Title: Total testosterone is not associated with muscle mass, function or exercise adaptations in pre-menopausal females

Short title: Testosterone and muscle in females

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Key points

- Bioavailable testosterone was positively related to exercise-induced muscle hypertrophy in pre-menopausal females.
- Nuclear localisation of the androgen receptor was positively related to muscle mass in pre-menopausal females before resistance training, but not with resistance-training induced hypertrophy.
- Total testosterone is not related to muscle mass or strength in pre-menopausal females.
- Testosterone treatment induced androgen receptor nuclear translocation, but did not induce mTOR signalling in myocytes from pre-menopausal females.

Abstract

Testosterone, the major androgen hormone, 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) underwent a 12-week resistance training program. Muscle strength, size, power and plasma and urine androgen hormone levels were measured. Skeletal muscle biopsies were collected before and after the training program to quantify the effect of resistance training on AR protein and mRNA 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.

Total testosterone was 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 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 conclusion, we found that, bioavailable testosterone and the proportion of nuclear-localised AR, but not total testosterone, with skeletal muscle mass and strength in pre-menopausal females.

Key words: androgens, sex hormones, muscle, female, women, exercise

1. 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) or weightlifting (Zaras *et al.*, 2020). Skeletal muscle dynamically reacts and adapts to external stimuli such as mechanical loading and unloading, or internal stimuli such as the hormonal milieu (Schiaffino *et al.*, 2013). Testosterone is 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 $nmol \cdot L^{-1}$ and 10-30 $nmol \cdot L^{-1}$, respectively) (Burger, 2002). The majority of testosterone circulates bound to carrier proteins, sex hormone binding globulin (SHBG; approximately 45%) or albumin (approximately 50%) (Burger, 2002). 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; Olea *et al.*, 1990; de Launoit *et al.*, 1991), or in the sarcolemma of myocytes linked to a G-protein coupled receptor (Dent *et al.*, 2012). AR exerts its effects via 2 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 (Wu *et al.*, 2010; White *et al.*, 2013) 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 become phosphorylated, dissociate from chaperone proteins and translocate 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 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). There is evidence of 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*=3,875 (Ye *et al.*, 2021)). Other, smaller studies 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; Morton *et al.*, 2018), is associated with increased muscle hypertrophy and function in young males.

Our knowledge of the association between endogenous testosterone and muscle mass is limited in females (Alexander *et al.*, 2022) and the available evidence stems from cross-sectional cohorts. Evidence from murine (Yoshioka *et al.*, 2007) and human (Pataky *et al.*, 2023; Hatt *et al.*, 2024) models however suggest that there are sex-specific differences in androgen action on the skeletal muscle transcriptome. We and others show that 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 (Gower & Nyman, 2000; Carmina *et al.*, 2009; van Geel *et al.*, 2009; Pöllänen *et al.*, 2011; Rariy *et al.*, 2011; Kogure *et al.*, 2015). 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 (Carmina *et al.*, 2009; Alexander *et al.*, 2021).

The aim of the current study was to investigate the association between total testosterone and muscle mass, strength and power in pre-menopausal females at baseline or with the changes in muscle mass, strength or power following a tightly controlled 12-week resistance exercise training protocol. A secondary aim was to identify whether the FAI is more closely associated with muscle mass, strength, power or anabolic potential. We also investigated whether markers of AR expression, activity or localisation, or markers of muscle protein synthesis or degradation were associated with muscle mass, strength, power or anabolic potential. An *in vitro* model was used to further our mechanistic understanding of the role of testosterone in female human primary myocytes.

2. Methods

2.1 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 was conducted in accordance with the Declaration of Helsinki (World Medical Organisation, 2018) and its later amendments.

2.2 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 program due to COVID-19-related interruptions in 2020, 2 participants withdrew for health-related reasons and 2 participants withdrew for personal reasons. Therefore, 27 females completed the training program. 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 and 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 1 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.

2.3 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) on their non-dominant wrist for 7 days, accompanied by a sleep diary that incorporates several validated sleep rating systems (Samn & Perelli, 1982; Jay *et al.*, 2006). Sleep quantity (total hours) and total daily energy expenditure (metabolic equivalent; METs) measurements were repeated for 24 hours on weeks 3, 6 and 9 of the trial to ensure participants' sleep and energy expenditure

158 remained consistent, as any changes to either of these variables could potentially affect the
159 outcome of this study. Sleep quantity and total daily energy expenditure (METs) were tested as
160 covariates in later statistical analysis and were added to the models if they were significantly
161 associated with both the independent and dependent variables of interest.

162 Protein intake, daily physical activity and sleep quantity were measured at baseline and every 3
163 weeks throughout the training program. Sleep and protein intake did not change significantly
164 during the program, suggesting the participants maintained their habitual diet, and sleep
165 patterns throughout the entire 12 weeks (Supplementary Figure 1A-B). Participants decreased
166 their total energy expenditure by 14% during week 6 (p<0.001) and by 10.5% during week 9
167 (p=0.041) when compared to baseline (Supplementary Figure 1C). Despite this change in total
168 energy expenditure, Akaike Information Criterion (AIC) tests revealed that total energy
169 expenditure was not a significant confounder of the linear models and was therefore not included
170 in subsequent analyses.

171 *2.4 Menstrual phase standardisation and hormonal contraception use*

172 The pre-post design of this study allowed each participant to act as her own control, therefore
173 we did not exclude participants based on hormonal contraceptive (HC) use. This study included
174 both normally menstruating females and females using HC. Some research suggests that
175 muscle strength may be greater in the late follicular phase (days 7-14) compared to other phases
176 (Knowles *et al.*, 2019). More recent research however suggests that there is no difference in
177 muscle strength between menstrual cycle phases (Colenso-Semple *et al.*, 2023). Despite this,
178 we aimed to minimise any potential confounding effect of the menstrual cycle on muscle
179 performance by avoiding the late follicular phase (days 7-14) of the menstrual cycle during pre-
180 and post-training testing in normally menstruating participants. The data collection period lasted
181 12 weeks, 3 full cycles of a typical menstrual cycle lasting 28 days, allowing each normally
182 menstruating participant to undergo pre- and post-testing during the same phase of their cycle.
183 Menstrual phases were verified through menstrual diaries and hormonal analysis, in line with
184 published guidelines for the inclusion of females in exercise physiology cohorts (Knowles *et al.*,
185 2019; Elliott-Sale *et al.*, 2021). Two separate researchers verified the menstrual phase of each
186 participant and consensus was reached in each case. The menstrual phase of each testing
187 timepoint is summarised in Supplementary Table 1. HC use and menstrual phase were tested as
188 covariates in all subsequent statistical analysis.

2.5 Familiarisation to the training program

Prior to beginning the training program, participants attended 3 familiarisation sessions at Deakin University. 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; Borg, 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.

2.6 Strength and power testing

Peak muscle power was assessed using a portable force plate (AMTI, Watertown, MA). Participants performed a countermovement jump (CMJ), without an arm swing. Four attempts were made, separated by 3 minutes rest and the highest values were recorded as peak muscle power.

Participants' repetition maximum (RM) was assessed for leg press, as well as all the exercises included in the training program. Leg press was included in the strength testing but not in the training program and represents the major measure of muscle strength in this study. Using an exercise that was not included in the training program minimised any learning effect, as participants did not train and therefore, learn the movement. Lower body 1RM was calculated from 5RM tests using the equation (Abadie & Wentworth, 2000): estimated 1RM = 4.67 + (1.14 × weight lifted. Upper body 1RM was calculated from 10RM tests using the equation (Abadie & Wentworth, 2000): estimated 1RM = 1.43 + (1.20 × weight lifted).

2.7 Plasma and urine collection

Plasma was collected from participants in the fasted state before and after the training program, as well as before exercise in weeks 2, 4, 6, 8 and 10. At 0700 h, 10 mL of venous blood was taken from the antecubital vein in vacutainer tubes containing 7.2 mg K2 EDTA (Becton Dickinson, Franklin Lakes, NJ). Blood was centrifuged immediately for 10 min at 1 500 *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.

2.8 Body composition analysis

Participants' body composition was assessed before and after the 12-week training program via bioelectrical impedance analysis (BIA; Tanita, Kewdale, WA) and dual-energy X-ray

absorptiometry (DXA; Lunar Prodigy Advance, GE Healthcare, Madison, WI). At the time of measurement, participants had abstained from vigorous exercise, caffeine, and alcohol for the previous 48 hours, minimising the chances of water-retention or dehydration that may occur, as per standard recommendations (Walter-Kroker *et al.*, 2011).

2.9 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 were assessed via peripheral quantitative computed tomography (pQCT) (XCT 3000, Stratec Medizintechnik GmBH, Pforzheim, Germany).

2.10 Collection of muscle tissue

Participants abstained from caffeine, alcohol and vigorous activity for 48 hours prior to the collection of muscle biopsies. The night before, participants consumed a low-protein, standardised meal of pasta and tomato-based sauce as 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 0700 h after an overnight fast from 2100 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 anaesthetised with 1% Lidocaine without epinephrine. 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.

2.11 Training programs

After all the baseline measures were assessed, the 12-week resistance training program commenced. Every Monday, Wednesday and Friday, participants arrived at Deakin University between 0600-0800 h after an overnight fast from 2100 h the previous evening. 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=11) to undertake a portion of their training sessions online. These training sessions were delivered via videoconferencing, replicating the time and days of the gym-based training sessions. Briefly, the gym-based training program consisted of squats, leg extensions, hamstring curls, shoulder

press, biceps curls and seated row exercises. Participants were provided with weights and performed 3 sets of 8-10 repetitions at 60-80% 1RM. Supplementary Table 2 outlines the 2 different training programs undertaken by participants. Both programs were designed by an Exercise and Sports Science Australia-accredited exercise scientist and an Australian Strength and Conditioning Association-accredited strength and conditioning coach. Progressive overload (add 5% load) was applied to each exercise when an individual was able to complete 2 additional repetitions in the last set of an exercise in 2 consecutive sessions.

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

2.12 Laboratory analysis

2.12.1 Hormone analysis

Testosterone (sensitivity 0.18 ng·mL^{-1}, intra-assay coefficient of variation (CV) 3.1-5.4%, inter-assay CV 4.2-7.4%), sex hormone binding globulin (SHBG; sensitivity 0.23 nmol·L^{-1}, intra-assay CV 2.3-4.8%, inter-assay CV 5.2-6.3%), dehydroepiandrosterone (DHEA; sensitivity 0.03 ng·mL^{-1}, intra-assay CV 3.9-7.6%, inter-assay CV 5.1-10.4%) and 5α-dihydrotestosterone (DHT; sensitivity 7.23 pg·mL^{-1}, intra-assay CV 3.33-6.25%, inter-assay CV 6.49-7.47%) were measured via Enzyme-Linked Immunosorbant Assay (ELISA; #IBRE52151, #IB30176808, #IBRE52221, #IBDB5202, Abacus Dx, Parkville, Australia), according to 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^{n}) in a WADA-accredited laboratory as described previously (Salamin *et al.*, 2022). Liquid chromatography mass spectrometry (LC/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:

$$\mathrm{Con_{adj=}}\ \frac{(1.020-1)}{\mathrm{SG_{samplemax}}-1}\ \cdot\ \mathrm{Con_{measured}},\ \text{where}\ \mathrm{SG_{samplemax}}\ =\ \mathrm{SG_{sample}}\ +\ 0.002$$

For validation of menstrual phase, oestradiol (E2; inter-assay CV 5.5-10.7%) and progesterone (P; inter-assay CV 6.4-19.2%) 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 2-step immune-enzymatic assay (inter-assay CV 5.2-7.8%) according to manufacturer's instructions (Beckman Coulter) and follicle stimulating hormone (FSH) was analysed via a microparticle enzyme immunoassay (MEIA) (inter-assay CV 3.8-4.3%) according to manufacturer's instructions (Beckman Coulter).

2.12.2 Protein extraction

Protein was extracted from 20-25 mg of skeletal muscle tissue via manual homogenisation in 15 $\mu L \cdot mg^{-1}$ muscle 1× RIPA lysis buffer 1 (#J62524-AE, Thermo Fisher Scientific) containing 10 $\mu L \cdot mL^{-1}$ phosphatase inhibitor and 1 $\mu L \cdot mL^{-1}$ protease inhibitor cocktail (#78440, Thermo Fisher Scientific). The homogenised protein lysates were then gently spun at 4°C for 1 hour. After 1 hour, samples were centrifuged at 13,000 g for 15 min at 4°C. The protein concentration of each sample was determined via Pierce Bicinchoninic Acid (BCA) assay (#23225, Thermo Fisher Scientific) according to manufacturer's instructions. Absorbance of samples was read at 562 nm using bovine serum albumin as a standard.

2.12.3 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. Following protein extraction, the samples were denatured for 5 min at 95°C with 1× Nupage sample reducing agent (#NP0004, Thermo Fisher Scientific) and 1× Nupage LDS sample buffer (#NP0007, Thermo Fisher Scientific). Thirty µg of total protein from each sample was loaded into a 4–15% gradient Criterion Tris-Glycine extended (TGX) Stain Free gel (#5678085, Bio-Rad, Gladesville, Australia), separated via electrophoresis at 200 V, 40 min and visualised on a Universal Hood II GelDoc (BioRad). The separated proteins were transferred to an Immobilon PVDF-FL membrane (#IPFL00005, Millipore, Billerica, MA) at 100 V, 60 min and blocked for 1 hour in 5% skim milk in Tris buffered saline plus 0.1% Tween-20 (TBST; #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 (#2920, 1:1000, mouse, Cell Signalling Technologies, Danvers, MA), p-Akt^{ser473} (#4060, 1:1000, rabbit, Cell Signalling Technologies), AR (#5153, 1:500, rabbit, Cell Signalling Technologies), p-AR^{ser213}

(#PA537478, 1:500, rabbit, Thermo Fisher Scientific), p-AR^{ser650} (#537479, 1:500, rabbit, Thermo Fisher Scientific), MAPK (#4696, 1:1000, mouse, Cell Signalling Technologies), p-MAPK$^{thr202/tyr204}$ (#9101, 1:500, rabbit, Cell Signalling Technologies), mTOR (#4517, 1:1000, mouse, Cell Signalling Technologies), p-mTOR^{ser2448} (#5536, 1:1000, rabbit, Cell Signalling Technologies), MuRF-1 (#MP3401, 1:1000, rabbit, ECM Biosciences), 4E-BP1 (#9452, 1:1000, rabbit, Cell Signalling Technologies), p-4E-BP1$^{thr37/46}$ (#2855, 1:500, rabbit, Cell Signalling Technologies), rpS6 (#2217, 1:1000, rabbit, Cell Signalling Technologies) and p-rpS6$^{ser235/236}$ (#4856, 1:1000, rabbit, Cell Signalling Technologies). The membranes were washed thrice for 10 min in TBST and incubated with the corresponding secondary antibody (#5151, 1:10,000, anti-rabbit IgG Dylight 800 or #5470, 1:10,000, anti-mouse IgG DyLight 680; Cell Signalling Technologies) for 1 hour at room temperature. Following 2× 10-min washes in TBST and 1× 10-min wash in 1× phosphate buffered saline (PBS), 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, Licor Biosciences). All blots were normalized against total protein load using the Bio-Rad Image Lab software (v6.0).

