick, MA, USA) (Schneider et al., 2012). The average number of myofibres per section was 753.8 ± 370.6.

In vivo AR localisation was also assessed via IHC. Eight-micrometre muscle sections were thawed and fixed in 4% paraformaldehyde (PFA) for 10 min. The muscle sections were permeabilised in 0.1% Triton X-100 for 5 min and blocked for 1 h in 5% bovine serum albumin/PBS. Following blocking, the muscle sections were incubated in an antibody against the AR (cat. no. 5153, 1:50, Cell Signaling Technology) in a blocking buffer at 4°C overnight. The following day, the sections were washed and incubated in a secondary antibody cocktail containing goat anti-rabbit IgG Alexa Fluor 488 (cat. no. A-11008, 1:500, Thermo Fisher Scientific) and wheat germ agglutinin (cat. no. W32466, 1:1000, Thermo Fisher Scientific) in blocking buffer for 1 h at room temperature. The sections were stained with 0.1 μg ml^{-1} 4′,6-diamidino-2-phenylindole (DAPI) stain (cat. no. 62248, 1:1000, Thermo Fisher Scientific) in PBS for 10 min. Ten images of each muscle section, with a mean of 29.9 ± 8.6 fibres per image, were obtained with dedicated software at ×40 magnification (Eclipse Ti2, Nikon, Tokyo, Japan).

RNA extraction and quantification. RNA was extracted from the homogenised skeletal muscle lysate (∼15 mg sample) using an Allprep DNA/RNA/miRNA Universal extraction kit (cat. no. 80224, Qiagen, Clayton, VIC, Australia) according to manufacturer's instructions, including a proteinase K and DNase treatment.

The quality and quantity of the RNA extracted was assessed using the TapeStation System according to the manufacturer's instructions (Agilent Technologies, Mulgrave, Australia). An RNA integrity number (RIN) of >7 was considered acceptable for downstream analysis. The average sample yield was 73.1 ± 22.5 ng μl^{-1} and the RIN average was 8.1 ± 0.8.

RNAseq. The RNAseq libraries were prepared using the Illumina TruSeq Stranded Total RNA with Ribo-Zero Gold protocol and sequenced with 150-bp paired-end reads on the Illumina Novaseq6000 (Macrogen Oceania Platform, Bella Vista, NSW, Australia). Reads underwent quality check with FastQC (v0.11.9); Kallisto (v0.46.1) was used to map reads to the human reference genome (*HomoSapien GRCh38*) and to generate trans-

cript counts. Genes with a mean across all samples of 10 reads per million (RPM) or fewer reads were removed (63%) from further analysis leaving a total of 14,979 for analysis. All RNA sequencing data generated or analysed during this study are publicly available (GEO: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE267512, submission number GSE267512). The R code used for the analysis is available at https://github.com/DaniHiam/TESTO_RNAseq.

In vitro experiments

Isolation of primary myocytes. To test whether there is a causal association between testosterone treatment and the Akt/mTOR pathway, we isolated myocytes from a sub-cohort of participants ($n = 6$) that underwent the 12-week resistance training programme. To ensure a heterogeneous sample, we selected participants from across a range of responses to the resistance training programme. A third biopsy was collected at rest from six participants from the *vastus lateralis* in exactly the same manner as described above approximately 6 months after the second muscle biopsy. Approximately 100−200 mg of muscle was placed in ice-cold serum free Ham's F10 nutrient mixture (cat. no. 11550043, Thermo Fisher Scientific). The tissue was manually minced and resuspended in a serum-free Ham's F10 nutrient mixture. Following three dissociation steps in warm (37°C) 0.05% Trypsin/EDTA (cat. no. 25300062, Thermo Fisher Scientific), the resulting cells were suspended in proliferation medium containing Ham's F10 nutrient mixture, 20% fetal bovine serum (FBS; cat. no. 10099141, Thermo Fisher Scientific), 1% penicillin–streptomycin (cat. no. 15140122, Thermo Fisher Scientific), 0.5% amphotericin B (cat. no. 15290018, Thermo Fisher Scientific) and 25 μg ml^{-1} fibroblast growth factor (fGFb; cat. no. PHG0026, Thermo Fisher Scientific). The cells were plated in flasks pre-coated with an extracellular matrix (ECM) gel from Engelbreth–Holm–Swarm murine sarcoma (cat. no. E1270, Sigma-Aldrich). The cells were maintained in humidified air at 37°C, 5% CO_2. Proliferation medium was changed every 48 h and the cells passaged once they had reached 70−80% confluence.

Purification of cultured human myoblasts. Once the cells had reached 70−80% confluence, myogenic satellite cells were purified using Magnetic Activated Cell Sorting (MACS) with anti-CD56^{+} microbeads (cat. no. 130-050-401, Miltenyi Biotec, Bergisch Gladbach, Germany) as previously described (Agley et al., 2013; McIlvenna et al., 2021).

Differentiation of cultured human myoblasts. Once the enriched myogenic cells reached 60−70% confluence, differentiation was induced by replacing the proliferation medium with Dulbecco's modified Eagle's medium (DMEM) without phenol red (cat. no. 21063029, Thermo Fisher Scientific) supplemented with 2% horse serum (cat. no. 16050122, Thermo Fisher Scientific) and 1% penicillin–streptomycin (cat. no. 15140122, Thermo Fisher Scientific). DMEM without phenol red was used to eliminate the oestrogenic effects of phenol red (Eriksen et al., 2014; Estrada et al., 2003; Wannenes et al., 2008). Cells were differentiated in either the presence of 100 nM testosterone dissolved in ethanol (cat. no. DRE-C17322500, Novachem, Heidelberg West, Australia) (henceforth referred to as testosterone treated, or TT) or the equivalent volume (2 μl well^{-1}) of the vehicle control, ethanol (henceforth referred to as control, or CON).

Protein was extracted from the cells at baseline (Day 0) and after 1 (D1), 4 (D4) and 7 days (D7) of differentiation, using 150 μl well^{-1} 1× RIPA lysis buffer (cat. no. J62524-AE, Thermo Fisher Scientific) containing 10 μl ml^{-1} phosphatase inhibitor and 1 μl ml^{-1} protease inhibitor cocktail (cat. no. 78440, Thermo Fisher Scientific). The protein concentration of each sample was determined by a Pierce Bicinchoninic Acid (BCA) assay (cat. no. 23225, Thermo Fisher Scientific) according to manufacturer's instructions. Western blots were completed under the same conditions as described above on the resultant cell protein lysate, loading 10 μg total protein well^{-1}.

Immunohistochemical analysis of androgen receptor location. In addition to western blot analyses, we also investigated the effect of testosterone treatment on the localisation of the AR in myocytes. At baseline, and after 1, 4 and 7 days of differentiation, human primary myocytes were fixed with 2% paraformaldehyde (PFA)/PBS for 10 min, permeabilised in 0.1% Triton X-100/PBS for 5 min and blocked with 3% BSA/PBS for 40 min at room temperature. Cells were incubated for 1 h at room temperature with the primary antibody against total AR (cat. no. 5153,1:50 in blocking buffer, rabbit, Cell Signaling Technology). Following this, the cells were incubated with AlexaFluor 488 goat anti-rabbit IgG (cat. no. A-11008, 1:5000, Thermo Fisher Scientific) and AlexaFluor 647 phalloidin (cat. no. A22287, 1:200, Thermo Fisher Scientific) in 1% BSA/PBS for 1 h at room temperature. The cells were then stained with 0.1 μg ml^{-1} DAPI (cat. no. 62248, 1:1000, Thermo Fisher Scientific) in PBS for 10 min. Cell images were obtained with dedicated software at ×100 magnification (Eclipse Ti2, Nikon, Tokyo, Japan).

Quantification of androgen receptor intensity. Quantification of AR content was performed using the open-source image analysis software CellProfiler

(version 4.2.5) and an analysis pipeline developed within this study (Stirling et al., 2021). For *in vivo* analysis, 5−10 images per participant for each time point were captured at ×40 magnification, imported into CellProfiler and split into individual greyscale images of sarcolemma, nuclei and AR staining based on RGB channels. DAPI-stained myonuclei were identified as objects with a diameter range between 8 and 50 pixels, as previously described (Sanz et al., 2019). To quantify the nAR/AR ratio, a binary mask of the DAPI-stained myonuclei was applied to the AR-stained images and the total intensity of AR expression within the nuclei was expressed as a ratio of total AR intensity per field. The percentage of AR+nuclei was quantified by counting the number of DAPI-stained myonuclei encompassed within a binary mask of the AR-stained images. The percentage of nuclei highly expressing AR was determined by creating a binary mask of regions expressing 3× the mean intensity of AR staining per field from the AR image and identifying the percentage of nuclei within this mask. The total number of muscle fibres per field was quantified using the previously described Muscle2View CellProfiler pipeline (Sanz et al., 2019). Each binary mask was manually reviewed by two independent analysts, and any masks containing apparent visual artifacts were excluded from analysis (an average of 3.8 images per participant were excluded).

For *in vitro* analysis, images of one field per well from six wells were imported into CellProfiler and split into individual greyscale images of actin, nuclei and AR staining based on RGB channels. A binary mask of the myocytes was created using the actin images, and the total area within each field occupied by this mask quantified. The total intensity of the AR stain was measured and expressed relative to the area occupied by myocytes per each field, as the measure 'AR intensity'. This CellProfiler pipeline was validated for the accurate and complete identification of both myocytes and AR protein through a manual review of 20% of the total images, performed by two independent analysts.

