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CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA INTERINSTITUICIONAL DE PÓS-GRADUAÇÃO EM CIÊNCIAS FISIOLÓGICAS ASSOCIAÇÃO AMPLA UFSCar/UNESP

JOÃO GUILHERME ALMEIDA BERGAMASCO

O PAPEL DAS ALTERAÇÕES DOS RECEPTORES ANDROGÊNICOS NA HIPERTROFIA MUSCULAR INDUZIDA PELO TREINAMENTO DE FORÇA

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Tese apresentada ao Programa Interinstitucional de Pós-graduação em Ciências Fisiológicas – Associação Ampla UFSCar/UNESP, como parte dos requisitos para a obtenção do título de Doutor em Ciências Fisiológicas.

Orientador: Prof. Dr. Cleiton Augusto Libardi.

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Folha de aprovação

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RESUMO

A expressão de receptores androgênicos (AR) tem sido apontada como potencial mecanismo associado à hipertrofia muscular. No entanto, pouco se sabe sobre as associações entre as mudanças agudas e crônicas nos conteúdos totais de AR, AR citoplasmático (cAR), AR nuclear (nAR) e conteúdo de AR ligado ao DNA (AR-DNA) induzidas pelo treinamento de força (RT) e a hipertrofia muscular em mulheres e homens. Adicionalmente, não se sabe se essas mudanças agudas e crônicas podem explicar diferenças nas magnitudes das adaptações hipertróficas entre não-respondedores e respondedores ao RT. O objetivo desta tese foi investigar os efeitos agudos e crônicos do RT nos conteúdos totais de AR, cAR, nAR e de AR-DNA no tecido muscular de mulheres e homens. Além disso, foi investigado se essas mudanças estão associadas à hipertrofia muscular em ambos os sexos. Finalmente, buscamos identificar possíveis diferenças entre não-respondedores e respondedores nos conteúdos basais, respostas agudas e crônicas nos marcadores de AR decorrentes do RT. Trinta e oito jovens destreinados (19 mulheres e 19 homens) realizaram 10 semanas de RT. Participantes que apresentaram aumentos na área de secção transversal muscular (mCSA) inferiores a dois erros típicos, considerando duas avaliações de mCSA, foram considerados não-respondedores e os maiores respondedores da nossa amostra foram pareados numericamente. Biópsias musculares foram realizadas no início (i.e., antes do período de treinos), 24 horas após a primeira sessão de RT (respostas agudas) e 96 horas após a última sessão de treino (respostas crônicas). Conteúdos proteicos de AR, cAR e nAR foram analisados por *Western blotting*, enquanto AR-DNA por ensaio oligonucleotídeo-ELISA. Imuno-histoquímica foi utilizada para determinar tipo e área de secção transversa das fibras (fCSA) e a ultrassonografia para determinar a mCSA. No período pré-treino, os homens demonstraram maiores conteúdos de nAR do que as mulheres. Além disso, houve associação significativa entre o cAR basal e a hipertrofia da fCSA do tipo II para os homens. Com relação aos resultados agudos, ambos os sexos apresentaram diminuição em AR, cAR e nAR, com maiores diminuições em nAR para os homens. Após 10 semanas de RT, os homens demonstraram maior conteúdo de cAR em comparação com as mulheres, enquanto ambos os sexos diminuíram os níveis de AR-DNA, e AR ou nAR permaneceram inalterados. As respostas agudas ou crônicas de AR, cAR, nAR e AR-DNA não se correlacionaram com a hipertrofia em mulheres ou homens. Não houve diferenças significantes entre não-respondedores e respondedores para a maioria dos marcadores de AR nos conteúdos basais, alterações agudas e crônicas decorrentes do RT. Entretanto, houve diferença significativa entre não-respondedores (+19.5%) e respondedores (-14.4%) na

atividade aguda de AR-DNA (ES = -1.39; 95% CI: -2.53 a -0.16). O conteúdo basal de cAR parece influenciar a hipertrofia nos homens, ao passo que mudanças agudas ou crônicas em AR ou suas frações não estão associadas à hipertrofia muscular em mulheres ou homens. Por fim, os achados do presente estudo não suportam os marcadores de AR enquanto mecanismo capaz de explicar diferenças na magnitude das respostas hipertróficas ao RT em mulheres e homens jovens destreinados.

Palavras-chave: Músculo esquelético; Receptores hormonais; Responsividade.