2.12.4 Immunohistochemical staining

Muscle fibre type composition and cross-sectional area were assessed via immunohistochemistry (IHC), staining for myosin heavy chain (MHC I and IIx) and laminin. Eight-micron 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 (#16210072, Thermo Fisher Scientific) in 1× PBS for 1 hour at room temperature. The muscle sections were incubated for 1 hour at room temperature in a primary antibody cocktail containing antibodies specific to anti-MHCI (#BA-F8, 1:20, Developmental Studies Hybridoma Bank; DSHB), anti-MHCIIx (#6H1, 1:20, DSHB) and anti-laminin (#L9393, 1:100, Sigma Aldrich) in 10% goat serum/PBS. The muscle sections were washed thrice in PBS and then incubated in a secondary antibody cocktail containing goat anti-mouse IgG2b Alexa Fluor 647 (#A-21242, 1:500, Thermo Fisher Scientific), goat anti-mouse IgM Alexa Fluor 555 (#A-21426, 1:500, Thermo Fisher Scientific) and Goat anti-rabbit IgG Alexa Fluor 405 (#A-31556, 1:500, Thermo Fisher Scientific) in 10% goat serum/PBS for 1 hour at room temperature. The muscle sections were washed thrice in PBS and then mounted using Vectashield fluorescent mounting medium (#H-1900, Vector Laboratories, Abacus Dx). One image of the entire muscle section was visualised using a Fluoview fv0i confocal microscope (Olympus) at 10× magnification and analysed using Semi-automatic Muscle Analysis using

Segmentation of Histology (SMASH) software (MATLAB application, Mathworks, 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-micron muscle sections were thawed for 10 minutes before they were fixed in 4% paraformaldehyde (PFA) for 10 min. The muscle sections were washed thrice in PBS and permeabilised in 0.1% Triton-X 100 for 5 min. After permeabilization, the muscle sections were blocked for 1 hour at room temperature in 5% bovine serum albumin/PBS. Following blocking, the muscle sections were incubated in an antibody against the androgen receptor (#5153, 1:50, Cell Signalling Technologies) in 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 (#A-11008, 1:500, Thermo Fisher Scientific) and wheat germ agglutinin (#W32466, 1:1000, Thermo Fisher Scientific) in blocking buffer for 1 hour at room temperature. The cells were washed thrice in PBS and stained with 0.1 $\mu g \cdot mL^{-1}$ DAPI stain (#62248, 1:1000, Thermo Fisher Scientific) in PBS for 10 minutes and mounted using Vectashield fluorescent mounting medium (#H-1900, Abacus Dx). Ten images of each muscle section, with an average of 29.9 ± 8.6 fibres per image were obtained with dedicated software at 40× magnification (Eclipse Ti2, Nikon, Tokyo, Japan).

2.12.5 RNA extraction and quantification

Frozen skeletal muscle (~15 mg) was combined with lysis buffer (#1053393, Qiagen, Clayton, Australia) and homogenised with 650-800 mg silica beads for 2× 30 second homogenisation steps at 6500 rpm (MagNA lyser, Roche Diagnostics, North Ryde, Australia). RNA was extracted from the homogenised lysate using an Allprep DNA/RNA/miRNA Universal extraction kit (#80224, Qiagen) 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 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 $ng \cdot \mu L^{-1}$ ± 22.5 $ng \cdot \mu L^{-1}$ and the RIN average was 8.1 ± 0.8.

2.12.6 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). 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 transcript counts. Genes with an average across all samples of 10 reads per million (RPM) or less 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 included in this published article, its supplementary information files and publicly available repositories (GEO: link pending). The R code used for the analysis is available at https://github.com/DaniHiam/TESTO_RNAseq

2.13 In vitro experiments

2.13.1 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 program. To ensure a heterogenous sample, we selected participants from across a range of responses to the resistance training program. A third biopsy was collected at rest from this subset of 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 Hams F10 nutrient mixture (#11550043, Thermo Fisher Scientific). The muscle was washed thrice in ice-cold serum-free Hams F10 nutrient mixture. The tissue was minced manually and resuspended in serum-free Hams F10 media and centrifuged at room temperature for 5 min, 230 *g*. The supernatant was removed, and the resulting pellet was resuspended in warm (37°C) 0.05% Trypsin/EDTA (#25300062, Thermo Fisher Scientific) and dissociated thrice on an orbital shaker for 20 min at 37°C. After the final dissociation, 10% v/v horse serum (#16050122, Thermo Fisher Scientific) was added to the tissue slurry and cells were filtered through a 75 µm cell strainer. The resultant flow-through was centrifuged at room temperature for 5 min, 530 *g* and the resulting pellet resuspended in proliferation media containing Hams F10 nutrient mixture, 20% foetal bovine serum (FBS; #10099141, Thermo Fisher Scientific), 1% penicillin-streptomycin (#15140122, Thermo Fisher Scientific), 0.5% Amphotericin B (#15290018, Thermo Fisher Scientific) and 25 $\mu g \cdot mL^{-1}$ fibroblast growth factor (fGFb; #PHG0026, Thermo Fisher Scientific). The cells were plated in flasks pre-coated with an extracellular matrix (ECM) gel from Engelbreth-Holm-Swarm murine sarcoma (#E1270, Sigma-Aldrich). The cells were maintained in humidified air at 37°C, 5% CO_2. Proliferation media was changed every 48 h and the cells passaged once they had reached 70-80% confluence.

406 *2.13.2 Purification of cultured human myoblasts*

407 Once the cells had reached 70-80% confluence, myogenic satellite cells were purified using
408 Magnetic Activated Cell Sorting (MACS) with anti-CD56+ microbeads (#130-050-401, Miltenyi
409 Biotec, Bergisch Gladbach, Germany) as previously described (Agley *et al.*, 2013; McIlvenna *et*
410 *al.*, 2021).

411 *2.13.3 Differentiation of cultured human myoblasts*

412 Once the enriched myogenic cells reached 60-70% confluence, differentiation was induced by
413 replacing the proliferation medium with Dulbecco's Modified Eagle's Medium (DMEM) without
414 phenol red (#21063029, Thermo Fisher Scientific) supplemented with 2% horse serum
415 (#16050122, Thermo Fisher Scientific) and 1% penicillin-streptomycin (#15140122, Thermo
416 Fisher Scientific). DMEM without phenol red was used to eliminate the oestrogenic effects of
417 phenol red (Estrada *et al.*, 2003; Wannenes *et al.*, 2008; Eriksen *et al.*, 2014). Cells were
418 differentiated either in the presence of 100 nM testosterone dissolved in ethanol (#DRE-
419 C17322500, Novachem, Heidelberg West, Australia) (henceforth referred to as testosterone
420 treated, or TT) or the equivalent volume (2 $\mu L \cdot well^{-1}$) of the vehicle control, ethanol (henceforth
421 referred to as control, or CON).

422 The cells were harvested for protein extraction at baseline (Day 0) and after 1 (D1), 4 (D4) and 7
423 days (D7) of differentiation, using 150 $\mu L \cdot well^{-1}$ 1× RIPA lysis buffer (#J62524-AE, Thermo Fisher
424 Scientific) containing 10 $\mu L \cdot mL^{-1}$ phosphatase inhibitor and 1 $\mu L \cdot mL^{-1}$ protease inhibitor cocktail
425 (#78440, Thermo Fisher Scientific). The homogenised protein lysates were then gently spun at
426 4°C for 1 hour. Samples were centrifuged at 13 000 *g* for 15 min at 4°C and the protein
427 concentration of each sample determined via Pierce Bicinchoninic Acid (BCA) assay (#23225,
428 Thermo Fisher Scientific) according to manufacturer's instructions. Western blots were
429 completed under the same conditions as described above on the resultant cell protein lysate,
430 loading 10 µg total $protein \cdot well^{-1}$.

431 *2.13.4 Immunohistochemical analysis of AR location*

432 In addition to western blot analyses, we also investigated the effect of testosterone treatment on
433 the localisation of the AR in myocytes. At baseline, and after 1, 4 and 7 days of differentiation,
434 human primary myocytes were washed thrice for 5 min in PBS. The myocytes were fixed with 2%
435 paraformaldehyde (PFA)/PBS for 10 min and washed thrice for 5 min in PBS. The fixed cells were
436 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 hour at room temperature with the primary antibody against total androgen receptor (#5153,1:50 in blocking buffer, rabbit, Cell Signalling Technologies). Following this, the cells were incubated with AlexaFluor 488 goat anti-rabbit IgG (#A-11008, 1:5000, Thermo Fisher Scientific) and AlexaFluor 647 phalloidin (#A22287, 1:200, Thermo Fisher Scientific) in 1% BSA/PBS for 1 hour at room temperature. The cells were washed thrice in PBS and stained with 0.1 $\mu g \cdot mL^{-1}$ DAPI (#62248, 1:1000, Thermo Fisher Scientific) stain in PBS for 10 minutes. Cell images were obtained with dedicated software at 100× magnification *(Eclipse Ti2, Nikon, Tokyo, Japan)*.

2.13.5 Quantification of androgen receptor intensity

Quantification of androgen receptor 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 grayscale 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 nuclei positive for AR expression 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 3x 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 2 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 1 field per well from 6 wells were imported into CellProfiler and split into individual grayscale 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 androgen receptor 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 androgen receptor protein through a manual review of 20% of the total images, performed by 2 independent analysts.

2.14 Statistical analysis

The statistical analyses for this study were performed using GraphPad Prism version 8 *(GraphPad Software, La Jolla, CA)* and R software version 4.0.2 using the packages *lmerTest* (Kuznetsova *et al.*, 2017), *tidyverse* (Hadley Wickham *et al.*, 2019), *car* (Fox and Weisberg, 2019), *AICcmodavg* (Mazerolle, 2020).

Two-tailed, paired t-tests in GraphPad were used to assess the effect of a 12-week resistance training program on the changes to participant anthropometric data, the protein expression of all measured proteins and thigh muscle size, strength and power. Leg press strength and muscle power were expressed as values relative to the individual's total body lean mass (kg) and the changes to muscle size, strength and power were expressed as delta percent change (Δ%). 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-AR^{ser213}= p-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 average of the pre- and post-training values was used for all post-training (delta change) linear models.

One-way, repeated measures analyses of variance (ANOVA) were used in GraphPad to assess the effect of 12 weeks of resistance training on the concentrations of testosterone, SHBG, DHT, DHEA, E2 and P 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 or p-AR, the nAR/AR ratio, or the proportion of AR+ nuclei. The model was of the form: $outcome = independent\ variable + covariate\ (if\ applicable)$. Before further analyses, the normality of all variables was assessed, and variables were log-transformed if necessary.

Possible covariates included age, BMI, E2, P, luteinising hormone, follicle stimulating hormone, chronotype, hormonal contraceptive use, average protein intake, average daily physical activity and menstrual phase. Before fitting the linear models, Akaike Information Criterion (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 3 set. 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 ~ ID + timepoint. ID was used to account for repeated measurements. ChIP-X enrichment 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 significant genes and transcription factors significant with an FDR adjusted p value <0.05. The following packages were also used in our analysis; *tidyverse* (Wickham *et al.*, 2019), *superheat* (Barter & Yu, 2018), *biomaRt* (Durinck *et al.*, 2009).

Graphpad software was used to performed 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 androgen receptor and markers of protein synthesis.

All values are presented and mean ± SD, unless otherwise stated. Significance for all statistical tests was set at $p<0.05$.

3. Results

Of the 35 females enrolled in the study, 27 females completed the 12-week resistance training program. Due to the SARS-CoV-2-related lockdowns experienced throughout Victoria, Australia in 2020 and 2021 (Dunstan, 2021), a sub-cohort of participants (n=11 of the 27 having completed the study) undertook a portion of their 36 training sessions at home (range: 2 to 6 sessions, average 4 sessions) delivered via videoconferencing. There were no differences in age, height, weight, BMI, ratio of hormonal contraceptive 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 1). There was no between-group difference in the trajectory of working weight progression for any exercise (Supplementary Figure 2). There was also no between-group difference in training-induced changes in working weight for any exercise, thigh muscle cross sectional area, power (Supplementary Figure 3), or pre- or post-training hormone levels (Supplementary Figure 4). The 2 training regimes were therefore considered equivalent and the results from both cohorts were pooled for all further analyses.

Table 1. *Baseline anthropometric and strength data separated for all participants that completed the gym-based training program (n=16) or participants who completed the blended gym- and home-based training program (n=11) and for all participants combined (n=27).*

	Participants undergoing normal training (*n*=16)	**Participants spending 2-6 sessions at home (*n*=11)**	**All participants (*n*=27)**
Age (years)	24.1 ± 5.0	21.9 ± 3.9 (*p* = 0.24)	23.5 ± 4.8
Height (m)	1.7 ± 0.1	1.6 ± 0.1 (*p* = 0.62)	1.7 ± 0.1
Weight (kg)	65.8 ± 13.1	66.3 ± 11.1 (*p* = 0.92)	65.9 ± 12.4
BMI (kg·m^{-2})	23.3 ± 3.7	24.5 ± 4.1 (*p* = 0.42)	23.6 ± 3.8
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* = 0.23)	2.0 ± 0.6
Calculated squat 1RM (AU)	104.7 ± 31.4	105.4 ± 24.3 (*p* = 0.97)	104.8 ± 30.0
Calculated leg press 1RM (AU)	179.8 ± 42.3	180.1 ± 27.5 (*p* = 0.89)	179.3 ± 39.1
Calculated leg extension 1RM (AU)	52.8 ± 12.2	45.9 ± 13.0 (*p* = 0.15)	51.0 ± 12.9
Calculated hamstring curl 1RM (AU)	35.9 ± 6.4	36.9 ± 6.9 (*p* = 0.74)	36.1 ± 6.7

Calculated seated row 1RM (kg)	40.4 ± 7.5	38.4 ± 7.3 (p = 0.42)	39.7 ± 7.5
Calculated shoulder press 1RM (kg)	9.4 ± 2.9	8.7 ± 2.4 (p = 0.75)	9.3 ± 2.8
Calculated biceps curl 1RM (kg)	8.2 ± 1.4	8.4 ± 1.4 (p = 0.97)	8.2 ± 1.4

Values are mean ± standard deviation. p-values shown in brackets are for pairwise comparisons to participants having completed normal training using unpaired t-tests.

3.1 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).

Muscle strength (measured via leg press 1RM) increased by 27.3% (p<0.001), thigh muscle cross-sectional area (CSA; measured via pQCT) by 5.9% (p<0.001) and muscle power (measured via vertical jump) by 13.0% (p<0.05) (Figure 1A-C).