Statistical analysis

The statistical analyses for this study were performed using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA) and R software version 4.0.2 using the packages *lmerTest* (Kuznetsova et al., 2017), *tidyverse* (Wickham et al., 2019), *car* (Fox & Weisberg, 2019) and *AICcmodavg* (Mazerolle, 2023).

Student's two-tailed, paired *t* test in GraphPad Prism was used to assess the effect of a 12-week resistance training programme on the changes to participant anthropometric data, the protein expression of all measured proteins, and thigh muscle size, strength and power. For the linear models, leg press strength and muscle power were expressed as values relative to the individuals' total body lean mass (kg) and the changes to muscle size, strength and power were expressed as delta percentage change (%). The phosphorylation status of all proteins is expressed as the amount of phosphorylation relative to the total protein content of that protein (e.g. $p\text{-}AR^{ser213}$ = $p\text{-}AR^{ser213}$/total AR content) and the changes in protein content from pre- to post-training is expressed as fold-change from the pre-training levels. If the protein content of a given protein did not change, the mean of the pre- and post-training values was used for all post-training (delta change) linear models.

One-way, repeated measures analysis of variance (ANOVA) was used in GraphPad Prism to assess the effect of 12 weeks of resistance training on the concentrations of testosterone, SHBG, DHT and DHEA and changes in dietary protein intake and total energy expenditure. If hormone concentrations did not significantly change, the area under the curve (AUC) using the trapezoidal method was used for all post-training (delta change) linear models. The AUC provides a surrogate measure for the total amount of hormone that participants were exposed to across the entire 12 weeks.

Linear models were used in RStudio to examine whether the outcome (muscle size, strength and power at baseline, or the delta change in these variables) was influenced by the independent variables of serum testosterone concentrations, the FAI, DHT, DHEA, the protein expression of AR, the nAR/AR ratio, or the proportion of AR+ nuclei. The model was of the form: outcome $\sim$ independent variable + covariate (if applicable). Before further analyses, the normality of all variables was assessed, and variables were log-transformed if necessary.

Possible covariates included age, body mass index (BMI), E2, P, luteinising hormone, FSH, chronotype, HC use, average protein intake, average daily physical activity and menstrual phase. Before fitting the linear models, AIC tests were run on linear models containing all possible combinations of the covariates to establish which covariates were required in the final model. The model with the lowest AIC that explained the largest proportion of variance in the association was chosen as the final model. The collinearity of linear models with appropriate covariates was assessed through variance inflation factors (VIF), with a threshold of three sets. The homoscedasticity of each model was assessed through residual and QQ plots.

Transcriptomic data were analysed using RStudio 4.1.3 (R Core Team, 2021). Differential gene analysis was conducted using the R package DeSeq2 (Love et al., 2014) using the model: Genes $\sim$ *ID* + timepoint. ID was used to account for repeated measurements. ChIP-X enrichment

Table 2. Baseline anthropometric and strength data separated for all participants that completed the gym-based training programme (n = 17) or participants who completed the blended gym- and home-based training programme (n = 10) and for all participants combined (n = 27)

	Participants undergoing normal training (n = 17)	Participants spending 2–6 sessions at home (n = 10)	All participants (n = 27)
Age (years)	24.5 ± 5.4	22.7 ± 4.5 (P = 0.38)	23.9 ± 5.1
Height (m)	1.7 ± 0.1	1.7 ± 0.1 (P = 0.82)	1.7 ± 0.1
Weight (kg)	63.6 ± 9.9	68.3 ± 12.4 (P = 0.28)	65.4 ± 10.9
BMI (kg m^{-2})	23.2 ± 3.4	24.1 ± 4.1 (P = 0.51)	23.5 ± 3.6
Hormonal contraceptive use	Users: 46% Non-users: 54%	Users: 67% Non-users: 33% (P = 0.30)	Users: 51% Non-users: 49%
Baseline testosterone concentrations (nmol l^{-1})	2.1 ± 0.5	2.0 ± 0.6 (P = 0.60)	2.0 ± 0.6
Calculated squat 1RM (AU)	102.1 ± 33.8	104.7 ± 25.6 (P = 0.83)	103.1 ± 30.6
Calculated leg press 1RM (AU)	172.2 ± 39.3	179.8 ± 28.9 (P = 0.60)	175.0 ± 35.4
Calculated leg extension 1RM (AU)	53.4 ± 11.3	45.0 ± 13.5 (P = 0.09)	50.3 ± 12.6
Calculated hamstring curl 1RM (AU)	36.0 ± 6.3	36.8 ± 7.2 (P = 0.78)	36.3 ± 6.5
Calculated seated row 1RM (kg)	40.2 ± 7.2	37.4 ± 7.2 (P = 0.33)	39.2 ± 7.2
Calculated shoulder press 1RM (kg)	9.4 ± 2.8	8.8 ± 2.5 (P = 0.60)	9.2 ± 2.7
Calculated biceps curl 1RM (kg)	8.2 ± 1.6	8.1 ± 1.4 (P = 0.86)	8.1 ± 1.5

Values are means ± standard deviation. P-values shown in brackets are for pairwise comparisons to participants having completed normal training using Student's unpaired t test.

analysis 3 (ChEA3) (https://maayanlab.cloud/chea3/) was used to perform transcription factor (TF) enrichment analysis on the differentially expressed genes (Keenan et al., 2019). The mean rank integration method was used to calculate the ranking of the most enriched TFs.

We considered genes and transcription factors significant with a false discovery rate (FDR) adjusted P-value <0.05. The following packages were also used in our analysis; *tidyverse* (Wickham et al., 2019), *superheat* (Barter & Yu, 2018) and *biomaRt* (Durinck et al., 2009).

GraphPad Prism was used to perform two-way ANOVAs with multiple comparisons to assess the effect of 7 days of testosterone treatment on myocyte diameter and the effect of acute testosterone treatment on the protein content of the AR and markers of protein synthesis.

All values are presented as means ± SD, unless otherwise stated. The significance for all statistical tests was set at $P < 0.05$.

Results

Of the 35 females enrolled in the study, 27 females completed the 12-week resistance training programme. Ten participants completed 11% (average 4/36 sessions) of their training via teleconferencing at home due to SARS-CoV-19-related lockdowns in Victoria, Australia. These participants completed the rest of their training sessions in the gym. There were no differences in age, height, weight, BMI, percentage of HC users or calculated baseline 1RM for any exercise between the participants who performed all of their training sessions in the gym and those that performed some sessions via video conferencing (Table 2). There was no between-group difference in the trajectory of working weight progression for any exercise. There was also no between-group difference in the training-induced changes in 1RM for any exercise, thigh muscle CSA, power or hormone levels. This is described in detail elsewhere (Alexander, Knowles, et al.,

2022). As there was no difference between the training programmes in training progression, hormone levels or any other outcome measure, the results from both cohorts were pooled for all further analyses.

Effect of 12 weeks of resistance training on body composition and muscle mass and function

Twelve weeks of resistance training increased body mass by 1.4% (pre-training: 65.4 ± 10.9 kg, post-training: 66.2 ± 10.5 kg, $P < 0.05$). Total body lean mass increased by 1.9% (pre-training: 42.1 ± 5.5 kg, post-training: 42.9 ± 5.7 kg, $P < 0.01$). Total body fat mass remained unchanged (pre-training 21.0 ± 7.8 kg, post-training: 21.5 ± 7.7 kg, $P = 0.125$).

The primary outcome measure of muscle strength (measured via leg press 1RM) increased by 27.3% ($P < 0.001$), the secondary outcome measure of thigh muscle cross-sectional area (CSA; measured via pQCT) increased by 5.9% ($P < 0.001$) and the tertiary outcome measure of muscle power (measured via vertical jump) increased by 13.0% ($P < 0.05$) (Fig. 2*A*–*C*). The calculated 1RM of the trained exercises squat, leg extension and hamstring curl increased by 49%, 47% and 34%, respectively ($P < 0.001$; Fig. 2*D*–*F*).

The primary outcome of leg press was not trained to minimise any learning effect that occurs with training a movement. This lack of learning effect was confirmed by the numerically lower percentage increase in leg press (+27%) compared to the trained movements of squat (+49%), leg extension (+47%) and hamstring curl (+34%).

Effect of 12 weeks of resistance training on the cellular markers of muscle hypertrophy

The average myofibre CSA (μm^2) did not change with resistance training (mixed fibre increase 7.7%, $P = 0.100$; type I fibre CSA increase: 8.7%, $P = 0.131$; type II fibre CSA increase: 5.7%, $P = 0.269$; Fig. 2*G*). ($P = 0.056$; Fig. 2*H*). The percentage of type I and type II fibres did not change with training (Fig. 2*I*) ($P = 0.725$). Representative images are shown in Fig. 2*J*–*M*.

Effect of 12 weeks of resistance training on androgen receptor protein content, phosphorylation status and nuclear localisation

Twelve weeks of resistance training did not induce any change in the protein ($P = 0.672$) content of the AR (Fig. 3*A*), or its phosphorylation status at either serine residue 213 (p-AR^{ser213}, $P = 0.730$; Fig. 3*B*) or serine residue 650 (p-AR^{ser650}, $P = 0.750$; Fig. 3*C*). *AR* mRNA expression also did not change with 12 weeks of resistance training (FDR = 0.956; Fig. 3*D*). Similarly, 12 weeks of resistance training did not induce any change in nAR *in vivo*. This was shown in both the ratio of nAR to total AR stain (nAR/AR ratio; Fig. 3*E*) and the percentage of nuclei that were AR positive (%AR+; Fig. 3*F*).