ABSTRACT

The expression of androgen receptors (AR) has been highlighted as a potential mechanism for muscle hypertrophy. However, little is known about the associations between acute and chronic changes in total AR, cytoplasmic AR (cAR), nuclear AR (nAR), and AR-DNA content induced by resistance training (RT) and muscle hypertrophy in women and men. Furthermore, it is unknown whether these acute and chronic changes can explain the differences in the magnitude of hypertrophic responses between non-responders and responders to RT. The aim of this thesis was to investigate the acute and chronic effects of RT on total AR, cAR, nAR, and AR-DNA content in women and men. Additionally, it was investigated whether these acute and chronic changes in AR molecular markers are associated with muscle hypertrophy in both sexes. Finally, we sought to identify if there are differences between nonresponders and responders in both baseline contents and acute and chronic changes in AR markers resulting from RT. Thirty-eight untrained young individuals (19 women and 19 men) underwent 10 weeks of RT. Participants that did not present increases in muscle cross-sectional area (mCSA) greater than 2 typical errors, considering two mCSA measurements, were considered nonresponders and responders were numerically matched accordingly. Biopsies of the vastus lateralis muscle were performed at baseline (i.e., before the training period), 24 hours after the first RT session (acute responses), and 96-120 hours after the last training session (chronic responses). AR, cAR, and nAR protein contents were analyzed by Western blotting, while AR-DNA was analyzed by oligonucleotide-ELISA assay. Immunohistochemistry was used for fiber type analysis and cross-sectional area (fCSA), and ultrasonography was used to determine the mCSA. At baseline, men showed greater nAR content compared to women. Additionally, there was a significant association between baseline cAR and type II fCSA hypertrophy for men. Regarding acute results, both sexes showed decreased AR, cAR, and nAR, while men demonstrated greater decreases in nAR. After 10 weeks of RT, men showed greater cAR compared to women, while both sexes decreased AR-DNA levels, and AR or nAR remained unchanged. Acute or chronic responses of AR, cAR, nAR, and AR-DNA did not correlate with hypertrophy in women or men. There were no significant differences between nonresponders and responders for most AR markers in baseline contents or acute and chronic changes induced by RT. However, there was a significant difference between nonresponders (+19.5%) and responders (-14.4%) in acute AR-DNA activity (ES = -1.39; 95% CI: -2.53 to -0.16). Baseline cAR content potentially influences hypertrophy in men, whereas acute or chronic changes in AR or its fractions are not associated with muscle hypertrophy in women or men. Finally, the present findings study do not support

AR markers as a mechanism capable of explaining differences in the magnitude of hypertrophic responses to RT in untrained young women and men.

Keywords: Skeletal muscle; Hormonal receptors; Responsiveness

LISTA DE FIGURAS

CAPÍTULO 1

CAPÍTULO 2

LISTA DE TABELAS

CAPÍTULO 1

CAPÍTULO 2

SUMÁRIO

CONTEXTUALIZAÇÃO

A trajetória que culminou com a elaboração da presente tese teve início em junho de 2019, com minha matrícula como aluno de doutorado no Programa Interinstitucional de Pós-Graduação em Ciências Fisiológicas UFSCar-Unesp (PIPGCF) – onde havia realizado o Mestrado. Nos primeiros seis meses de processo, cursei os créditos necessários para cumprimento dos requisitos do PIPGCF e pude colaborar com a coleta de dados e na escrita de estudos que vieram a ser publicados (Biazon *et al*., 2019, doi: 10.3389/fphys.2019.00446; Angleri *et al*., 2020, doi: 10.3389/fphys.2019.01576 e Soligon *et al*., 2020, doi: 10.1007/s00421-020-04446-x). Além disso, finalizei a escrita para submissão e posterior publicação do estudo realizado em meu mestrado (Bergamasco *et al*., doi: 10.1519/JSC.0000000000003632). Adicionalmente, juntamente ao Prof. Dr. Cleiton Augusto Libardi, foi elaborado o delineamento experimental do que, à época, seria o projeto que daria origem a minha tese de doutorado. Entretanto, em março de 2020, foi decretado o fechamento das universidades devido à pandemia do Covid-19, o que impossibilitou a coleta de dados em nosso laboratório, uma vez que as intervenções realizadas em nosso laboratório incluem a participação de seres humanos.