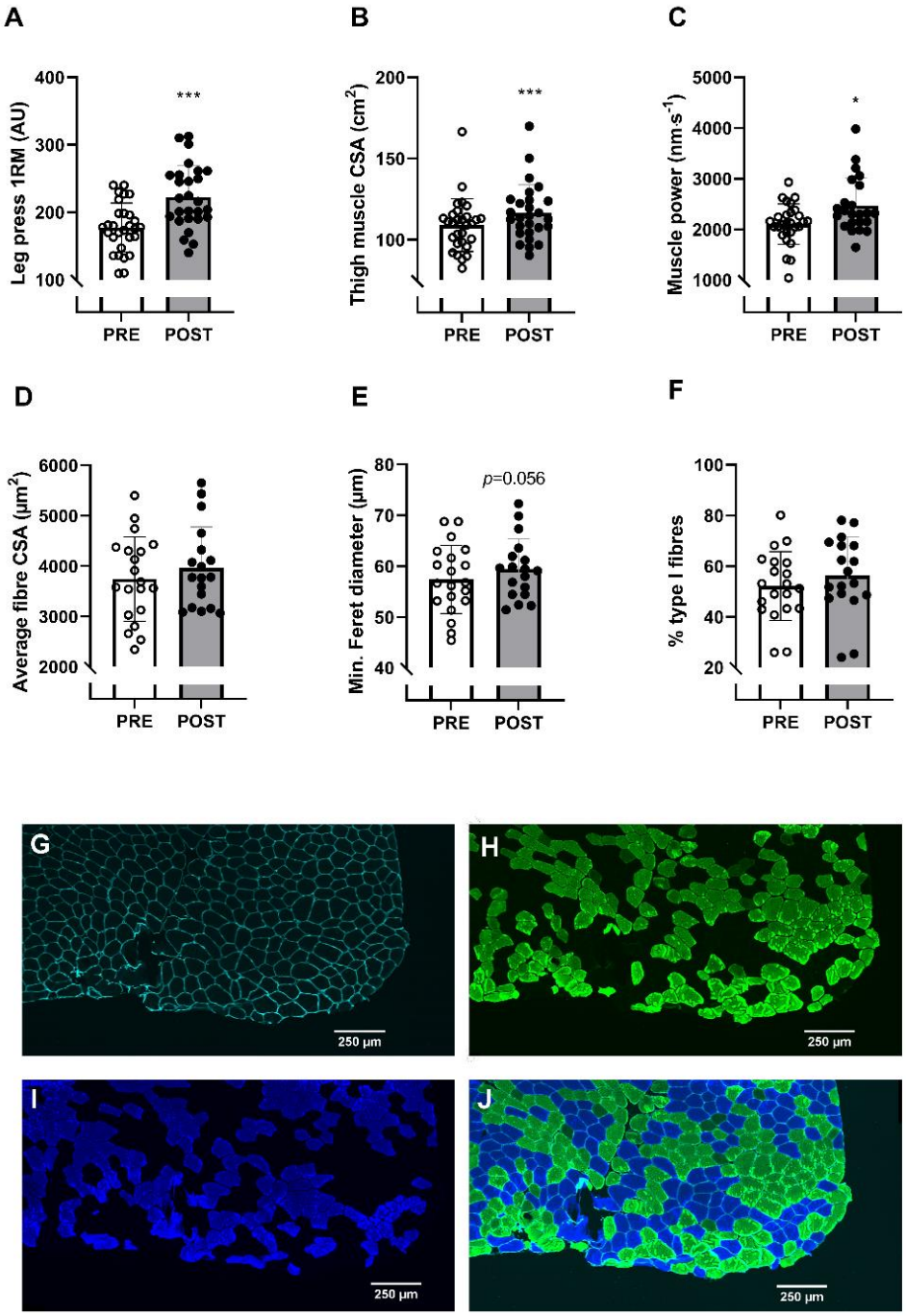

Figure 1. *A) Leg press 1RM, B) (B) thigh muscle cross sectional area (CSA) (C) muscle power D) mixed muscle myofibre CSA, E) minimum Feret diameter and F) percentage of type I fibres in untrained, pre-menopausal females before and ager 12 weeks of resistance training (n=27). G) Representative images showing the laminin-stained sarcolemma of a muscle section, H) type I fibres, I) type II fibres and J) a composite image of both. Scale for representative images is 1.24 µm·pixel^{-1} for all images shown. n=753.8 ± 370.6 fibres per slice. White scale bar represents 250 µm. Pre-training (PRE) values are indicated by clear bars and post-training (POST) values are indicated by dark bars. *indicates p<0.05, ***indicates p<0.001. Data were analysed using two-tailed, paired t-tests. Values are represented as mean ± SD.*

3.2 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). Similarly, the minimum Feret diameter, which is indicative of the circularity of the fibre and robust against experimental errors such as the orientation of the fibre upon cutting and therefore a more reliable indication of fibre CSA, increased by 4% but did not reach statistical significance (p=0.056). The percentage of type I and type II fibres did not change with training (Figure 1D-F) (p=0.725). Representative images are shown in Figure 1G-J.

3.3 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 androgen receptor (Figure 2A), or its phosphorylation status at either serine residue 213 (p-AR^{ser213}, p=0.730; Figure 2B) or serine residue 650 (p-AR^{ser650}, p=0.750; Figure 2C). *AR* mRNA expression also did not change with 12 weeks of resistance training (FDR=0.956; Figure 2D). 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; Figure 2E) and the percentage of nuclei that were AR positive (%AR+; Figure 2F).

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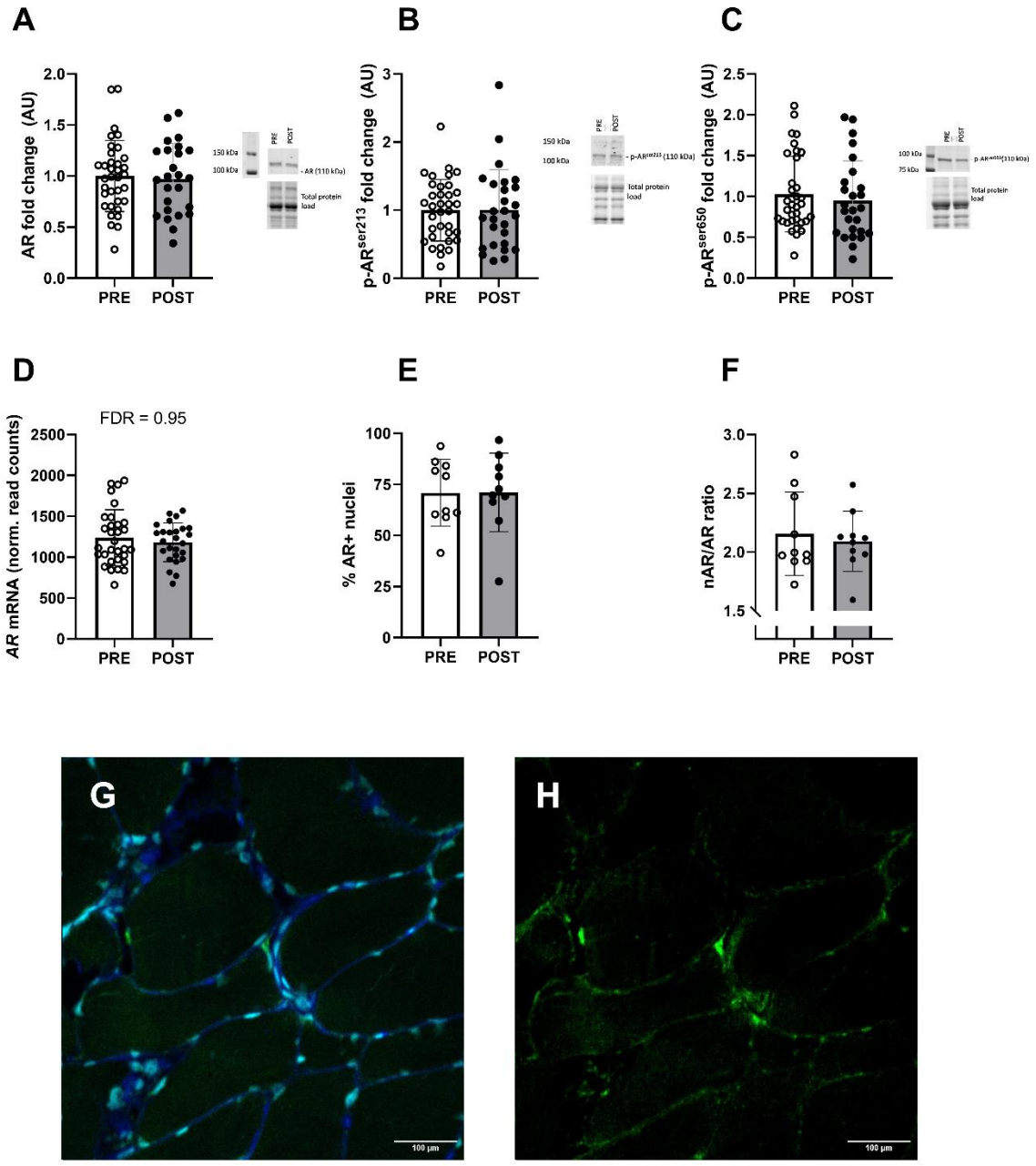

577 ***Figure 2***. *The protein content of A) total AR protein, B) p-AR^{ser213}, C) p-AR^{ser650}, D) AR mRNA expression*
578 *(n=27), E) the percentage of AR+ nuclei or F) the nAR/AR ratio (n=10 participants) before and after 12 weeks*
579 *of resistance training in previously untrained, pre-menopausal females. Western blots for each protein are*
580 *resented beside the corresponding graph. Pre-training (PRE) values are indicated by clear bars and post-*
581 *training (POST) values are indicated by dark bars. G) Representative composite image of a muscle section*
582 *stained with DAPI (cyan; stains the nucleus), wheat germ agglutinin (blue; stains the sarcolemma) and α-*
583 *AR (green) at 40x magnification. H) Representative image of α-AR (green) stain at 40x magnification. Scale*
584 *for representative images is 0.62 µm·pixel^{-1}. White scale bar represents 100 µm. n=5-10 images per*

section, average 29.9 ± 8.6 fibres per image. Data were analysed via two-way, paired t-tests. Values are represented as mean ± SD.

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

Twelve weeks of resistance training increased the levels of muscle protein synthesis signalling molecules total Akt protein by 13% (p<0.05) (Figure 3A). 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) (Supplementary Figure 5).

mRNA expression of *RPS6KC1* mRNA increased by 11% (FDR<0.01; Figure 3B). 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) (Supplementary Figure 5).

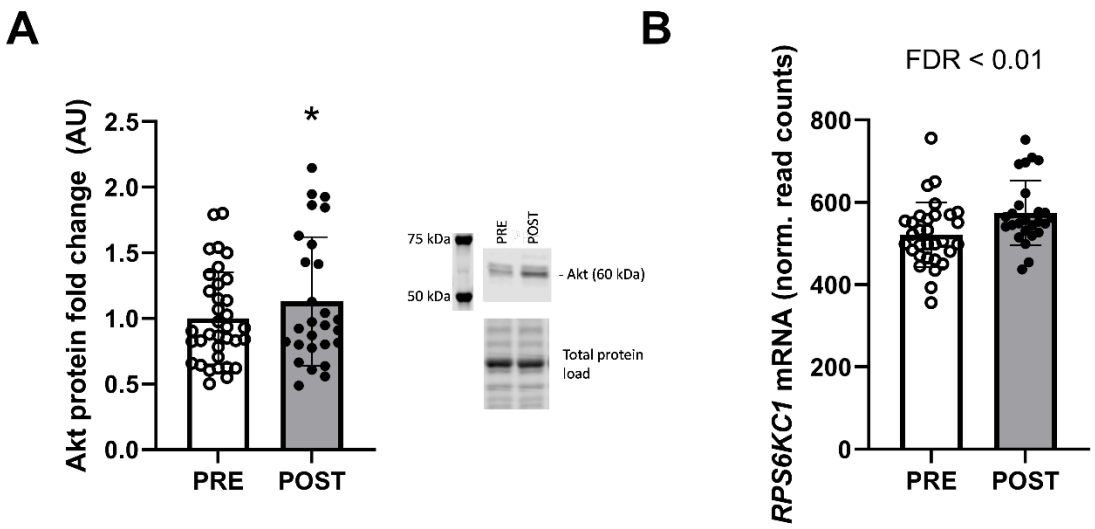

Figure 3. *(A) total Akt protein and (B) RPS6Kc1 mRNA before and after 12 weeks of resistance training in pre-menopausal, previously untrained females (n=27). Representative Western blots for total Akt are presented beside the corresponding graph. Pre-training (PRE) values are indicated by clear bars and post-training (POST) values are indicated by dark bars. Values are represented as mean ± SD.*

3.5 *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 (Figure 4A-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 (Supplementary Figure 6). 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.

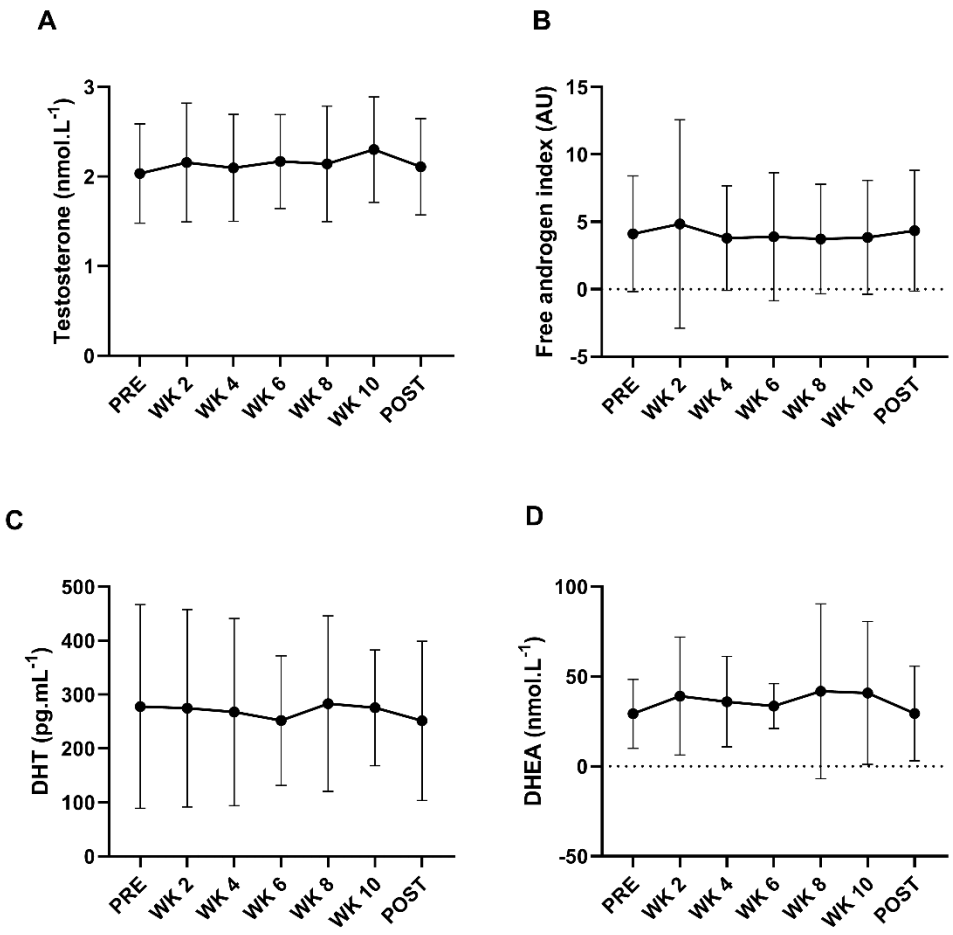

Figure 4*. Twelve weeks of resistance training did not affect concentrations of (A) total testosterone (nmol·L^{-1}), (B) the free androgen index (FAI; AU), (C) dihydrotestosterone (DHT; pg·mL^{-1}) or (D)*

dehydroepiandrosterone (DHEA; nmol·L^{-1}) in plasma of previously untrained, pre-menopausal females (n=27). Data were analysed using a one-way ANOVA. Values are represented as mean ± SD.