Effect of 12 weeks of resistance training on molecular markers of protein synthesis and degradation

Twelve weeks of resistance training increased the levels of total Akt protein by 13% ($P < 0.05$) (Fig. 4*A*). There was no change in the basal total or phospho-protein content of other markers of protein synthesis or degradation: p-Akt^{ser473} ($P = 0.972$), mTORC1 ($P = 0.365$), p-mTORC1^{ser2448} ($P = 0.976$), MAPK ($P = 0.330$), p-MAPK$^{thr202/tyr204}$ ($P = 0.191$), rpS6 ($P = 0.890$), p-rpS6$^{ser235/236}$ ($P = 0.533$), 4E-PB1 ($P = 0.830$), p-4E-PB1$^{thr37/46}$ ($P = 0.977$) and MuRF1 (TRIM63) ($P = 0.209$) (Fig. 4*B*–*K*).

mRNA expression of *RPS6KC1* mRNA increased by 11% (FDR < 0.01; Fig. 5*A*). There was no change in the mRNA levels of protein degradation markers *TRIM63* (FDR = 0.688), *FBXO32* (FDR = 0.757), *TRAF6* (FDR = 0.443), *FOXO1* (FDR = 0.430) or *FOXO3* (FDR = 0.268) (Fig. 5*B*–*F*).

Effect of 12 weeks of resistance training on plasma and urine sex hormone concentrations

The average plasma testosterone concentration at baseline was 2.0 ± 0.6 nmol l^{-1}, ranging between 1.1 and 3.1 nmol l^{-1}. Twelve weeks of resistance training did not change the plasma levels of testosterone, DHT, DHEA or the FAI (Fig. 6*A*–*D*). The androgen profile from urine measured via LC-MS, which included testosterone, epitestosterone, androsterone, etiocholanolone, 5α-adiol, 5β-adiol, DHEA and DHT, did not change across 12 weeks of resistance training and confirmed what was observed in plasma (data not shown). As circulating hormones directly reflect the form that is utilised by the muscle, plasma hormone concentrations were used over urine concentrations in all further analyses. Since there were no training-induced changes at any time point, the AUC of these hormones, which is indicative of the total exposure to this hormone during the 12-week training period, was used in subsequent analyses, where stated.

Effect of 12 weeks of resistance training on the muscle transcriptomic profile

Two hundred and fourteen transcripts were differentially expressed between pre- and post-training (122 up-regulated, 92 down-regulated, FDR < 0.05) (Fig. 7*A*).

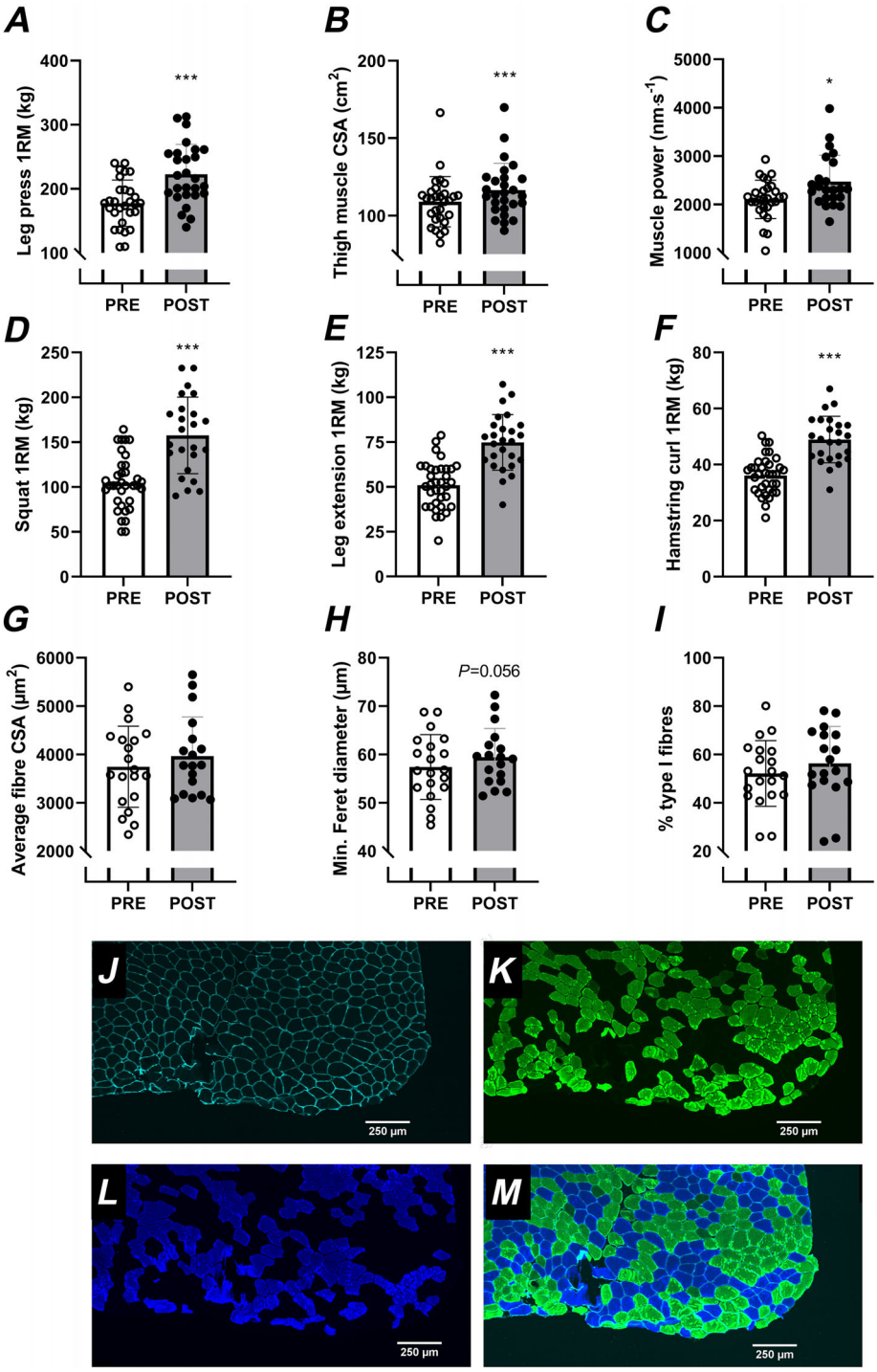

Figure 2. The effect of a 12-week resistance training programme on muscle size, strength and power in pre-menopausal females

A–I: Leg press 1RM (*A*), thigh muscle cross-sectional area (CSA) (*B*), muscle power (*C*), squat 1RM (*D*), leg extension 1RM (*E*), hamstring curl 1RM (*F*), mixed muscle myofibre CSA (*G*), minimum Feret diameter (*H*) and percentage of type I fibres (*I*) in untrained, pre-menopausal females before and after 12 weeks of resistance training. Pre-training (PRE) values are indicated by open bars and post-training (POST) values are indicated by filled bars. $n = 27$ for *A–F*, $n = 17$ for *G–K*. $^{*}P < 0.05$, $^{***}P < 0.001$. Data were analysed using two-tailed, paired *t* tests. Values are presented as means ± SD. *J–M* Representative images showing laminin-stained sarcolemma of a muscle section (*J*), type I fibres (*K*), type II fibres (*L*) and a composite image of both (*M*). Scale for representative images is 1.24 µm pixel^{-1} for all images shown. $n = 753.8 \pm 370.6$ fibres per slice. White scale bar represents 250 µm.