Durante o período de atividades remotas, nosso grupo de pesquisa passou por um processo de adaptação até retornar ao funcionamento de algumas de suas atividades, tais quais: i) grupo de estudos semanal, ii) reuniões constantes com o Prof. Dr. Cleiton para reajustar o cronograma ao passo que os prazos eram reduzidos gradualmente pela pandemia, iii) reuniões para ajustar o delineamento experimental do projeto. Além disso, durante o período de atividades remotas, pude publicar estudos contendo dados previamente coletados em nosso laboratório (Bergamasco *et al*., 2022, doi: 10.1123/jsr.2021-0101 e Scarpelli *et al*., 2021, doi: 10.3389/fspor.2021.671764), realizar cursos e ministrar aulas de maneira remota como professor convidado em curso de graduação e em curso de pós-graduação *lato sensu*.

No ano de 2022, obtivemos a antecipação do retorno das atividades presenciais em nosso laboratório, através do comprometimento em cumprir medidas sanitárias junto ao Núcleo Executivo de Vigilância em Saúde (NEVS), vinculado ao comitê gestor da pandemia da UFSCar. Entre março e junho pude colaborar com a coleta de dados de um estudo conduzido por nosso grupo – que atualmente encontra-se em fase de elaboração de carta de resposta aos revisores – até que em junho, demos início ao recrutamento de participantes para a coleta dos dados que deram origem ao presente trabalho.

Por conta do afastamento devido à pandemia e da necessidade de cumprimento dos prazos, optamos em comum acordo por realizar um único projeto capaz de comportar perguntas suficientes para compor três Teses de Doutorado. A coleta de dados ocorreu entre julho e outubro de 2022 e, até o presente momento, três estudos foram publicados (Chaves *et al*., 2024, doi: 10.1055/a-2256-5857; e Scarpelli *et al*., 2024, doi: 10.1007/s00421-024-05484-5; Bergamasco *et al*. 2024, doi: 10.1249/MSS.0000000000003509).

Concomitante à coleta de dados, me inscrevi para o edital Capes-PrInt, na tentativa de que – ainda que em fase final do doutorado – pudesse experienciar um período sanduíche em um laboratório internacional, e obtive aprovação. Previamente à minha ida, preparamos a documentação necessária para o envio de um total de 690 amostras para o laboratório do Prof. Dr. Michael D. Roberts. Amostras coletadas no Musculab e armazenadas separadamente em duas alíquotas: tecido puro e tecido preservado em OCT.

Assim, entre fevereiro e julho de 2023, realizei um período de Doutorado Sanduíche na Universidade de Auburn, localizado na cidade de Auburn (Alabama, EUA), no laboratório *Nutrabolt Applied and Molecular Sciences Laboratory*, liderado pelo Prof. Dr. Michael D. Roberts. Neste período, tive oportunidade de aprender diversas técnicas laboratoriais de análise, as quais me permitiram realizar as análises biomoleculares do presente estudo, tais quais: imuno-histoquímica, *Western Blotting* e ensaios enzimáticos (ELISA), além de todos os preparativos necessários para a condução destas técnicas (e.g., corte de tecido muscular preservado em *optimum cutting temperature* (OCT) em criostato e sua preparação em lâminas para microscopia, homogeneização de tecido muscular, condução e leitura de ensaio realizado para determinar o conteúdo proteico das amostras (BCA), preparos para *Western Blotting* e separação e extração das frações de interesse após preparo com kits específicos). Além disso, tive oportunidade de aprender e realizar as análises dos resultados obtidos a partir destes ensaios através de equipamentos e softwares específicos, como: construção e sobreposição de imagens de imuno-histoquímica através de microscopia óptica, posterior análise de quantificação de área de fibras musculares, células satélites e mionúcleos via ImageJ e Myovision, análise de quantificação do conteúdo proteico via software ImageLab e leitura e análise de ELISA via espectrofotômetro.

Após meu retorno, finalizei as análises de conteúdo proteico e as últimas imagens obtidas pela análise de imuno-histoquímica, ambos os procedimentos previamente realizados em Auburn. Em seguida, conduzi a elaboração dos dois manuscritos que integram a presente Tese de Doutorado.