3.6 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) (Figure 5A). 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 (Figure 5B). The AR was ranked 284 of 1632 TFs ranked, indicating that the androgen receptor and its binding to androgen response elements (ARE) 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.

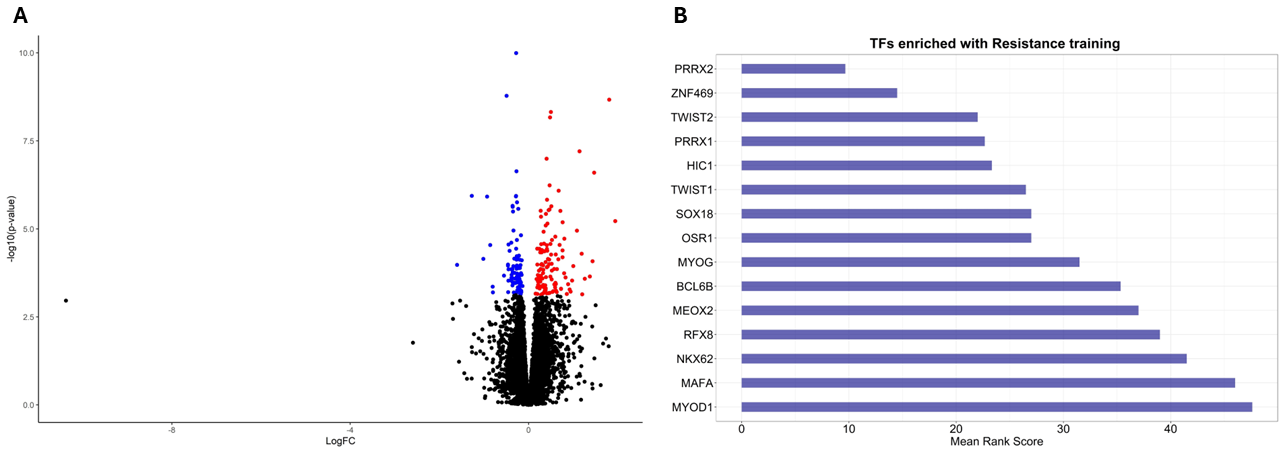

***Figure 5**. (A) Volcano Plot displaying 122 up-regulated and 92 down-regulated transcripts in response to 12 weeks of resistance training in pre-menopausal females (n=35 pre-training, 27 post training). Each point represents a transcript. Red points indicate an increase in mRNA expression following exercise. Blue points indicate a decrease in mRNA expression following exercise. 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.*

3.7 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 2). These results were replicated with testosterone precursor and metabolite DHEA and DHT, respectively (all p-values >0.05) (Supplementary 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 2). The AUC of the FAI across 12-weeks of resistance training was positively associated to the changes in muscle strength (β=0.05, SE=0.02, p=0.044) that occurred with 12 weeks of resistance training (Figure 6A).

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).

Table 2. *Linear mixed models of the association between total testosterone, the free androgen index, and markers of AR signalling and muscle size, strength and power before and after 12 weeks of resistance training in pre-menopausal females. n=35 pre-training, 27 change with training.*

PRE-TRAINING					Δ% CHANGE WITH TRAINING				
Variable	β	SE	*p*	Model adjusted for	Variable	β	SE	*p*	Model adjusted for
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*	-
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

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*	-

665 ** indicates p<0.05. CSA: cross sectional area, E2: oestradiol, AUC: area under the curve, AR: androgen receptor,*

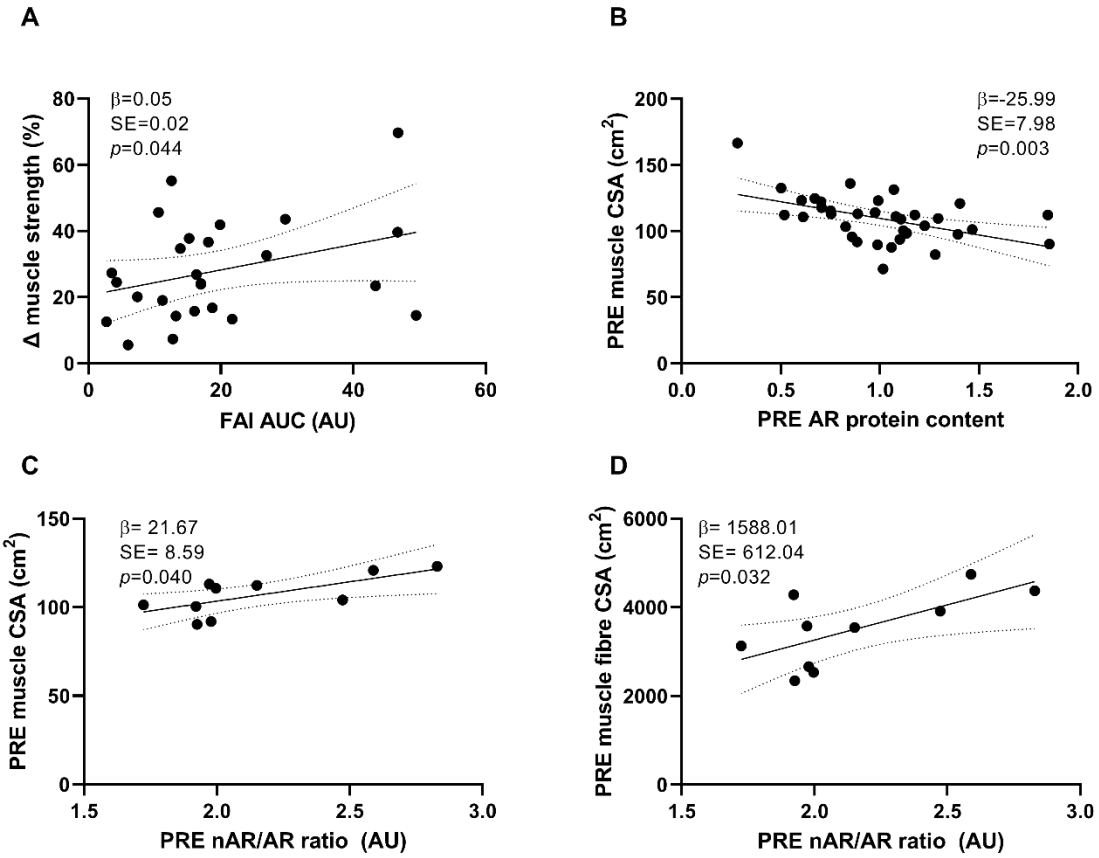

Figure 6. *Linear associations between A) the free androgen index area under the curve (FAI AUC; AU) and the changes in muscle strength, (B) baseline AR protein content (AU) and baseline thigh muscle CSA (cm^2) (C) pre-training nAR/AR ratio (AU) and pre-training muscle CSA (cm^2) and (D) pre-training nAR/AR ratio (AU) and pre-training mixed muscle fibre CSA (cm^2) in pre-menopausal females. Dashed lines represent 95% confidence intervals.*

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

In young males, the protein content of the androgen receptor (AR) is positively associated with resistance training-induced hypertrophy (Morton *et al.*, 2018). We therefore tested the association between the total or phospho-protein content of the AR and muscle CSA, strength, power and fibre CSA prior to, or in response to a 12-week resistance training program. Total androgen receptor content was negatively associated with thigh muscle CSA pre-training (β=-25.99, SE=7.98, *p*=0.003; Table 2, Figure 6B), but this association was not maintained after resistance training. There was no significant association between the phospho-AR content and any outcomes as any time point (Supplementary Table 3).

3.9 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 program (Table 2). 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; Figure 6C) and muscle fibre CSA (β=1588.01, SE=612.04, p=0.032; Figure 6D), 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 2). There were trends (*p=0.05-0.08*) for the percentage of nuclei that were AR+ to be positively associated with pre-training muscle CSA, strength and myofibre CSA, but these did not reach statistical significance (Supplementary Table 3).

3.10 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 6 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; Figure 7A-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; Figure 7C.) However, 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; Supplementary Figure 7). Instead, immunohistochemical staining showed that, in myocytes treated with a vehicle control, the AR is distributed throughout the cytosol of the cell with no or little nuclear localisation (Figure 7D, upper panel). After 24 hours of 100 nM testosterone treatment, there was a striking translocation of the AR to the nucleus of myoblasts (Figure 7D, lower panel) 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 (Figure 7E).

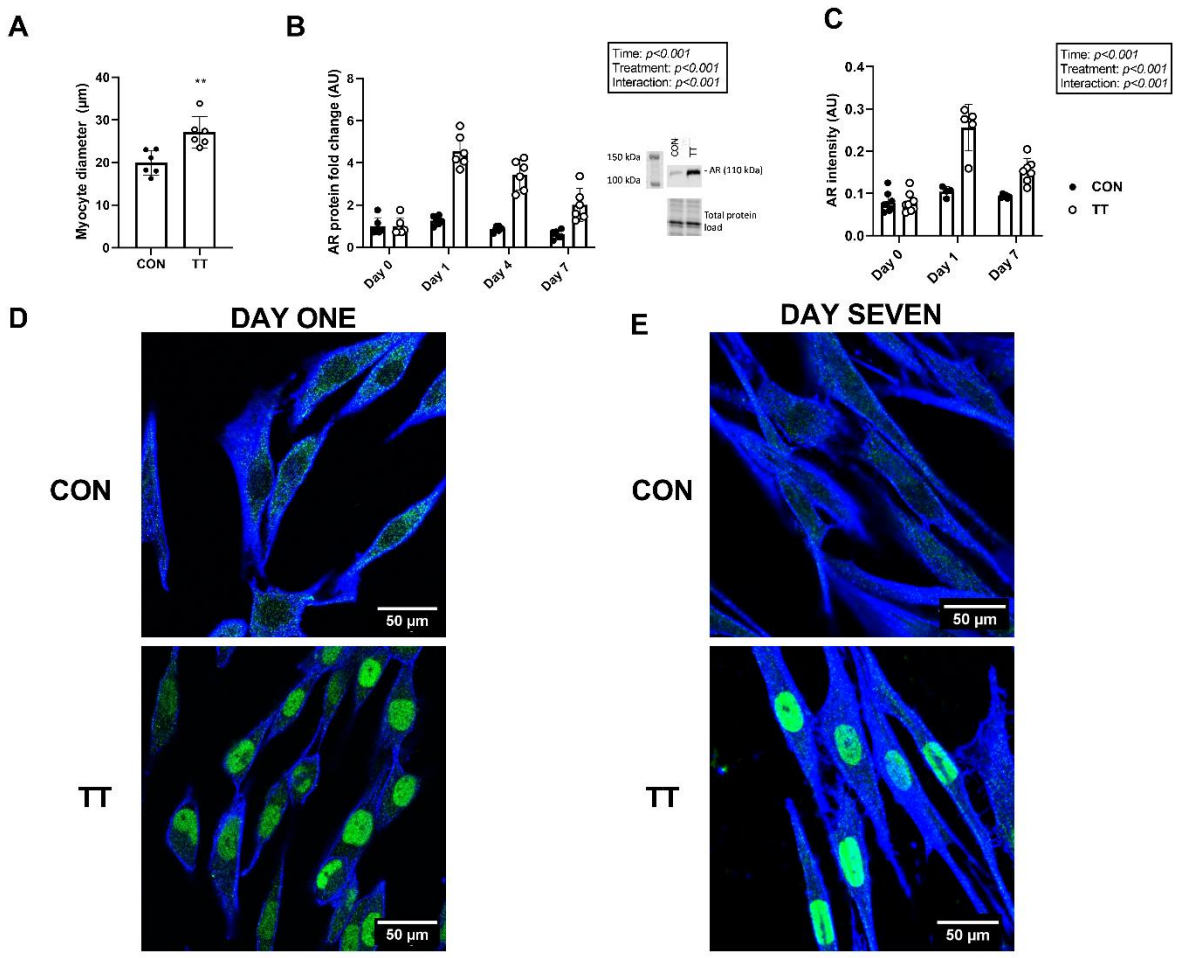

Figure 7*. Testosterone treatment over 7 days of differentiation increased (A) myotube diameter, (B) AR protein content and (C) AR intensity relative to the proportion of the visual field occupied by myocytes. AR cellular localisation in primary muscle cell lines treated with vehicle (CON) or 100 nM testosterone (TT) after D) 1 day or E) 7 days 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. Data were analysed using two-tailed, paired t-tests and two-way ANOVA. Values are represented mean ± SD. **indicates p<0.01.*

4. Discussion

We showed that 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 androgen response element, does not play a significant role in determining the muscle transcriptomic profile pre- or post-resistance training. In contrast, the bioavailable fraction of testosterone and the nAR/AR ratio were positively associated with muscle mass and strength at baseline, but not

with resistance-training induced changes in muscle mass or strength. 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 (Gower & Nyman, 2000; Carmina *et al.*, 2009; van Geel *et al.*, 2009; Pöllänen *et al.*, 2011; Rariy *et al.*, 2011; Kogure *et al.*, 2015). 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 training in males (Morton et al., 2016; Morton et al., 2018). These results suggest that the association between testosterone and skeletal muscle differs between endogenous and exogenous testosterone. We posit that there are only positive associations between total testosterone and muscle mass and function when homeostasis is perturbed and concentrations are manipulated pharmacologically to above (Ferrando *et al.*, 1998; Sheffield-Moore *et al.*, 1999; Bhasin *et al.*, 2001; Sinha-Hikim *et al.*, 2004; Hirschberg *et al.*, 2020) or below (Mauras *et al.*, 1998; Overkamp *et al.*, 2023) physiological concentrations in both males and females.

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 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 were small (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 wildtype littermates (MacLean *et al.*, 2008). Conversely, female ARKO mice did not display any differences in muscle mass or strength compared to their wildtype 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 program (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 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 hours 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

791 pathways such as the Akt/mTOR or MAPK as previously suggested (Wu *et al.*, 2010; Basualto-
792 Alarcón *et al.*, 2013; White *et al.*, 2013).