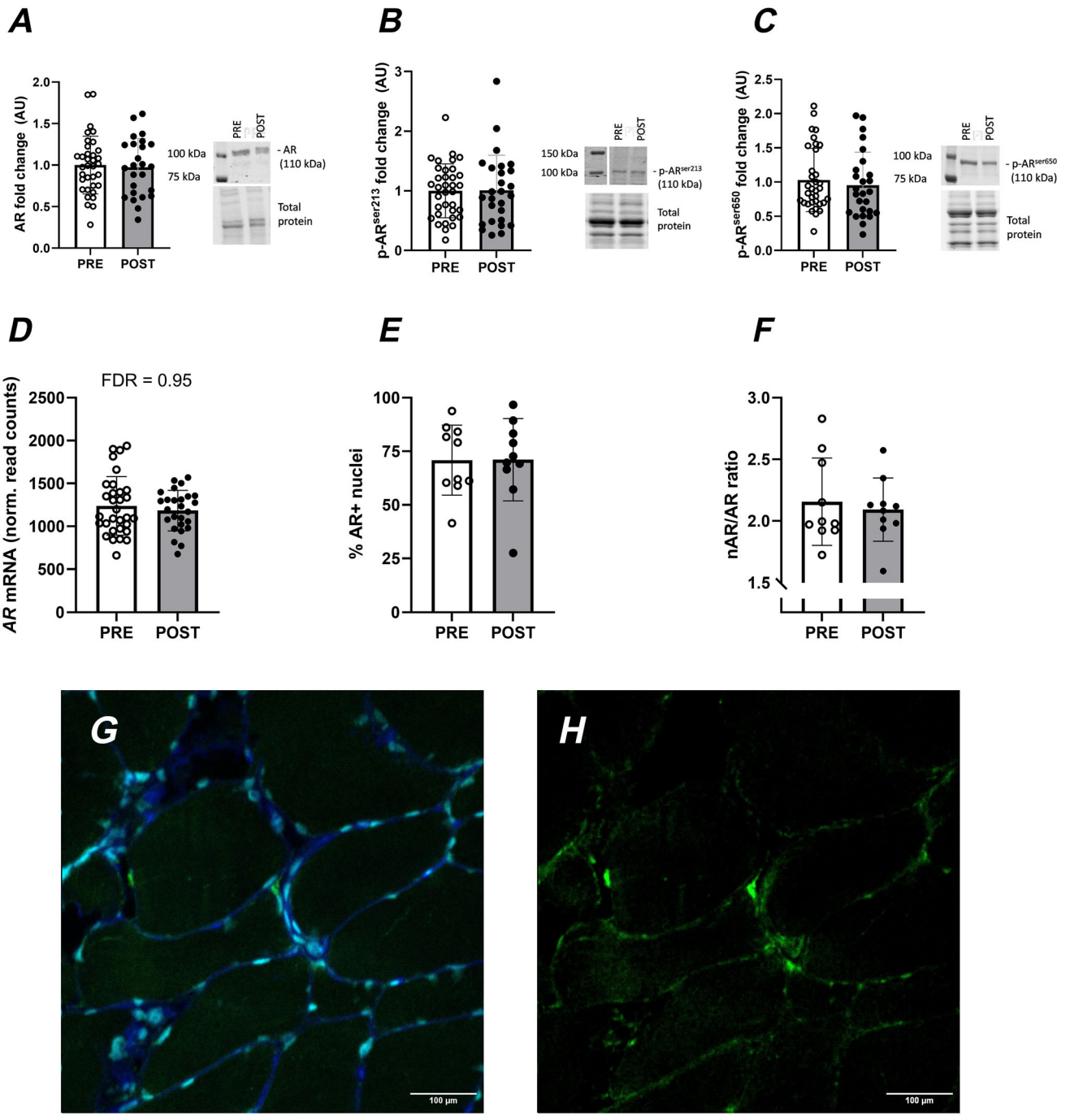

Figure 3. The effect of a 12-week resistance training programme on skeletal muscle androgen receptor protein content and signalling in pre-menopausal females
The protein content of total AR protein (*A*), p-AR^{ser213} (*B*), p-AR^{ser650} (*C*), AR mRNA expression (n = 27) (*D*), the percentage of AR+ nuclei (*E*) or the nAR/AR ratio (n = 10 participants) (*F*) before and after 12 weeks of resistance training in previously untrained, pre-menopausal females. Western blots for each protein are presented beside the corresponding graph. Pre-training (PRE) values are indicated by open bars and post-training (POST) values are indicated by filled bars. Data were analysed by Student's two-way, paired t test. Values are presented as means ± SD. *G*. Representative composite image of a muscle section stained with DAPI (cyan; stains the nucleus), wheat germ agglutinin (blue; stains the sarcolemma) and α-AR (green) at ×40 magnification. *H*. Representative image of α-AR (green) stain at ×40 magnification. Scale for representative images is 0.62 µm pixel^{-1}. White scale bar represents 100 µm. n = 5–10 images per section, mean 29.9 ± 8.6 fibres per image.

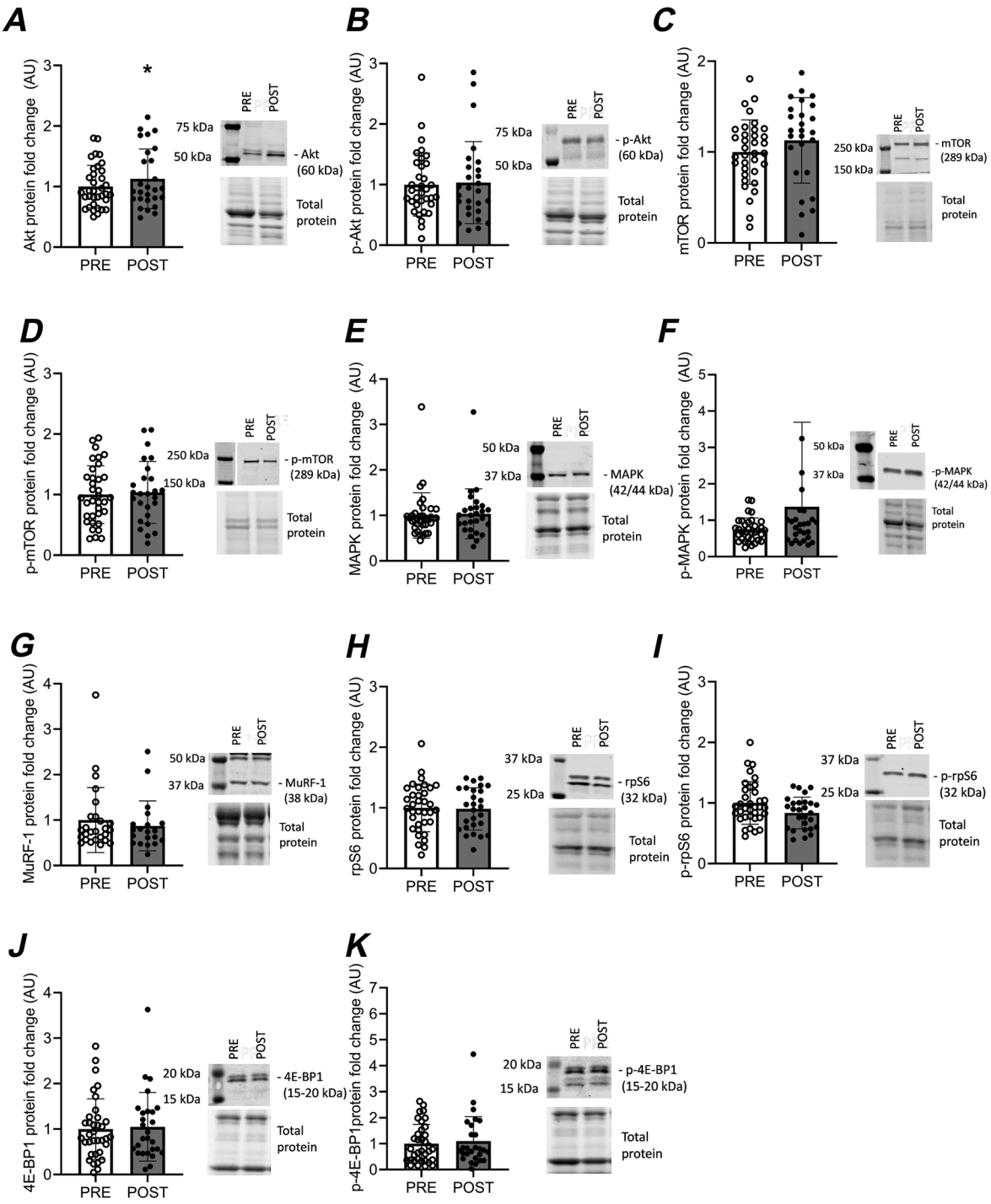

Figure 4. The effect of a 12-week resistance training programme on the protein content of total Akt (*A*), p-Akt (*B*), total mTOR (*C*), p-mTOR (*D*), MAPK (*E*), p-MAPK (*F*), MuRF-1 (*G*), rpS6 (*H*), p-rpS6 (*I*), 4E-BP1 (*J*) and p-4E-BP1 (*K*) in pre-menopausal females

Representative western blots for proteins are presented beside the corresponding graph. Pre-training (PRE) values are indicated by open bars and post-training (POST) values are indicated by filled bars. Protein was normalised to total protein load. Values are represented as fold change compared to PRE values. Bars represent means ± SD. $n = 27$.

We then investigated the putative role of androgens in the regulation of the muscle transcriptome, by conducting transcription factor enrichment (TF) analysis on the differentially expressed genes using the mean rank integration method to rank the most enriched TFs. The top 15 TFs included muscle-specific transcription factors MYOG, MYOD1 and MEOX2 (Fig. 7*B*). The AR was ranked 284 of 1632 TFs ranked, indicating that the AR and its binding to AREs may only play a minor role, if any, in the female global muscle transcriptomic response to anabolic stimulation. In line with this finding, none of the 14,979 individual detected transcripts were significantly associated with total testosterone concentrations.