793 No markers of protein synthesis or degradation were associated with total or bioavailable
794 testosterone in our human cohort. This is mirrored by our findings that testosterone treatment
795 did not promote Akt/mTOR or MAPK signalling in myocytes taken from female donors, despite
796 significant increases in myotube diameter with testosterone treatment. In support of this,
797 exogenous testosterone administration did not change molecular regulators of muscle mass and
798 mitochondrial biosynthesis, including markers of mTOR signalling from resting biopsies in males
799 (*n*=50) (Howard *et al.*, 2020) and females (*n*=48) (Horwath *et al.*, 2022) *in vivo*, or female primary
800 myocytes *in vitro* (Pataky *et al.*, 2023). Instead, testosterone administration to primary myotubes
801 from female donors induced changes of proteins within the sarcoplasmic compartment,
802 including myosin and titin, both of which play important roles in the contractile apparatus and
803 muscle hypertrophy (Pataky *et al.*, 2023). This suggests that, in contrast to *in vitro* data from rat
804 (Wu *et al.*, 2010; White *et al.*, 2013) and murine (Basualto-Alarcón *et al.*, 2013) myocytes, the
805 primary mechanism of action of testosterone in humans may not be through the upregulation of
806 the mTOR pathway. Instead, the changes in lean mass seen with exogenous testosterone
807 administration in males (Bhasin *et al.*, 2001; Howard *et al.*, 2020) and females (Horwath *et al.*,
808 2022) may stem from a net positive protein turnover in favour of protein accretion driven by an
809 increase in the genomic signalling of the AR rather than through activation of the Akt/mTOR non-
810 genomic signalling pathways. This genomic signalling may lead to an increase in transcription of
811 target genes and eventually in translational capacity, as evidenced by increases in total ribosome
812 number (Mobley *et al.*, 2018) and muscle RNA content (Howard *et al.*, 2020).

813 *4.1 Limitations*

814 While this study included 4 participants (~15%) who had been diagnosed with polycystic ovary
815 syndrome (PCOS), there was only 1 participant who consistently had total testosterone
816 concentrations above the typical female reference range (testosterone levels >2.5 $nmol \cdot L^{-1}$
817 (Burger, 2002)). This limited range of testosterone concentrations restrict the generalisability of
818 these results. Our previous research (Alexander *et al.*, 2021) suggests that the association
819 between the FAI and lean mass is not linear but quadratic in nature, where the association
820 plateaus and eventually becomes negative with increasing testosterone concentrations.
821 Including females with a wider range of testosterone levels, including hyperandrogenic females
822 and individuals with DSD, would increase the generalisability of our findings and allow to validate

the association between testosterone and muscle across a larger spectrum of androgen concentrations.

4.2 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 plays a significant role in the maintenance of muscle mass and strength in pre-menopausal females. This suggests that findings from studies demonstrating a large anabolic effect of testosterone administration on muscle are not generalisable to the association between endogenous testosterone concentrations and muscle mass and function and should therefore be interpreted with caution.

Availability of data and materials

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: link pending). The R code used for the analysis is available at https://github.com/DaniHiam/TESTO_RNAseq

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Authors' contributions

SEA was involved in the design and conception of the study, and performed data collection, laboratory and scientific analyses and preparation the manuscript. OEK was involved in the design of the training program and the delivery of the training program. RMW was involved with

laboratory and statistical analyses. BG and KF were involved with data collection. PJ performed body composition scans. PDG was involved with laboratory analyses and the design of the *in vitro* experiments. AG performed all muscle biopsies. SeL was involved in the design and conception of the studies and preparation of the manuscript. DH performed statistical and transcriptomic analyses. GDW and BA were involved in the design and conception of the study. All authors contributed to the editing and reviewing of the manuscript. All authors approved the manuscript in its final form.

Competing interests

The authors declare no competing interests.

References

Abadie BR & Wentworth MC. (2000). Prediction of one repetition maximal strength from a 5-10 repetition submaximal strength test in college-aged females. *Journal of Exercise Physiology Online* **3**.

Agley CC, Rowlerson AM, Velloso CP, Lazarus NR & Harridge SDR. (2013). Human skeletal muscle fibroblasts, but not myogenic cells, readily undergo adipogenic differentiation. *Journal of Cell Science* **126,** 5610-5625.

Ahtiainen JP, Hulmi JJ, Kraemer WJ, Lehti M, Nyman K, Selänne H, Alen M, Pakarinen A, Komulainen J, Kovanen V, Mero AA & Häkkinen K. (2011). Heavy resistance exercise training and skeletal muscle androgen receptor expression in younger and older men. *Steroids* **76,** 183-192.

Alexander SE, Abbott G, Aisbett B, Wadley GD, Hnatiuk JA & Lamon S. (2021). Total testosterone is not associated with lean mass or handgrip strength in pre-menopausal females. *Scientific Reports* **11,** 10226.

Alexander SE, Pollock AC & Lamon S. (2022). The effect of sex hormones on skeletal muscle adaptation in females. *Eur J Sport Sci* **22,** 1035-1045.

Exercise and Sports Science Australia. (2019). Adult pre-exercise screening system.

Barbieri D, Zaccagni L, Babić V, Rakovac M, Mišigoj-Duraković M & Gualdi-Russo E. (2017). Body composition and size in sprint athletes. *J Sports Med Phys Fitness* **57,** 1142-1146.

Barter RL & Yu B. (2018). Superheat: An R package for creating beautiful and extendable heatmaps for visualizing complex data. *Journal of Computational and Graphical Statistics* **27,** 910-922.

Basualto-Alarcón C, Jorquera G, Altamirano F, Jaimovich E & Estrada M. (2013). Testosterone signals through mTOR and androgen receptor to induce muscle hypertrophy. *Medicine and science in sports and exercise* **45,** 1712-1720.

Bergstrom J. (1962). Muscle electrolytes in man. *Scandinavian Journal of Clinical and Laboratory Investigation* **14,** 511-513.

Berns EM, de Boer W & Mulder E. (1986). Androgen-dependent growth regulation of and release of specific protein(s) by the androgen receptor containing human prostate tumor cell line LNCaP. *Prostate* **9,** 247-259.

Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R & Storer TW. (2001). Testosterone dose-response relationships in healthy young men. *American Journal of Physiology-Endocrinology and Metabolism* **281,** E1172-E1181.

Borg G. (1982a). A category scale with ratio properties for intermodal and interindividual comparisons. *Psychophys Judg Proc Percept* 25-34.

Borg GA. (1982b). Psychophysical bases of perceived exertion. *J Med Sci Sports Exerc* **14,** 377-381.

Burger HG. (2002). Androgen production in women. *Fertility and Sterility* **77,** 3-5.

Carmina E, Guastella E, Longo RA, Rini GB & Lobo RA. (2009). Correlates of increased lean muscle mass in women with polycystic ovary syndrome. *European journal of endocrinology* **161,** 583-589.

Informed Choice (2021). Informed Choice Certification Process. https://choice.wetestyoutrust.com/about/certification-process

Colenso-Semple LM, D'Souza AC, Elliott-Sale KJ & Phillips SM. (2023). Current evidence shows no influence of women's menstrual cycle phase on acute strength performance or adaptations to resistance exercise training. *Front Sports Act Living* **5,** 1054542.

de Launoit Y, Veilleux R, Dufour M, Simard J & Labrie F. (1991). Characteristics of the biphasic action of androgens and of the potent antiproliferative effects of the new pure antiestrogen EM-139 on cell cycle kinetic parameters in LNCaP human prostatic cancer cells. *Cancer Res* **51,** 5165-5170.

929 Dent JR, Fletcher DK & McGuigan MR. (2012). Evidence for a Non-Genomic Action of Testosterone in
930 Skeletal Muscle Which may Improve Athletic Performance: Implications for the Female
931 Athlete. *J Sports Sci Med* **11,** 363-370.

932

933 Dunstan J. (2021). Melbourne marks 200 days of COVID-19 lockdowns since the pandemic began. In
934 *ABC news*. Melbourne.

935

936 Durinck S, Spellman PT, Birney E & Huber W. (2009). Mapping identifiers for the integration of genomic
937 datasets with the R/Bioconductor package biomaRt. *Nature protocols* **4,** 1184-1191.

938

939 Elliott-Sale KJ, Minahan CL, de Jonge X, Ackerman KE, Sipilä S, Constantini NW, Lebrun CM & Hackney
940 AC. (2021). Methodological Considerations for Studies in Sport and Exercise Science with
941 Women as Participants: A Working Guide for Standards of Practice for Research on Women.
942 *Sports Med* **51,** 843-861.

943

944 Eriksen MB, Glintborg D, Nielsen MF, Jakobsen MA, Brusgaard K, Tan Q & Gaster M. (2014).
945 Testosterone treatment increases androgen receptor and aromatase gene expression in
946 myotubes from patients with PCOS and controls, but does not induce insulin resistance.
947 *Biochemical and biophysical research communications* **451,** 622-626.

948

949 Estrada M, Espinosa A, Müller M & Jaimovich E. (2003). Testosterone stimulates intracellular calcium
950 release and mitogen-activated protein kinases via a G protein-coupled receptor in skeletal
951 muscle cells. *Endocrinology* **144,** 3586-3597.

952

953 Ferrando AA, Tipton KD, Doyle D, Phillips SM, Cortiella J & Wolfe RR. (1998). Testosterone injection
954 stimulates net protein synthesis but not tissue amino acid transport. *Am J Physiol* **275,** E864-
955 871.

956

957 Gower BA & Nyman L. (2000). Associations among oral estrogen use, free testosterone concentration,
958 and lean body mass among postmenopausal women. *J Clin Endocrinol Metab* **85,** 4476-4480.

959

960 Hadley Wickham, Mara Averick, Jennifer Bryan, Winston Chang, Lucy D'Agostino McGowan, Romain
961 François, Garrett Grolemund, Alex Hayes, Lionel Henry, Jim Hester, Max Kuhn, Thomas Lin
962 Pedersen, Evan Miller, Stephan Milton Bache, Kirill Müller, Jeroen Ooms, David Robinson,
963 Dana Paige Seidel, Vitalie Spinu, Kohske Takahashi, Davis Vaughan, Claus Wilke, Kara Woo &
964 Yutani H. (2019). Welcome to the Tidyverse. *Journal of Open Sources Software* **4,** 1686.

Häkkinen K, Pakarinen A & Kallinen M. (1992). Neuromuscular adaptations and serum hormones in women during short-term intensive strength training. *European Journal of Applied Physiology and Occupational Physiology* **64,** 106-111.

Hatt AA, Kamal M, Mikhail AI, Fortino SA, Wageh M, Kumbhare D & Parise G. (2024). Nuclear-localized androgen receptor content following resistance exercise training is associated with hypertrophy in males but not females. *The FASEB Journal* **38,** e23403.

Hirschberg AL, Elings Knutsson J, Helge T, Godhe M, Ekblom M, Bermon S & Ekblom B. (2020). Effects of moderately increased testosterone concentration on physical performance in young women: a double blind, randomised, placebo controlled study. *British journal of sports medicine* **54,** 599-604.

Horne JA & Östberg O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *International Journal of Chronobiology* **4,** 97-110.

Horwath O, Moberg M, Hirschberg AL, Ekblom B & Apró W. (2022). Molecular Regulators of Muscle Mass and Mitochondrial Remodeling Are Not Influenced by Testosterone Administration in Young Women. *Front Endocrinol (Lausanne)* **13,** 874748.

Howard EE, Margolis LM, Berryman CE, Lieberman HR, Karl JP, Young AJ, Montano MA, Evans WJ, Rodriguez NR, Johannsen NM, Gadde KM, Harris MN, Rood JC & Pasiakos SM. (2020). Testosterone supplementation upregulates androgen receptor expression and translational capacity during severe energy deficit. *Am J Physiol Endocrinol Metab* **319,** E678-e688.

Huang G, Basaria S, Travison TG, Ho MH, Davda M, Mazer NA, Miciek R, Knapp PE, Zhang A, Collins L, Ursino M, Appleman E, Dzekov C, Stroh H, Ouellette M, Rundell T, Baby M, Bhatia NN, Khorram O, Friedman T, Storer TW & Bhasin S. (2014). Testosterone dose-response relationships in hysterectomized women with or without oophorectomy: effects on sexual function, body composition, muscle performance and physical function in a randomized trial. *Menopause* **21,** 612-623.

Jay SM, Dawson D & Lamond N. (2006). Train drivers' sleep quality and quantity during extended relay operations. *Chrono Biol Int* **23,** 1241-1252.

1001 Jin HJ, Kim J & Yu J. (2013). Androgen receptor genomic regulation. *Transl Androl Urol* **2,** 157-177.

1002

1003 Keenan AB, Torre D, Lachmann A, Leong AK, Wojciechowicz ML, Utti V, Jagodnik KM, Kropiwnicki E,
1004 Wang Z & Ma'ayan A. (2019). ChEA3: transcription factor enrichment analysis by orthogonal
1005 omics integration. *Nucleic acids research* **47,** W212-w224.

1006

1007 Knowles OE, Aisbett B, Main LC, Drinkwater EJ, Orellana L & Lamon S. (2019). Resistance Training and
1008 Skeletal Muscle Protein Metabolism in Eumenorrheic Females: Implications for Researchers
1009 and Practitioners. *Sports Med* **49,** 1637-1650.

1010

1011 Kogure GS, Silva RC, Picchi Ramos FK, Miranda-Furtado CL, Lara LA, Ferriani RA & Dos Reis RM. (2015).
1012 Women with polycystic ovary syndrome have greater muscle strength irrespective of body
1013 composition. *Gynecol Endocrinol* **31,** 237-242.

1014

1015 Krakowsky Y & Grober ED. (2015). Testosterone Deficiency - Establishing A Biochemical Diagnosis.
1016 *Ejifcc* **26,** 105-113.

1017

1018 Kuznetsova A, Brockhoff PB & Christensen RHB. (2017). lmerTest Package: Tests in Linear Mixed Effects
1019 Models. *2017* **82,** 26.

1020

1021 Lamon S, Morabito A, Arentson-Lantz E, Knowles O, Vincent GE, Condo D, Alexander SE, Garnham A,
1022 Paddon-Jones D & Aisbett B. (2021). The effect of acute sleep deprivation on skeletal muscle
1023 protein synthesis and the hormonal environment. *Physiological reports* **9,** e14660-e14660.

1024

1025 Leung JK & Sadar MD. (2017). Non-Genomic Actions of the Androgen Receptor in Prostate Cancer.
1026 *Front Endocrinol (Lausanne)* **8,** 2.

1027

1028 Love MI, Huber W & Anders S. (2014). Moderated estimation of fold change and dispersion for RNA-
1029 seq data with DESeq2. *Genome Biology* **15,** 550.

1030

1031 MacLean HE, Chiu WS, Notini AJ, Axell AM, Davey RA, McManus JF, Ma C, Plant DR, Lynch GS & Zajac
1032 JD. (2008). Impaired skeletal muscle development and function in male, but not female,
1033 genomic androgen receptor knockout mice. *Faseb j* **22,** 2676-2689.

1034

1035 Mauras N, Hayes V, Welch S, Rini A, Helgeson K, Dokler M, Veldhuis JD & Urban RJ. (1998).
1036 Testosterone Deficiency in Young Men: Marked Alterations in Whole Body Protein Kinetics,
1037 Strength, and Adiposity1. *The Journal of Clinical Endocrinology & Metabolism* **83,** 1886-1892.

1038

1039 McIlvenna LC, Patten RK, McAinch AJ, Rodgers RJ, Stepto NK & Moreno-Asso A. (2021). Transforming
1040 Growth Factor Beta 1 Alters Glucose Uptake but Not Insulin Signalling in Human Primary
1041 Myotubes From Women With and Without Polycystic Ovary Syndrome. *Frontiers in*
1042 *Endocrinology* **12**.

1043

1044 McLeod M, Breen L, Hamilton DL & Philp A. (2016). Live strong and prosper: the importance of skeletal
1045 muscle strength for healthy ageing. *Biogerontology* **17,** 497-510.

1046

1047 Mitchell CJ, Churchward-Venne TA, Bellamy L, Parise G, Baker SK & Phillips SM. (2013). Muscular and
1048 systemic correlates of resistance training-induced muscle hypertrophy. *PLoS One* **8,** e78636.

1049

1050 Mazerolle MJ. (2020). *AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c)*. R
1051 package version 2.3-1, .

1052

1053 Mobley CB, Haun CT, Roberson PA, Mumford PW, Kephart WC, Romero MA, Osburn SC, Vann CG,
1054 Young KC, Beck DT, Martin JS, Lockwood CM & Roberts MD. (2018). Biomarkers associated
1055 with low, moderate, and high vastus lateralis muscle hypertrophy following 12 weeks of
1056 resistance training. *PLoS One* **13,** e0195203.

1057

1058 Morton RW, Oikawa SY, Wavell CG, Mazara N, McGlory C, Quadrilatero J, Baechler BL, Baker SK &
1059 Phillips SM. (2016). Neither load nor systemic hormones determine resistance training-
1060 mediated hypertrophy or strength gains in resistance-trained young men. *Journal of Applied*
1061 *Physiology* **121,** 129-138.

1062

1063 Morton RW, Sato K, Gallaugher MPB, Oikawa SY, McNicholas PD, Fujita S & Phillips SM. (2018). Muscle
1064 Androgen Receptor Content but Not Systemic Hormones Is Associated With Resistance
1065 Training-Induced Skeletal Muscle Hypertrophy in Healthy, Young Men. *Frontiers in Physiology*
1066 **9**.

1067

1068 Mouser JG, Loprinzi PD & Loenneke JP. (2016). The association between physiologic testosterone
1069 levels, lean mass, and fat mass in a nationally representative sample of men in the United
1070 States. *Steroids* **115,** 62-66.

Olea N, Sakabe K, Soto AM & Sonnenschein C. (1990). The proliferative effect of "anti-androgens" on the androgen-sensitive human prostate tumor cell line LNCaP. *Endocrinology* **126,** 1457-1463.

World Medical Organisation. (2018). WMA Declaration of Helsinki - Ethical principles for medical research involvoing human subjects.

Overkamp M, Houben LHP, van der Meer S, van Roermund JGH, Bos R, Kokshoorn APJ, Larsen MS, van Loon LJC, Beelen M & Beijer S. (2023). Onset of androgen deprivation therapy leads to rapid deterioration of body composition, physical performance, cardiometabolic health and quality-of-life in prostate cancer patients. *Scand J Urol* **57,** 60-66.

Pataky MW, Dasari S, Michie KL, Sevits KJ, Kumar AA, Klaus KA, Heppelmann CJ, Robinson MM, Carter RE, Lanza IR & Nair KS. (2023). Impact of biological sex and sex hormones on molecular signatures of skeletal muscle at rest and in response to distinct exercise training modes. *Cell Metabolism* **35,** 1996-2010.e1996.

Pöllänen E, Sipilä S, Alen M, Ronkainen PH, Ankarberg-Lindgren C, Puolakka J, Suominen H, Hämäläinen E, Turpeinen U, Konttinen YT & Kovanen V. (2011). Differential influence of peripheral and systemic sex steroids on skeletal muscle quality in pre- and postmenopausal women. *Aging Cell* **10,** 650-660.

R Core Team. (2021). R: A Language and Environment for Statistical Computing.

Rariy CM, Ratcliffe SJ, Weinstein R, Bhasin S, Blackman MR, Cauley JA, Robbins J, Zmuda JM, Harris TB & Cappola AR. (2011). Higher Serum Free Testosterone Concentration in Older Women Is Associated with Greater Bone Mineral Density, Lean Body Mass, and Total Fat Mass: The Cardiovascular Health Study. *The Journal of Clinical Endocrinology & Metabolism* **96,** 989-996.

Russell AP, Lamon S, Boon H, Wada S, Güller I, Brown EL, Chibalin AV, Zierath JR, Snow RJ, Stepto N, Wadley GD & Akimoto T. (2013). Regulation of miRNAs in human skeletal muscle following acute endurance exercise and short-term endurance training. **591,** 4637-4653.

Fox J, Weisberg S. (2019). *An R companion to Applied Regression*. Sage, Thousand Oaks CA.

Salamin O, Nicoli R, Langer T, Boccard J, Grundisch CS, Xu C, Rudaz S, Kuuranne T, Pitteloud N & Saugy M. (2022). Longitudinal evaluation of multiple biomarkers for the detection of testosterone gel administration in women with normal menstrual cycle. *Drug Test Anal* **14,** 833-850.

Samn SW & Perelli LP. (1982). Estimating aircrew fatigue: a technique with application to airlift operations. School of Aerospace Medicine Brooks Afb tx.

Sanz G, Martínez-Aranda LM, Tesch PA, Fernandez-Gonzalo R & Lundberg TR. (2019). Muscle2View, a CellProfiler pipeline for detection of the capillary-to-muscle fiber interface and high-content quantification of fiber type-specific histology. *J Appl Physiol (1985)* **127,** 1698-1709.

Schiaffino S, Dyar KA, Ciciliot S, Blaauw B & Sandri M. (2013). Mechanisms regulating skeletal muscle growth and atrophy. *Febs j* **280,** 4294-4314.

Schneider CA, Rasband WS & Eliceiri KW. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9,** 671-675.

Sheffield-Moore M, Urban RJ, Wolf SE, Jiang J, Catlin DH, Herndon DN, Wolfe RR & Ferrando AA. (1999). Short-term oxandrolone administration stimulates net muscle protein synthesis in young men. *J Clin Endocrinol Metab* **84,** 2705-2711.

Sinha-Hikim I, Taylor WE, Gonzalez-Cadavid NF, Zheng W & Bhasin S. (2004). Androgen receptor in human skeletal muscle and cultured muscle satellite cells: up-regulation by androgen treatment. *J Clin Endocrinol Metab* **89,** 5245-5255.

Xyris Software. (2019). Easy Diet Diary. FoodWorks.

Stirling DR, Swain-Bowden MJ, Lucas AM, Carpenter AE, Cimini BA & Goodman A. (2021). CellProfiler 4: improvements in speed, utility and usability. *BMC Bioinformatics* **22,** 433.

Storer TW, Basaria S, Traustadottir T, Harman SM, Pencina K, Li Z, Travison TG, Miciek R, Tsitouras P, Hally K, Huang G & Bhasin S. (2017). Effects of Testosterone Supplementation for 3 Years on Muscle Performance and Physical Function in Older Men. *J Clin Endocrinol Metab* **102,** 583-593.

Tieland M, Trouwborst I & Clark BC. (2018). Skeletal muscle performance and ageing. *J Cachexia Sarcopenia Muscle* **9,** 3-19.

van Geel TA, Geusens PP, Winkens B, Sels JP & Dinant GJ. (2009). Measures of bioavailable serum testosterone and estradiol and their relationships with muscle mass, muscle strength and bone mineral density in postmenopausal women: a cross-sectional study. *European journal of endocrinology* **160,** 681-687.

Walter-Kroker A, Kroker A, Mattiucci-Guehlke M & Glaab T. (2011). A practical guide to bioelectrical impedance analysis using the example of chronic obstructive pulmonary disease. *Nutr J* **10,** 35-35.

Wannenes F, Caprio M, Gatta L, Fabbri A, Bonini S & Moretti C. (2008). Androgen receptor expression during C2C12 skeletal muscle cell line differentiation. *Molecular and cellular endocrinology* **292,** 11-19.

White JP, Gao S, Puppa MJ, Sato S, Welle SL & Carson JA. (2013). Testosterone regulation of Akt/mTORC1/FoxO3a signaling in skeletal muscle. *Mol Cell Endocrinol* **365,** 174-186.

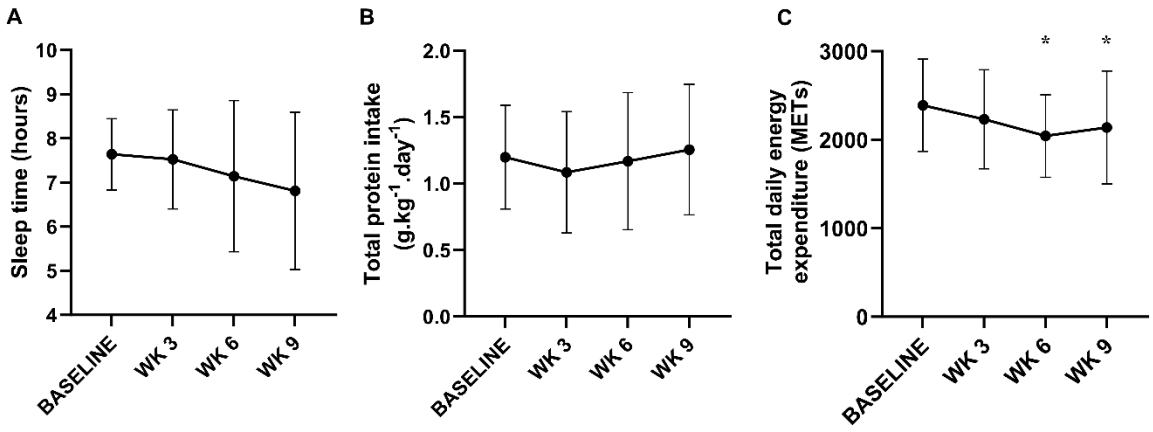
Wickham H, Averick M, Bryan J, Chang W, McGowan LDA, François R, Grolemund G, Hayes A, Henry L & Hester J. (2019). Welcome to the Tidyverse. *Journal of Open Source Software* **4,** 1686.

Wu Y, Bauman WA, Blitzer RD & Cardozo C. (2010). Testosterone-induced hypertrophy of L6 myoblasts is dependent upon Erk and mTOR. *Biochem Biophys Res Commun* **400,** 679-683.

Ye J, Zhai X, Yang J & Zhu Z. (2021). Association between Serum Testosterone Levels and Body Composition among Men 20-59 Years of Age. *Int J Endocrinol* **2021,** 7523996.

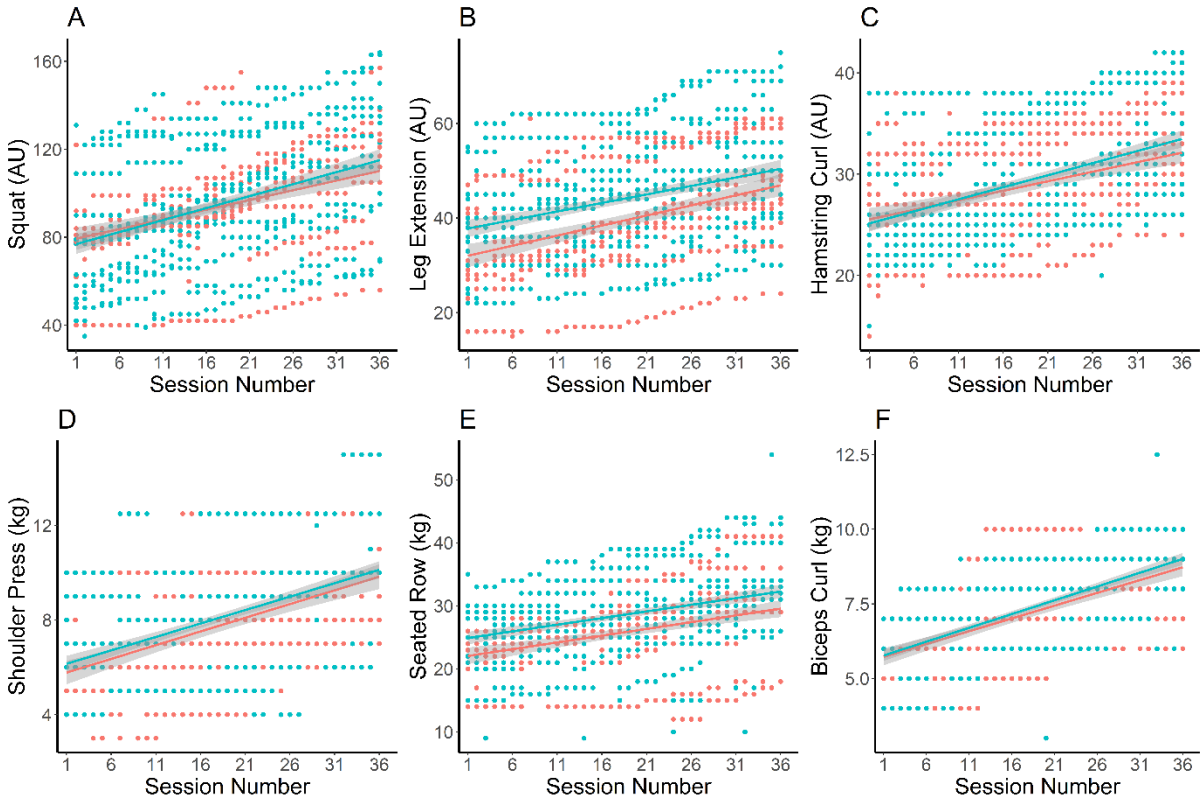
Yoshioka M, Boivin A, Bolduc C & St-Amand J. (2007). Gender difference of androgen actions on skeletal muscle transcriptome. *Journal of Molecular Endocrinology* **39,** 119-133.

Zaras N, Stasinaki AN, Spiliopoulou P, Hadjicharalambous M & Terzis G. (2020). Lean Body Mass, Muscle Architecture, and Performance in Well-Trained Female Weightlifters. *Sports (Basel)* **8**.



Supplementary Figure 1. A) total sleep time did not differ across 12 weeks of resistance training in pre-menopausal females. B) Total protein intake ($g \cdot kg^{-1} \cdot day^{-1}$) did not fluctuate with 12 weeks of resistance training in pre-menopausal females. C) The total daily energy expenditure (METs) decreased at 6 weeks compared to baseline, but at no other timepoints across 12 weeks of

resistance training in pre-menopausal females. Data were analysed via one-way ANOVA *denotes significant post-hoc test (p<0.05 compared to baseline).