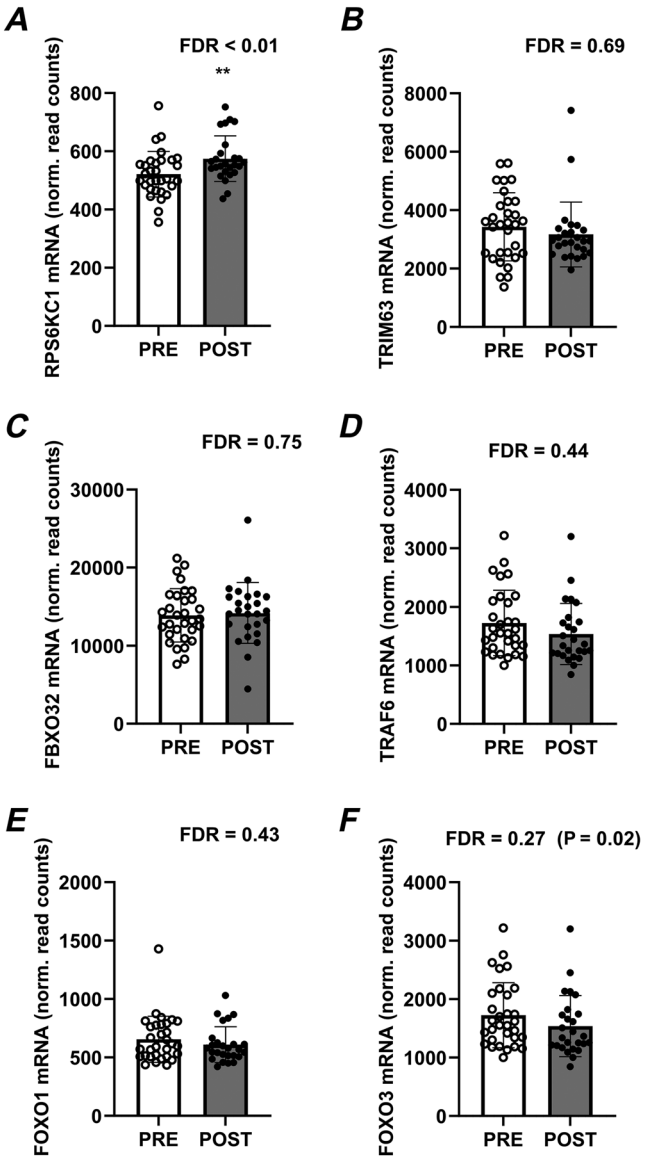

Figure 5. The effect of a 12-week resistance training programme on the mRNA expression of (*A*) RPS6K1, (*B*) TRIM63, (*C*) FBX032, (*D*) TRAF6, (*E*) FOXO1 and (*F*) FOXO3 in pre-menopausal females
Pre-training (PRE) values are indicated by clear bars and post-training (POST) values are indicated by dark bars. mRNA was normalised to counts per million. Values are represented as mean ± SD. n = 27.

Associations between androgen hormone concentrations and muscle strength, size and power pre- and post-training

We next used linear models to test the association between baseline androgen concentrations, or total exposure to androgens during the 12-week training period, and baseline or training-induced changes in muscle size and function, respectively. AIC tests were used to identify the moderators to be included in each model. There was no evidence of an association between baseline total testosterone and pre-training muscle strength ($P = 0.445$), CSA ($P = 0.417$), power ($P = 0.929$) and fibre CSA ($P = 0.147$). Similarly, there was no evidence of an association between the AUC of testosterone (indicative of the total exposure to testosterone across 12 weeks) and the training-induced changes in muscle CSA ($P = 0.969$), strength ($P = 0.744$), power ($P = 0.279$) and muscle fibre CSA ($P = 0.534$) (Table 3). These results were replicated with testosterone precursor and metabolites DHEA and DHT, respectively (all P-values >0.05) (Table 3).

The same model was then used to investigate the association between the FAI, indicative of the amount of bioavailable testosterone, and whole thigh muscle CSA, strength, power and fibre CSA. There was no evidence of an association between pre-training FAI and muscle CSA, strength, fibre CSA and power at baseline (Table 3). The AUC of the FAI across 12 weeks of resistance training was positively associated to the changes in muscle strength ($\beta = 0.05$, SE $= 0.02$, $P = 0.044$) that occurred with 12 weeks of resistance training (Fig. 8*A*).

There were no correlations between total testosterone or the FAI and total or phospho-protein content of markers of protein synthesis Akt, mTOR, MAPK, rpS6 or 4E-BP1 before or after 12 weeks of resistance training (data not shown).

Associations between androgen receptor content and muscle strength, size and power

In young males, the protein content of the AR is positively associated with resistance training-induced hypertrophy (Morton et al., 2018). We therefore tested the association between the total AR protein content and muscle CSA, strength, power and fibre CSA prior to or in response to a 12-week resistance training programme. Total AR content was negatively associated with thigh muscle CSA pre-training ($\beta = -25.99$, SE $= 7.98$, $P = 0.003$; Table 3, Fig. 8*B*), but this association was not maintained after resistance training.

Table 3. Linear mixed models of the association between total testosterone, the free androgen index (FAI), DHEA, DHT and markers of AR signalling and muscle size, strength and power before and after 12 weeks of resistance training in pre-menopausal females

Pre-training					Δ% Change with training				
Variable	β	SE	P	Model adjusted for	Variable	β	SE	P	Model adjusted for
Pre-training total testosterone							**Testosterone AUC**		
Pre-training muscle CSA	−5.18	6.30	0.417	Pre-training E2	Δ% muscle CSA	0.00	0.00	0.207	E2 AUC
Pre-training thigh muscle strength	0.25	0.32	0.445	Pre-training E2	Δ% thigh muscle strength	0.00	0.01	0.744	E2 AUC
Pre-training muscle power	0.30	3.44	0.929	Pre-training E2	Δ% muscle power	0.02	0.02	0.279	—
Pre-training muscle fibre CSA	514.20	336.10	0.147	—	Δ% muscle fibre CSA	0.08	0.12	0.534	—
Pre-training free androgen index							**Free androgen index AUC**		
Pre-training muscle CSA	−1.05	3.05	0.734	Pre-training E2	Δ% muscle CSA	0.00	0.01	0.799	E2 AUC
Pre-training thigh muscle strength	0.29	0.15	0.095	Pre-training E2	Δ% thigh muscle strength	0.05	0.02	0.044*	E2 AUC
Pre-training muscle power	0.98	1.33	0.469	—	Δ% muscle power	−0.01	0.05	0.912	E2 AUC
Pre-training muscle fibre CSA	2.94	159.2	0.985	—	Δ% muscle fibre CSA	0.03	0.03	0.378	E2 AUC
Pre-training DHEA							**DHEA AUC**		
Pre-training muscle CSA	−6.05	4.23	0.162	Pre-training E2	Δ muscle CSA	0.47	0.32	0.159	—
Pre-training thigh muscle strength	0.06	0.22	0.777	Pre-training E2	Δ thigh muscle strength	−0.02	0.04	0.623	—
Pre-training muscle power	−1.648	2.31	0.481	Pre-training E2	Δ muscle power	0.09	0.07	0.208	—
Pre-training muscle fibre CSA	22.76	243.60	0.927	—	Δ muscle fibre CSA	−0.08	0.05	0.157	—
Pre-training DHT							**DHT AUC**		
Pre-training muscle CSA	−3.81	4.86	0.439	Pre-training E2	Δ muscle CSA	−0.47	0.270	0.097	—
Pre-training thigh muscle strength	0.34	0.25	0.187	Pre-training E2	Δ thigh muscle strength	0.00	0.03	0.980	—

(*Continued*)

Table 3. (Continued)

Pre-training					Δ% Change with training				
Variable	β	SE	*P*	Model adjusted for	Variable	β	SE	*P*	Model adjusted for
Pre-training muscle power	−1.37	2.58	0.600	Pre-training E2	Δ muscle power	0.03	0.06	0.655	—
Pre-training muscle fibre CSA	55.23	279.57	0.846	—	Δ muscle fibre CSA	−0.02	0.05	0.607	—
Pre-training AR protein content					**Average AR protein content**				
Pre-training muscle CSA	−25.99	7.98	0.003*	Pre-training TT	Δ% muscle CSA	0.01	0.04	0.202	TT AUC
Pre-training thigh muscle strength	0.26	0.44	0.561	Pre-training TT	Δ% thigh muscle strength	0.01	0.09	0.943	TT AUC
Pre-training muscle power	5.45	5.09	0.292	Pre-training TT	Δ% muscle power	−0.08	0.194	0.688	TT AUC
Pre-training muscle fibre CSA	−552.7	522.9	0.305	—	Δ% muscle fibre CSA	−0.02	0.13	0.081	—
Pre-training nAR/AR ratio					**Average nAR/AR ratio**				
Pre-training muscle CSA	21.67	8.59	0.040*	Pre-training E2	Δ% muscle CSA	−0.16	2.36	0.949	E2 AUC
Pre-training thigh muscle strength	0.71	1.21	0.266	Pre-training E2	Δ% thigh muscle strength	−0.18	0.27	0.529	E2 AUC
Pre-training muscle power	−9.50	4.42	0.075	Pre-training E2	Δ% muscle power	−0.17	0.36	0.655	E2 AUC
Pre-training muscle fibre CSA	1588.01	612.04	0.032*	—	Δ% muscle fibre CSA	0.01	0.28	0.974	—
Pre-training AR+ nuclei (%)					**Average AR+ nuclei (%)**				
Pre-training muscle CSA	1.12	0.49	0.058	Pre-training E2	Δ muscle CSA	0.06	0.13	0.654	E2 AUC
Pre-training thigh muscle strength	0.06	2.01	0.085	Pre-training E2	Δ thigh muscle strength	−0.03	0.01	0.059	E2 AUC
Pre-training muscle power	−0.32	−1.01	0.353	Pre-training E2	Δ muscle power	0.01	0.02	0.696	E2 AUC
Pre-training muscle fibre CSA	99.19	49.06	0.078	—	Δ muscle fibre CSA	0.01	0.28	0.974	—

n = 35 pre-training, 27 change with training. *$P < 0.05$. AR, androgen receptor; AUC, area under the curve; CSA, cross-sectional area; E2, oestradiol; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; nAR, nuclear localised androgen receptor; TT, total testosterone.

Associations between androgen receptor nuclear localisation and muscle strength, size and power

Linear models assessed the association between AR cellular localisation and muscle mass, strength and power prior to or in response to a 12-week resistance training programme (Table 3). The ratio of nAR to total AR intensity (nAR/AR ratio) was positively associated with whole muscle CSA (β = 21.67, SE = 8.59, P = 0.04; Fig. 8*C*) and muscle fibre CSA (β = 1588.01, SE = 612.04, P = 0.032; Fig. 8*D*), but not strength or power pre-training. There were no significant associations between the nAR/AR ratio and resistance-training-induced changes in muscle mass, strength or power (Table 3). There were trends (P = 0.05–0.08) for the percentage of AR+ nuclei to be positively associated with pre-training whole muscle CSA (P = 0.058) and myofibre CSA (P = 0.078), and resistance training-induced change in muscle strength (P = 0.059), but these did not reach statistical significance (Table 3).