Supplementary Figure 2. The trajectory of working weight progression for (A) squat, (B) leg extension, (C) hamstring curl, (D) shoulder press, (E) seated row and (F) biceps curl. Participants undertaking a 12-week gym-based training program are denoted in blue (n=16), and participants undertaking the blended resistance training program are denoted in red (n=11).

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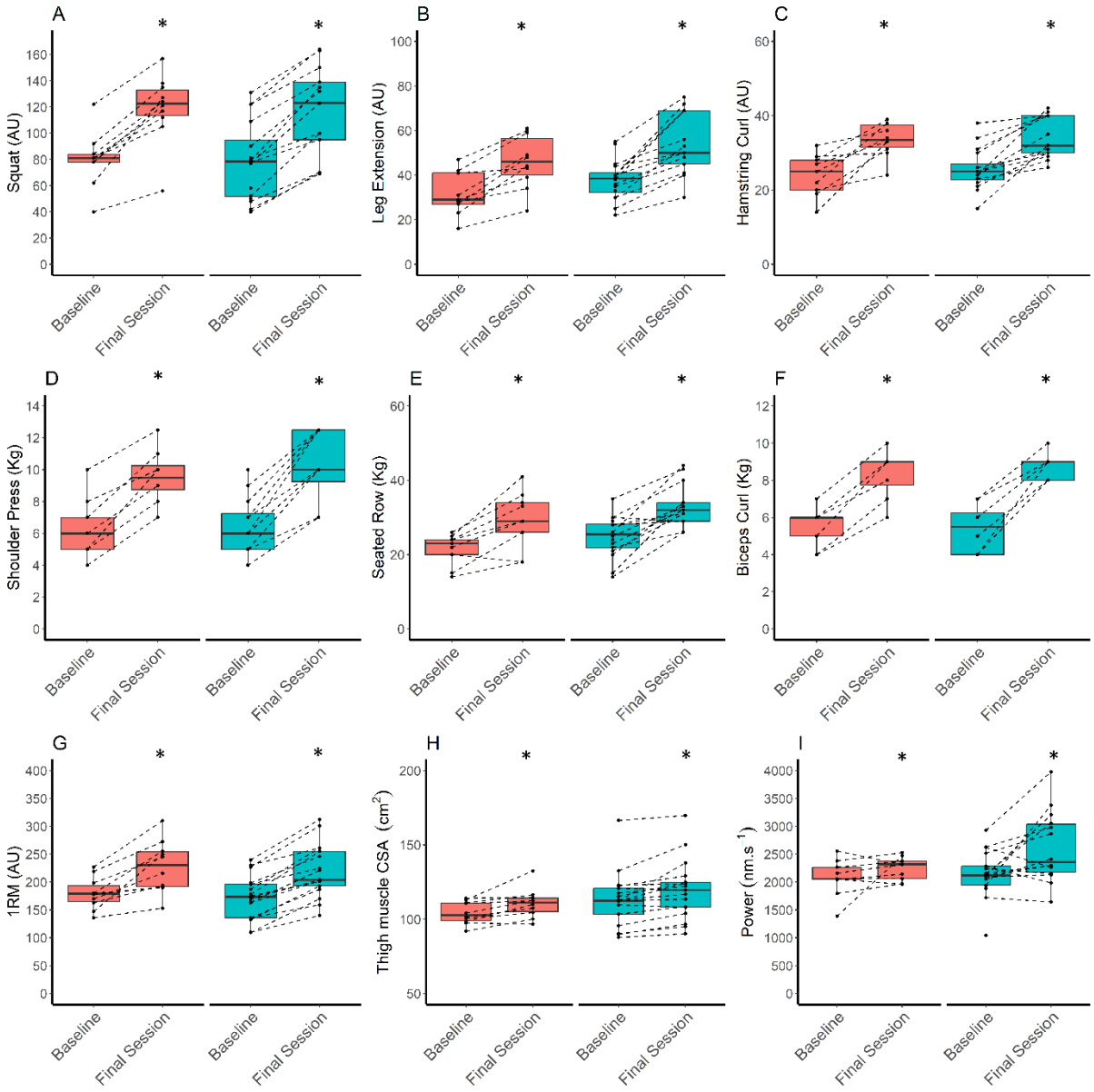

1199 **Supplementary Figure 3**. Working weight used in the first and final session for each participant.
1200 (A) squat, (B) leg extension, (C) hamstring curl, (D) shoulder press, (E) seated row and (F) biceps
1201 curl. (G) Lower limb strength (measured via leg press 1RM), (H) thigh muscle cross sectional area
1202 (CSA; cm^2) and (I) muscle power (measured via counter movement jump) increased with 12
1203 weeks of resistance training but this was not different between participants who undertook either
1204 a gym-based or blended training program. Participants undertaking a 12-week gym-based
1205 training program are denoted in blue (n=16), and participants undertaking the blended resistance
1206 training program are denoted in red (n=11). *indicates $p<0.05$ compared to baseline.

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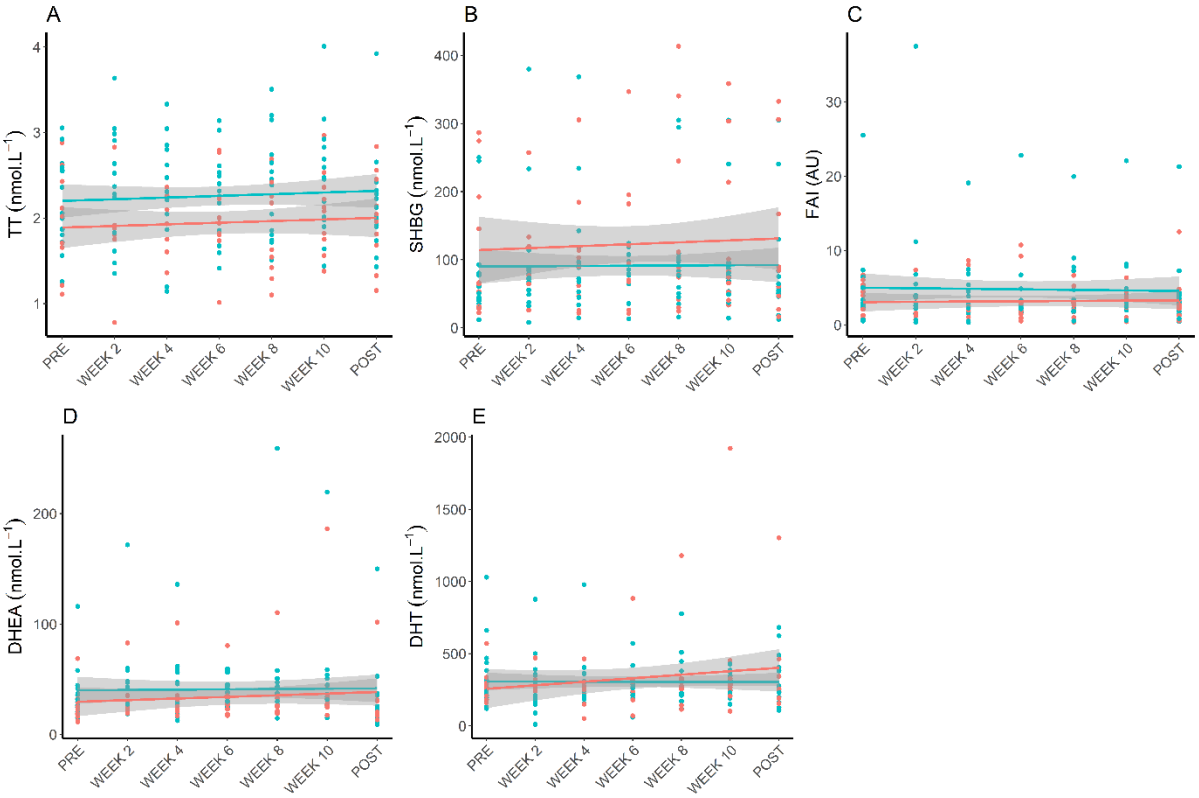
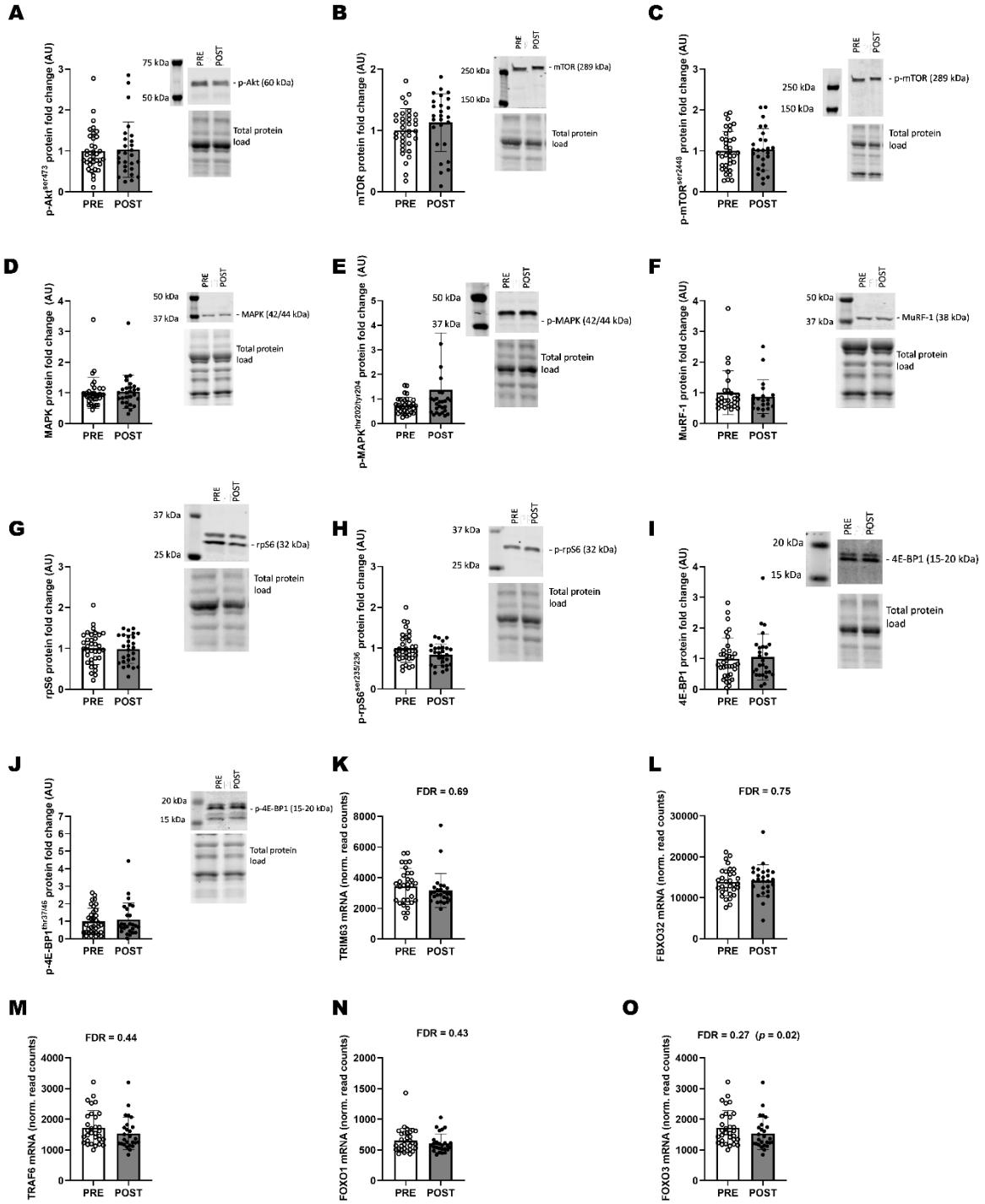
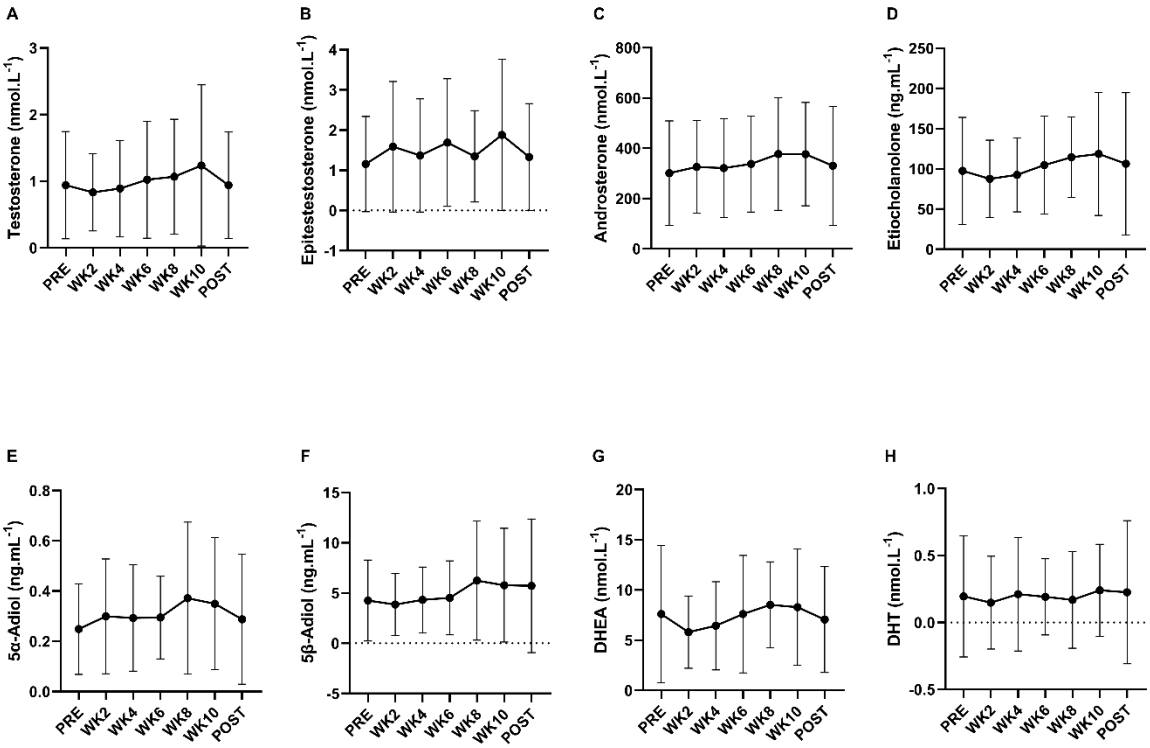


1208 **Supplementary Figure 4.** Hormone levels across 12 weeks of resistance training. (A) Total
1209 testosterone (TT), (B) sex hormone binding globulin (SHBG) and (C) free androgen index (FAI), (D)
1210 DHEA, (E) DHT concentrations. Participants undertaking a 12-week gym-based training program
1211 are denoted in blue (n=16), and participants undertaking the blended resistance training program
1212 are denoted in red (n=10).