Testosterone treatment increases female human primary myotube diameter through the nuclear translocation of the AR but does not activate the Akt/mTOR pathway

To further examine whether testosterone plays a direct, regulatory role in female skeletal muscle, we cultured human primary myocytes from six donor participants from the human study. Seven days of testosterone treatment increased myotube diameter by 37% compared to a vehicle control (P < 0.01) and increased total AR protein content by over 4-fold, 3-fold and 2-fold after 1, 4 and 7 days of testosterone treatment, respectively (P < 0.001; Fig. 9*A* and *B*). This result was replicated using immunohistochemical staining, where the intensity of the AR (indicative of AR content) was significantly greater after 1 and 7 days of testosterone treatment, compared to a vehicle control (P < 0.01; Fig. 9*C*). Immunohistochemical staining showed that, in myocytes treated with a vehicle control, the AR is distributed throughout the cytosol of

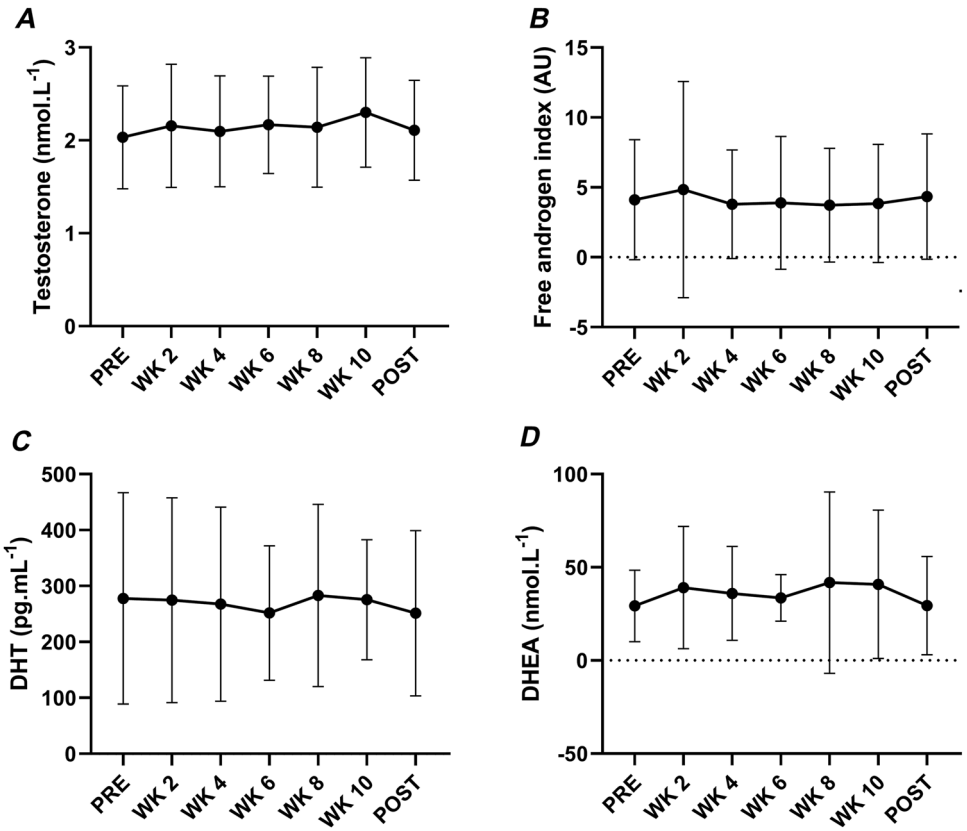

Figure 6. The effect of a 12-week resistance training programme on the androgen profile in pre-menopausal females
Resistance training did not change basal concentrations of testosterone (nmol·L^{-1}) (*A*), the free androgen index (FAI; AU) (*B*), dihydrotestosterone (DHT; pg·mL^{-1}) (*C*) or dehydroepiandrosterone (DHEA; nmol·L^{-1}) (*D*) in previously untrained, pre-menopausal females (n = 27). Data were analysed using a one-way ANOVA. Values are presented as means ± SD.

the cell with no or little nuclear localisation (Fig. 9*D*, upper panel; CON). After 24 h of 100 nM testosterone treatment, there was a striking translocation of the AR to the nucleus of myoblasts (Fig. 9*D*, lower panel; TT) paralleled by an increase in AR protein content in the cell. The AR protein then remained in the nucleus across the 7 days of differentiation of the testosterone treated myotubes (Fig. 9*E*, lower panel; TT).

Despite this, the protein content of Akt, p-Akt, mTOR, p-mTOR and p-MAPK did not change with testosterone treatment at any time point, despite their expression levels fluctuating across the differentiation time course ($P > 0.05$; Fig. 10).

Discussion

We showed that the bioavailable fraction of testosterone was positively related to resistance training-induced changes in muscle mass in pre-menopausal females. The proportion of nuclear-localised AR was positively associated with muscle mass and strength in pre-menopausal females at baseline, but not with resistance training-induced changes in muscle mass or strength. Conversely, total testosterone was not associated with muscle CSA, strength, power or the muscle anabolic response to 12 weeks of resistance training in pre-menopausal females. Transcriptomic data support the hypothesis that androgen genomic signalling, via the ARE, does not play a significant role in determining the muscle transcriptomic profile pre- or post-resistance training. These findings shed light on the limited body of knowledge regarding the role of testosterone in the regulation of female skeletal muscle.

Our results demonstrate that total testosterone is not a direct determinant of muscle mass, strength, or the muscle response to anabolic stimulation in pre-menopausal females. These results are in accordance with previous cross-sectional data showing that total testosterone is not associated with muscle mass or strength in pre- and post-menopausal females (Alexander et al., 2021; Carmina et al., 2009; Gower & Nyman, 2000; Kogure et al., 2015; Pöllänen et al., 2011; Rariy et al., 2011; van Geel et al., 2009). While there is some cross-sectional evidence of a positive relationship between testosterone and muscle mass in males (Mouser et al., 2016), there is also no relationship between testosterone and resistance-training-induced adaptations in males (Morton et al., 2016, 2018).

The bioavailable fraction of testosterone measured via the FAI was positively associated with resistance training-induced muscle strength, suggesting that the bioavailable rather than the total fraction of testosterone may play a regulatory role in the anabolic response of the female skeletal muscle. In line with these findings, the FAI was positively associated with resistance training-induced thigh muscle hypertrophy in a small study of pre-menopausal females ($n = 5$) (Häkkinen et al., 1992), and to muscle mass in a large cross-sectional cohort of pre-menopausal females conducted by our group ($n = 706$) (Alexander et al., 2021). However, it is important to bear in mind that the effect sizes in this study and our previous work (Alexander et al., 2021) were small

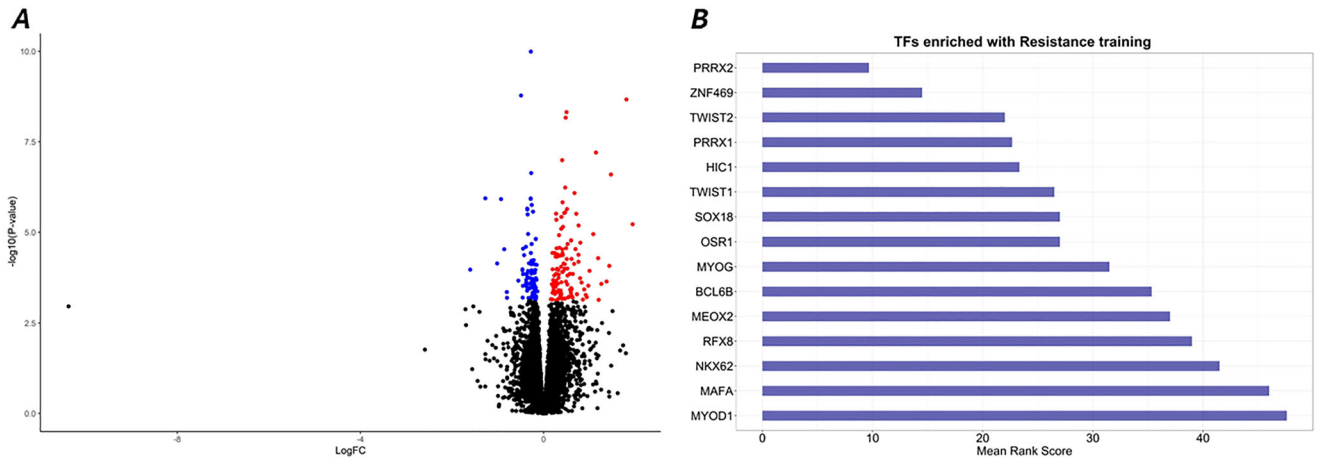

Figure 7. The effect of a 12-week resistance training programme on the skeletal muscle transcriptome in previously untrained, pre-menopausal females (n = 27)
A, Volcano plot displaying 122 up-regulated and 92 down-regulated transcripts in response to 12 weeks of resistance training in pre-menopausal females (n = 35 pre-training, 27 post training). Each point represents a transcript. Red points indicate an increase in mRNA expression following resistance training. Blue points indicate a decrease in mRNA expression following resistance training. Black dots represent genes which were not significantly differentially expressed. Significance was set at a false discovery rate (FDR) adjusted P-value <0.05. *B*, Top 15 transcription factors regulating the differentially expressed genes were ranked according to ChIP-X enrichment analysis 3 (ChEA3) using the mean rank integration method. Significance was set at FDR-adjusted P-value <0.05.

(β = 0.05 and 0.01, respectively). While the association was significant, the bioavailable fraction of testosterone only explains a small proportion of the variance in muscle mass in pre-menopausal females.

We show, for the first time, a negative association between total AR protein content and whole muscle CSA in pre-menopausal females. This is in contrast to males, where the AR protein content was positively associated with resistance training-induced hypertrophy and strength in healthy young (Ahtiainen et al., 2011; Mitchell et al., 2013; Morton et al., 2018) and older (Ahtiainen et al., 2011) males, further suggesting sex-specific differences in the role of the AR in skeletal muscle regulation. In support of this finding, male AR knockout (ARKO) mice displayed significant reductions in muscle mass and strength compared to their wild-type littermates (MacLean et al., 2008). Conversely, female ARKO mice did not display any differences in muscle mass or strength compared to their wild-type controls (MacLean et al., 2008), further demonstrating sex-specific differences in the role of the AR in the maintenance of muscle mass and function. We also confirm that resistance training does not affect AR protein content, phosphorylation status or nuclear localisation in female skeletal muscle, in agreement with previous work showing no change in AR protein content or nuclear localisation following 10 weeks of resistance training (n = 13 females) (Hatt et al., 2024). Males, in contrast, display significant increases in both AR protein content and nuclear localisation after the same resistance training programme (Hatt et al., 2024), suggesting further sex-specific AR regulation with chronic resistance exercise.

The proportion of AR localised to the nucleus (nAR/AR ratio) was positively associated with muscle size at both the fibre and whole-muscle level. Therefore, the ability to recruit the AR and translocate it to the nucleus rather than total AR content or testosterone concentrations may be more physiologically relevant to the maintenance of muscle mass in females. Taken together, these results point towards a negative feedback loop between total AR and muscle mass regulation. The positive association between nAR/AR and muscle size suggests that individuals with increased AR sensitivity, and therefore a greater ability to recruit and translocate AR to the nucleus, may require less total AR protein content to maintain their muscle mass. This is supported by our *in vitro* findings showing that, after an initial increase in total AR protein content with testosterone treatment, AR protein content begins to return to baseline levels after 4 and 7 days of treatment. Despite this decrease in total AR protein content, the

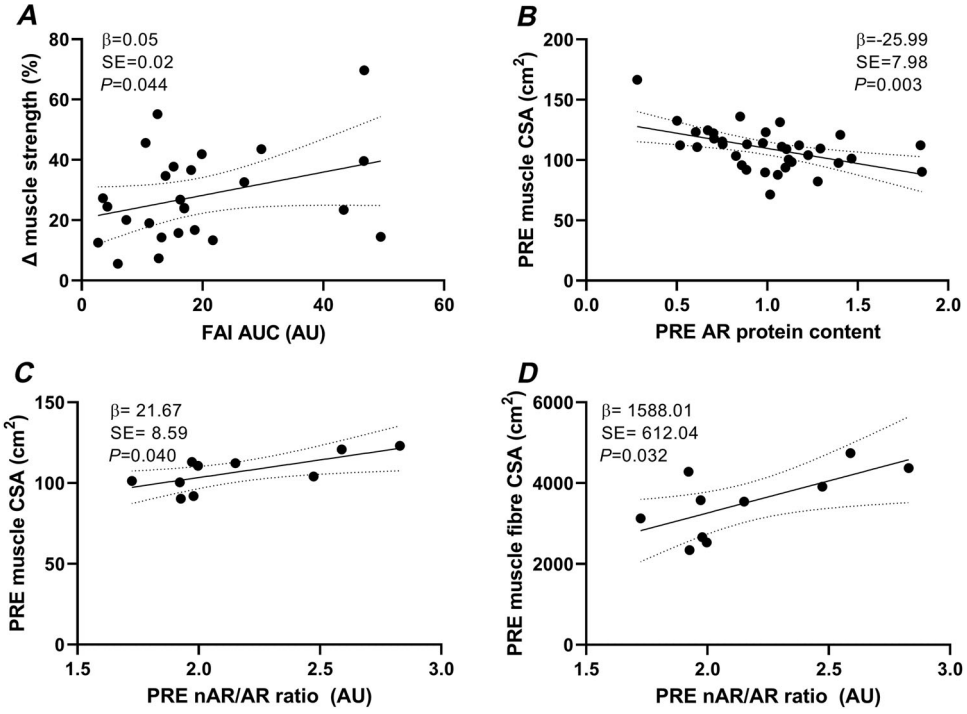

Figure 8. Linear associations between the free androgen index area under the curve (FAI AUC; AU) and the changes in muscle strength (leg press 1RM) (*A*), baseline AR protein content (AU) and baseline thigh muscle CSA (cm^2) (*B*), pre-training nAR/AR ratio (AU) and pre-training muscle CSA (cm^2) (*C*) and pre-training nAR/AR ratio (AU) and pre-training mixed muscle fibre CSA (cm^2) (*D*) in pre-menopausal females
Dashed lines represent 95% confidence intervals.

amount of AR in the nucleus was sustained across 7 days of treatment, suggesting a negative regulation of the AR by testosterone or with increased AR sensitivity.

Our data showing a rapid increase in AR protein content and nAR within 24 h of testosterone treatment *in vitro* are in line with previous findings showing that 6 days of testosterone treatment increased AR protein content in primary muscle cell cultures from male donors *in vitro* (Sinha-Hikim et al., 2004) and in muscle biopsies obtained from healthy, young males *in vivo* ($n = 6$) after 20 weeks of treatment with 600 mg week^{-1} testosterone (Sinha-Hikim et al., 2004). Taken together, our data and others' indicate that testosterone treatment primarily increases myotube diameter through genomic AR signalling and induces a marked and sustained translocation of the AR into myonuclei *in vitro* and *in vivo* (Bhasin et al., 2001), rather than non-genomic signalling pathways such as the Akt/mTOR or MAPK as previously suggested (Basualto-Alarcón et al., 2013; White et al., 2013; Wu et al., 2010).

No markers of protein synthesis or degradation were associated with total or bioavailable testosterone in our human cohort. This is mirrored by our findings that testosterone treatment did not promote Akt/mTOR or MAPK signalling in myocytes taken from female donors, despite significant increases in myotube diameter with testosterone treatment. In support of this, exogenous testosterone administration did not change molecular regulators of muscle mass and mitochondrial biosynthesis, including markers of mTOR signalling from resting biopsies in males ($n = 50$) (Howard et al., 2020) and females ($n = 48$) (Horwath et al., 2022) *in vivo*, or female primary myocytes *in vitro* (Pataky et al., 2023). Instead, testosterone administration to primary myotubes from female donors induced changes of proteins within the