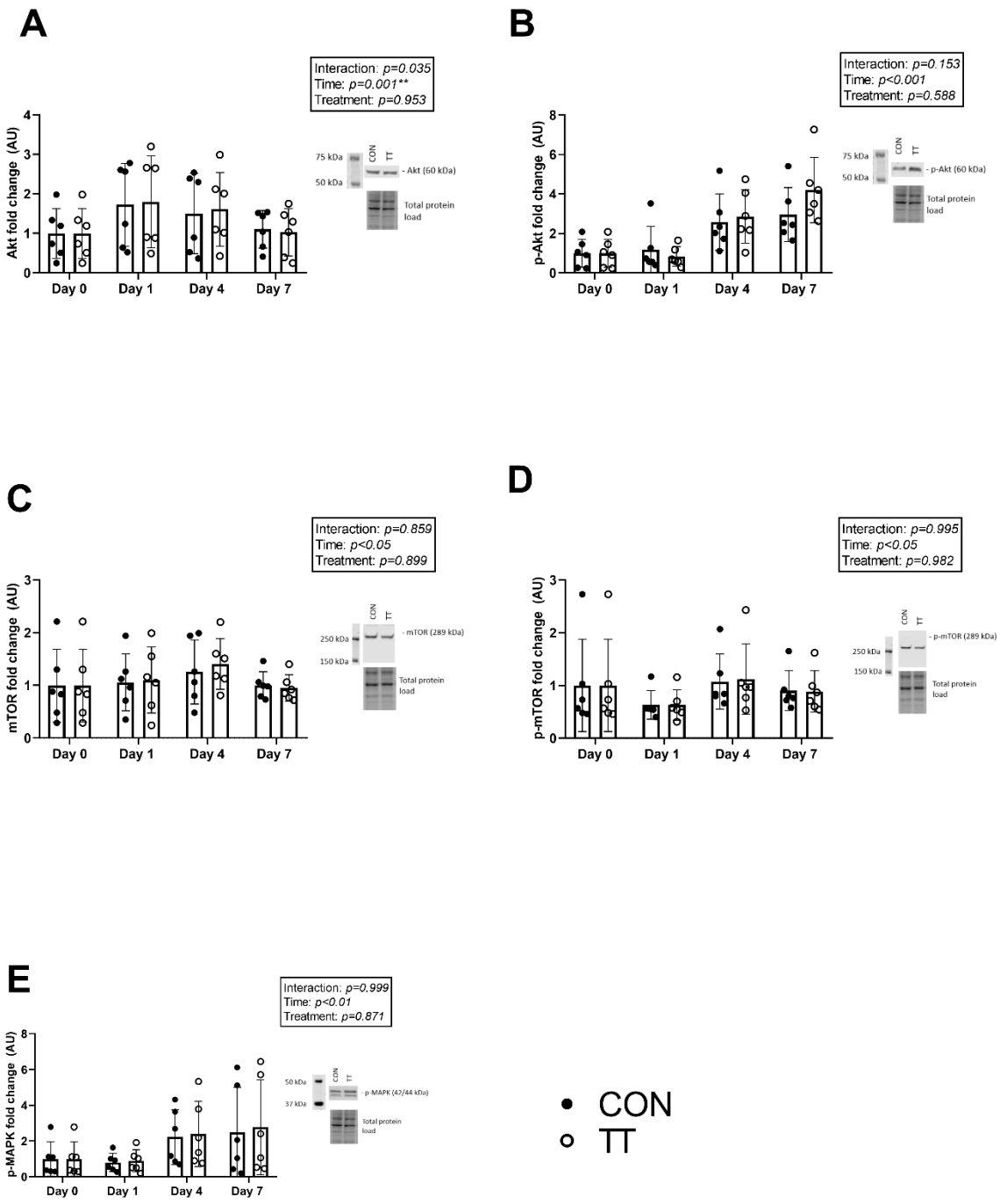


Supplementary Figure 5. Twelve weeks of resistance training did not influence the protein content of markers of protein synthesis and degradation in (A) p-Akt^{ser473}, (B) total mTOR, (C) p-mTOR^{ser2448}, (D) total MAPK, (E) p-MAPK$^{thr202/tyr204}$, (F) MuRF-1, (G) S6 ribosomal protein, (H) p-S6 ribosomal protein$^{ser235/236}$, (I) total 4E-BP, (J) p-4E-BP1$^{thr37/46}$ or the mRNA content of markers of protein degradation in (K) TRIM63, (L) FBXO32, (M) TRAF6, (N) FOXO1 and (O) FOXO3 in biopsies

taken at rest from previously untrained, pre-menopausal females (n=35 baseline, n=27 post training). Data were analysed via two-tailed, paired t-tests. Representative western blots for each protein are presented next to the corresponding graph. All proteins were normalised against total protein content, mRNA was normalised to read counts per million. Pre-training (PRE) values are indicated by clear bars and post-training (POST) values are indicated by dark bars. Bars indicate mean ± SD.



Supplementary Figure 6. The androgen profile measured by LC/MS from urine in pre-menopausal females is not influenced by resistance training. The concentrations of a) testosterone (nmol·L^{-1}), b) epitestosterone (nmol·L^{-1}), c) androsterone (nmol·L^{-1}), d) etiocholanolone (ng·mL^{-1}), e) 5α-adiol (ng·mL^{-1}), f) 5β-adiol (ng·mL^{-1}), g) DHEA (nmol·L^{-1}) and h) DHT (nmol·L^{-1}) were stable across twelve weeks of resistance training in our cohort (n=27). Data were analysed with one-way ANOVA. Bars indicate mean ± SD.



Supplementary Figure 7. Treatment with 100 nM testosterone did not significantly change the expression of A) Akt, B) p-Akt, C) mTOR, D) p-mTOR or E) p-MAPK compared to a vehicle control across 1, 4 or 7 days of differentiation. Black circles represent vehicle control condition (CON), white circles represent testosterone treated (TT) condition. Data were analysed via two-way, repeated measures ANOVA. Bars represent mean ± SD.

Supplementary Table 1. Summary of the menstrual phases of each participant at pre- and post-training testing timepoints. The menstrual phase was informed by a menstrual calendar collected by the participant and circulating concentrations of oestradiol (E2), progesterone, luteinising hormone (LH) and follicle stimulating hormone (FSH). Two independent researchers determined the menstrual phase for each entry and agreement was met at each case. Note: This study aimed to avoid testing and biopsy times during the late follicular (LF) phase of the menstrual cycle. Seven out of 62 (11%) testing times were taken during the late follicular phase. Statistical analyses will include the menstrual phase as a covariate to account for these times.

Participant	Timepoint	Menstrual calendar timepoint	E2 (pmol·L^{-1})	Progesterone (nmol·L^{-1})	LH (IU·L^{-1})	FSH (IU·L^{-1})	Menstrual phase (informed by calendar and hormones)
P01	PRE	Day 11 (out of 29)	514.1	6.7	5.3	4.5	**LF**
P01	POST	Day 18 (out of 26)	325.6	7.81	7.2	5.8	EL
P02	PRE	N/A	96.6	3.82	8.2	14	EF
P02	POST	N/A	169.5	4.337	1.2	5.7	EF
P03	PRE	N/A	92.3	7.62	9.6	8.3	ACTIVE PILL
P03	POST	N/A	28.3	7.3	2.4	2.9	ACTIVE PILL
P04	PRE	N/A	106.3	4.56	1.7	4.9	ACTIVE PILL
P04	POST	N/A	49.3	2.97	4.4	7.3	ACTIVE PILL
P05	PRE	N/A	111.6	4.36	3.6	8	ACTIVE PILL
P05	POST	N/A	127.1	4.58	3.1	7.5	ACTIVE PILL

P06	PRE	N/A	30.4	3.54	0	0.3	ACTIVE PILL
P06	POST	N/A	16.3	3.02	0	0.4	ACTIVE PILL
P07	PRE	Day 2 (out of 43)	158.3	5.11	6.5	7	EF
P07	POST	Day 22 (out of 30)	112.9	5.36	21.7	7.2	EL
P08	PRE	Day 31 (out of 34)	93.8	10.73	7.4	7	ML
P08	POST	Day 33 (out of 35)	526.1	67.83	6.8	2.2	ML
P09	PRE	Day 9 (out of 24)	287.4	10.13	3.6	5.9	**LF**
P09	POST	Day 22 (out of 27)	157.6	8.33	2.6	7.6	ML
P10	PRE	N/A	95.2	5	7.1	13.3	EF
P10	POST	N/A	433.7	64.5	1.1	3.9	ML
P11	PRE	Day 7 (out of 31)	248	6.44	7.2	4.7	**LF**
P11	POST	Day 26 (out of 29)	159.3	9.84	6.8	6	ML
P12	PRE	N/A	63.2	9.3	0	0.1	ACTIVE PILL
P12	POST	N/A	81.6	9.2	0	0	ACTIVE PILL
P13	PRE	Day 27 (out of 34)	955.5	14.7	71.1	10.6	EL
P13	POST	Day 14 (out of 30)	151	5.3	7.7	9.4	EF
P14	PRE	N/A	110	6.6	4.2	2	ACTIVE PILL
P14	POST	N/A	51.6	8.7	0	0.2	ACTIVE PILL

P15	PRE	Day 7 (out of 21)	215.3	8.5	4.7	6	**LF**
P15	POST	Day 20 (out of 22)	254.3	8.1	4.2	5.4	ML
P16	PRE	Day 48 (out of 84)	162.4	4.4	2	5.5	EF
P16	POST	Day 22 (out of 43)	218.7	5.1	3.1	7.2	EF
P17	PRE	N/A	50.3	13.4	0.2	0.2	ACTIVE PILL
P17	POST	N/A	71.3	10	0.4	0.4	ACTIVE PILL
P18	PRE	N/A	329.3	35.9	1.1	3.4	ML
P18	POST	N/A	765.6	8.4	10.1	6.1	**LF**
P19	PRE	N/A	81.9	8.3	8.9	4.9	ACTIVE PILL
P19	POST	N/A	152.2	7.8	8.7	5.7	ACTIVE PILL
P20	PRE	Day 7 (out of 25)	135.7	12.3	2.8	3.9	EF
P20	POST	Day 15 (out of 25)	725.3	10.9	8	2.6	**LF**
P21	PRE	N/A	107.4	16.4	6.5	7.3	ACTIVE PILL
P22	PRE	Day 20 (out of 28)	604.1	7.4	3.6	3.5	ML
P23	PRE	N/A	183.3	5.2	6.8	8.8	EF
P24	PRE	N/A	62.9	7.2	0.2	0.5	ACTIVE PILL
P25	PRE	Day 16 (out of 29)	N/A	N/A	N/A	N/A	EL
P26	PRE	N/A	50.7	12.9	0.2	0.6	ACTIVE PILL

P27	PRE	N/A	83.5	8.5421	0.2	0.5	ACTIVE PILL
P27	POST	N/A	62.1	9.8872	0.6	1.4	ACTIVE PILL
P28	PRE	Day 6 (of 28)	157.2	5.1909	9.6	6.7	EF
P28	POST	Day 5 (of 27)	1296.7	72.0561	8.1	2	**LF**
P29	PRE	N/A	183.5	4.189	4.1	7	**PLACEBO PILL**
P29	PRE	N/A	69.5	7.016	3.3	5.1	ACTIVE PILL
P30	PRE	Day 1 (out of 31)	438.3	10.3182	19.9	8.7	EF
P30	POST	Day 28 (out of 30)	175.1	5.4004	12.9	7.7	ML
P31	PRE	Day 3 (of 25)	85.6	7.77	5.6	5.6	EF
P31	POST	Day 3 (of 33)	84	5.8703	5.6	6.6	EF
P32	PRE	N/A	212.2	5.1581	11.9	7	EF
P33	PRE	N/A	273.1	29.3536	3.2	2.4	ML
P34	PRE	Day 4 (of 24)	140.1	6.1849	4.8	7.6	EF
P34	POST	Day 5 (of N/A)	112.9	5.0798	8.3	6.8	EF
P35	PRE	Day 29 (of 29)	107.2	7.9207	3.3	8.7	ML
P35	POST	Day 13 (of 29)	195.6	4.2692	3.2	5.4	EF

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Supplementary Table 2. The resistance training program prescribed to participants (n=27). The gym-based program 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 programs using the home-based resistance training program when access to the training facility was not possible. *last set was prescribed as many repetitions as possible (AMRAP).

Gym-based resistance training program						
Exercise	**Sets**	**Reps**	**Intensity (%RM)**	**Rest between sets (s)**	**Tempo (s)**	**Volume load (reps × sets × intensity)**
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 program						
Exercise	**Sets**	**Reps**	**Intensity (%RM)**	**Rest between sets (s)**	**Tempo (s)**	**Volume load (reps × sets × intensity)**
Squats	3	15*	Approximately 45% RM	90	2,0,2,0	20.25

Forward lunges	3	15·leg^{-1*}	Approximately 45% RM	90	2,0,2,0	20.25
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

Supplementary Table 3. Linear mixed models assessing the association between markers of the androgen profile and AR activity and muscle size, strength and power before and after 12 weeks of resistance training in pre-menopausal females (n=35 pre-training, 27 change with training).

PRE-TRAINING					CHANGE WITH TRAINING				
Variable	**β**	**SE**	***p***	**Model adjusted for**	**Variable**	**β**	**SE**	***p***	**Model adjusted for**
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*	-
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*	-
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*	-
p-AR (ser213) PROTEIN CONTENT					**AVERAGE p-AR (ser213) PROTEIN CONTENT**				
pre-training muscle CSA	-5.41	3.95	*0.180*	Pre-training TT	**Δ% muscle CSA**	**-0.03**	**0.01**	***0.040****	**TT AUC**
pre-training thigh muscle strength	0.34	0.19	*0.078*	Pre-training TT	Δ% thigh muscle strength	-0.06	0.04	*0.098*	TT AUC
pre-training muscle power	2.78	2.28	*0.736*	Pre-training TT	Δ% muscle power	-0.01	0.07	*0.938*	TT AUC

pre-training muscle fibre CSA	-352.70	233.10	*0.148*	-	Δ% muscle fibre CSA	-0.08	0.04	*0.087*	-
p-AR (ser650) PROTEIN CONTENT					**AVERAGE p-AR (ser650) PROTEIN CONTENT**				
pre-training muscle CSA	-6.56	6.44	*0.316*	Pre-training TT	Δ% muscle CSA	-0.03	0.02	*0.116*	TT AUC
pre-training thigh muscle strength	0.59	0.30	*0.055*	Pre-training TT	Δ% thigh muscle strength	0.01	0.05	*0.881*	TT AUC
pre-training muscle power	-3.66	3.60	*0.317*	Pre-training TT	Δ% muscle power	0.02	0.09	*0.808*	TT AUC
pre-training muscle fibre CSA	-76.05	482.13	*0.876*	-	Δ% muscle fibre CSA	-0.08	0.10	*0.443*	-
AR+ nuclei (%)					**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*	-

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