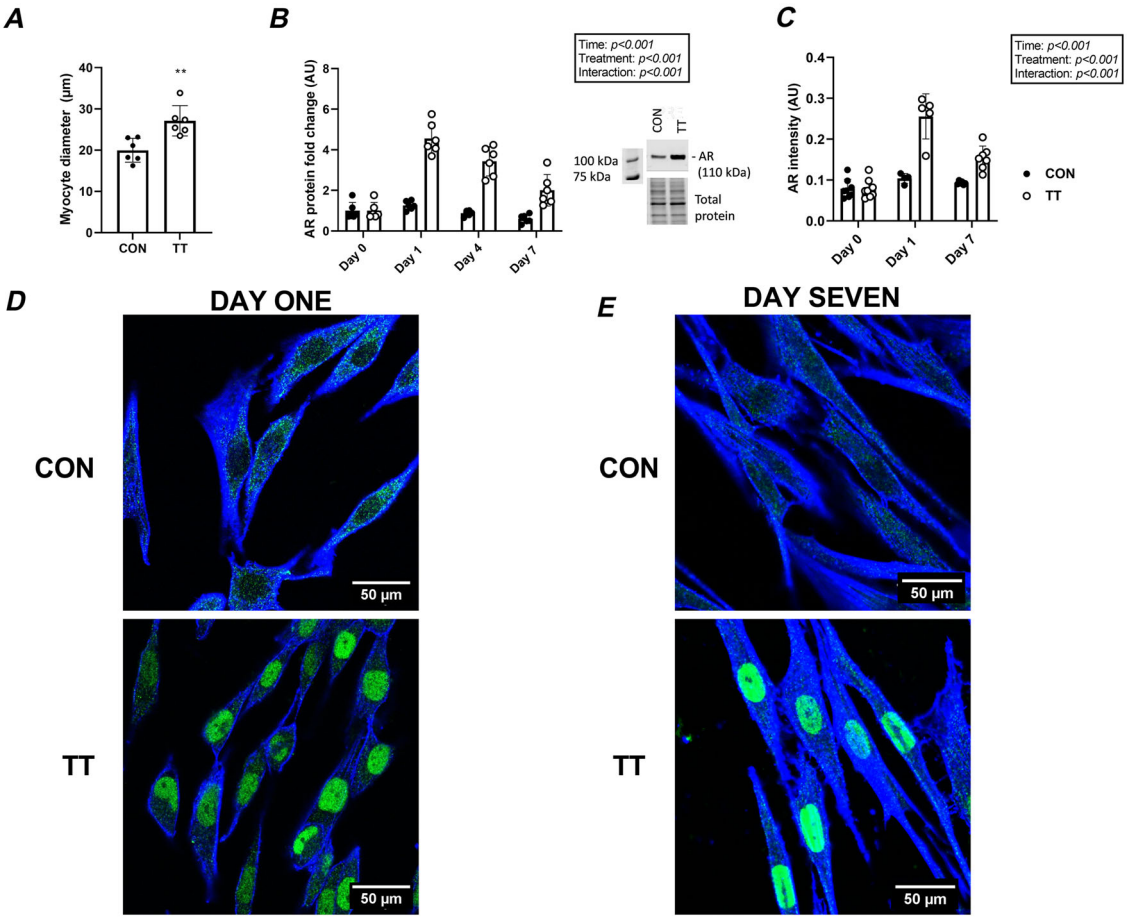

Figure 9. Testosterone treatment increases myocytes diameter, AR protein content and AR nuclear localisation *in vitro*
A–C, testosterone treatment over 7 days of differentiation increased myotube diameter (*A*), AR protein content (*B*) and AR intensity relative to the proportion of the visual field occupied by myocytes (*C*). Data were analysed using Student's two-tailed, paired *t* test and two-way ANOVA. Values are presented means ± SD. $^{**}P < 0.01$. *D* and *E*, AR cellular localisation in primary muscle cell lines treated with vehicle (CON) or 100 nM testosterone (TT) after 1 day (*D*) or 7 days (*E*) of treatment ($n = 6$ female donors). Phalloidin (stains actin) appears in blue. AR appears in green. Scale is 0.25 μm pixel^{-1} for all images. White scale bar represents 50 μm.

sarcoplasmic compartment, including myosin and titin, both of which play important roles in the contractile apparatus and muscle hypertrophy (Pataky et al., 2023). This suggests that, in contrast to *in vitro* data from rat (White et al., 2013; Wu et al., 2010) and murine (Basualto-Alarcón et al., 2013) myocytes, the primary mechanism of action of testosterone in humans may not be through the upregulation of the mTOR pathway. Instead, the accretion in lean mass seen with exogenous testosterone administration in males (Bhasin et al., 2001; Howard et al., 2020) and females (Horwath et al., 2022) may stem from a net positive protein turnover in favour of protein accretion driven by an increase in the genomic signalling of the AR rather than through activation of the Akt/mTOR non-genomic signalling pathways. This genomic signalling may lead to an increase in transcription of target genes and eventually in translational capacity, as evidenced by increases in total ribosome number (Mobley et al., 2018) and muscle RNA content (Howard et al., 2020).

Limitations

While this study included four participants (∼15%) who had been diagnosed with polycystic ovary syndrome (PCOS), there was only one participant who consistently had total testosterone concentrations above the typical female reference range (testosterone levels >2.5 nmol l^{-1}; Burger, 2002). This limited range of testosterone concentrations restricts the generalisability of these results. Our previous research (Alexander et al., 2021) suggests that the association between the FAI and lean mass is not linear but quadratic in nature, where the association plateaus and eventually becomes negative

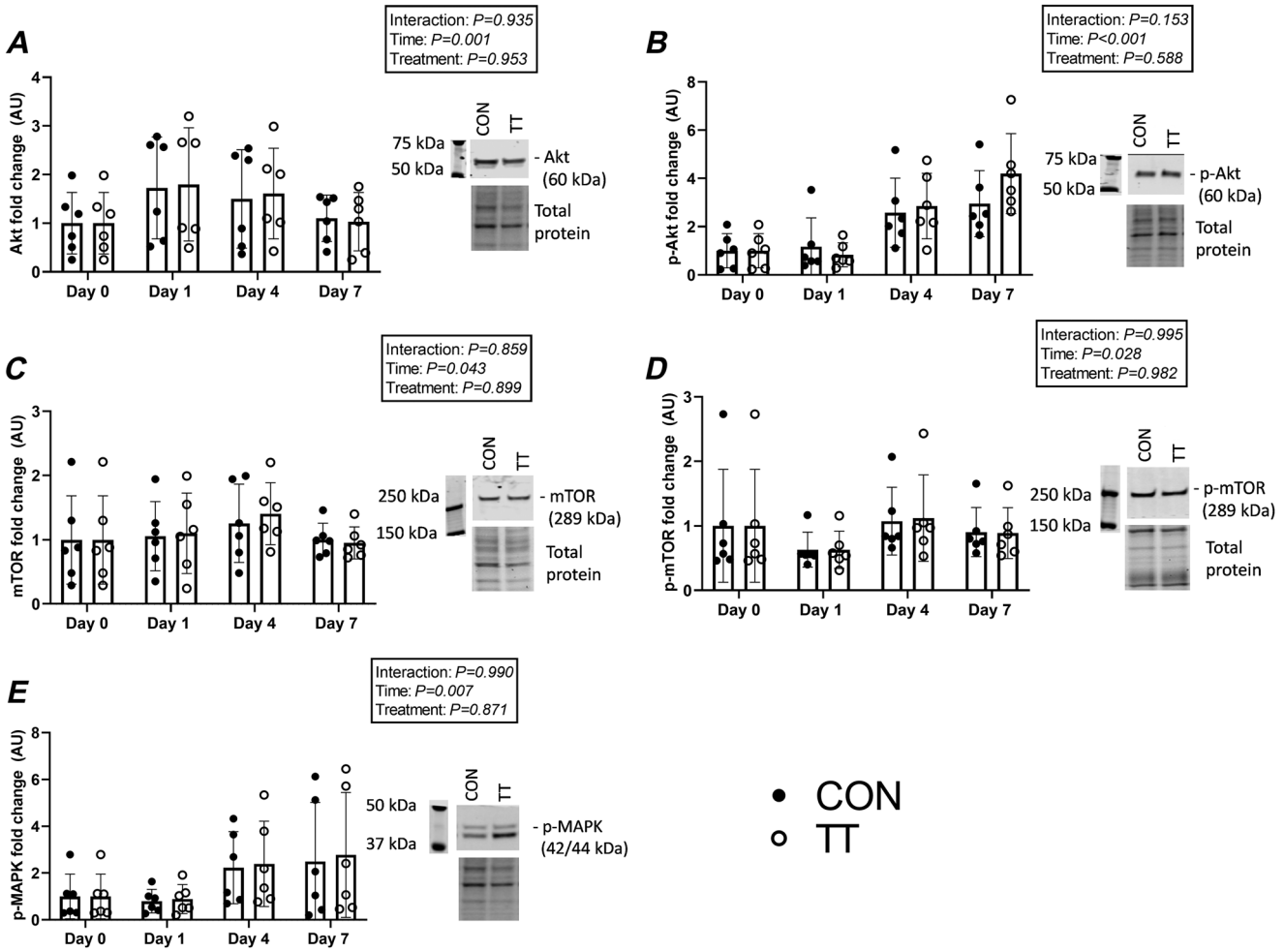

Figure 10. Treatment with 100 nM of testosterone did not change the protein content of total Akt (*A*), p-Akt (*B*), total mTOR (*C*), p-mTOR (*D*) or p-MAPK (*E*) compared to a vehicle control across 1, 4 or 7 days of differentiation
Filled circles represent vehicle control condition (CON), open circles represent testosterone treated (TT) condition. Data were analysed via two-way, repeated measures ANOVA and presented as fold change compared to Day 0. Protein was normalised to total protein load. Bars represent means ± SD, n = 6 female donors.

with increasing testosterone concentrations. Including females with a wider range of testosterone levels, including hyperandrogenic females and individuals with differences of sex development (DSD), would increase the generalisability of our findings and allow validation of the association between testosterone and muscle across a larger spectrum of androgen concentrations. Similarly, this study investigated individuals with an XX karyotype and the results should not be extrapolated to those who have experienced elevated levels of testosterone during male puberty. Finally, the effect sizes of the associations found in this study and our previous work (Alexander et al., 2021) were significant but small ($\beta = 0.03–0.05$), which should be kept in mind when interpreting the significance of these findings in real-world situations.

This study provides foundational knowledge regarding the relationship between testosterone and skeletal muscle. While we would not expect the relationship between androgens and skeletal muscle to critically differ between trained and untrained individuals, it should be noted that this study was conducted in untrained females from the general population. The results should, therefore, be interpreted with caution when considering elite athletes.

Conclusions

Our results suggest that, rather than total circulating testosterone concentrations, an individual's sensitivity to bioavailable androgens and their ability to recruit the AR to the nucleus might play a small, yet significant role in the maintenance of muscle mass and strength in pre-menopausal females.

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Additional information

Data availability statement

All RNA sequencing data generated or analysed during this study are included in this published article, its supplementary information files and publicly available repositories (GEO: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE267512, submission number GSE267512). The R code used for the analysis is available at https://github.com/DaniHiam/TESTO_RNAseq

Competing interests

None declared.

Author contributions

S.E.A. was involved in the design and conception of the study, and performed data collection, laboratory and scientific analyses and preparation the manuscript. O.E.K. was involved in the design of the training programme and the delivery of the training programme. R.M.W. was involved with laboratory and statistical analyses. B.G. and K.F. were involved with data collection and laboratory analyses. P.J. performed body composition scans. P.D.G. was involved with laboratory analyses and the design of the *in vitro* experiments. A.G. performed all muscle biopsies. S.L., G.D.W. and B.A. wereinvolved in the design and conception of the studies and preparation of the manuscript. D.H. performed statistical and transcriptomic analyses. N.E. wasinvolved in the design and conception of the study. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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