Por fim, a presente tese é composta por uma síntese do trabalho realizado, em língua portuguesa, seguido por dois capítulos, onde em cada um deles consta um dos artigos que resultaram do meu projeto de doutorado, e, portanto, estarão em língua inglesa. O capítulo 1, é composto pelo manuscrito do artigo intitulado: "*Acute and chronic changes in muscle androgen receptor markers are not associated with muscle hypertrophy in women and men*", aceito para publicação no periódico "*Medicine & Science in Sports & Exercise*" – ISSN: 0195-9131. Periódico classificado como A1 de acordo com o Qualis Capes para a área da Ciências Biológicas II, com fator de impacto: 4.1. O capítulo 2, é composto pelo manuscrito do artigo intitulado: "*Androgen receptor markers do not differ between nonresponders and responders to resistance training-induced muscle hypertrophy*", encontra-se em fase de revisão (R1) para publicação em edição especial endereçada à identificação de fatores que impulsionam respostas heterogêneas ao exercício, do periódico: "*Jounal of Applied Physiology"*. Esse periódico é classificado como A2 de acordo com o Qualis Capes para a área da Ciências Biológicas II, com fator de impacto: 3.3.

SÍNTESE GERAL

Introdução

Os hormônios androgênicos, assim como seus receptores desempenham papel fundamental na manutenção da massa muscular dos seres humanos (DAVEY *et al.*, 2016; JANSSEN *et al.*, 1994; RUIZEVELD DE WINTER *et al.*, 1991). Além disso, foi sugerido por décadas que alterações transientes (i.e., agudas) nos níveis séricos dos hormônios anabólicos e no conteúdo proteico de receptores androgênicos (AR) promovidas pelo treinamento de força (RT) contribuiriam para a hipertrofia muscular (BAMMAN *et al.*, 2001; HULMI *et al.*, 2008; KRAEMER, 1988; KRAEMER *et al.*, 2006; RATAMESS *et al.*, 2005; SPIERING *et al.*, 2009; VINGREN *et al.*, 2009; WILLOUGHBY *et al.*, 2004). Entretanto, ao passo que esse paradigma foi quebrado para os hormônios circulantes (MORTON *et al.*, 2016; MORTON *et al.*, 2018; WEST *et al.*, 2010; WEST *et al.*, 2009), está por ser determinado se as alterações agudas promovidas pelo RT nos AR podem estar associadas à hipertrofia muscular.

Diferentemente das alterações agudas, tem sido demonstrada associação significante entre o conteúdo basal de AR, bem como as alterações de longo-prazo (i.e., crônicas) nesse conteúdo promovidas pelo RT, e a hipertrofia muscular (AHTIAINEN *et al.*, 2011; MITCHELL *et al.*, 2013; MORTON *et al.*, 2018). Entretanto, além de alguns estudos refutarem essas associações (HATT *et al.*, 2024; MOBLEY *et al.*, 2018), tem sido sugerido que analisar frações subcelulares dos AR pode proporcionar uma compreensão mais abrangente de seu potencial papel na hipertrofia muscular. Isso porque os AR podem estar localizados em diferentes frações celulares. Considerando a via de sinalização canônica dos AR, os AR são ativados após interação com os hormônios androgênicos, e assim, translocam-se do citoplasma (cAR) para o núcleo das células (nAR), onde atuam como fatores de transcrição gênica através de ligação ao DNA (AR-DNA) (CLAESSENS *et al.*, 2001; DAVEY *et al.*, 2016; EDER *et al.*, 2001; EVANS, 1988; WYCE *et al.*, 2010).

Até o presente momento, a literatura acerca dos efeitos do RT nas respostas agudas e crônicas dos AR tem estudado este fenômeno exclusivamente em homens (AHTIAINEN *et al.*, 2011; CARDACI *et al.*, 2020; HULMI *et al.*, 2008; KRAEMER *et al.*, 2006; MOBLEY *et al.*, 2018; MORTON *et al.*, 2018; RATAMESS *et al.*, 2005; ROBERTS *et al.*, 2009; SPIERING *et al.*, 2009; SPILLANE *et al.*, 2015; WILLOUGHBY *et al.*, 2004). Entretanto, sabe-se que mulheres e homens possuem notória discrepância entre os níveis séricos de hormônios

androgênicos (NEALE *et al.*, 2013), bem como no conteúdo de AR (HATT *et al.*, 2024; VINGREN *et al.*, 2009). Embora as diferenças hormonais não impactem significantemente na hipertrofia muscular relativa (REFALO *et al*., 2024; ROBERTS *et al*., 2020), é possível que o mesmo não seja válido para o conteúdo de AR. Nesse sentido, Hatt *et al.* (HATT *et al.*, 2024) demonstrou associação significativa entre alterações no conteúdo de nAR e hipertrofia da fibra muscular apenas para homens. A partir desses achados, é possível sugerir que outras frações subcelulares dos AR, bem como sua associação à hipertrofia muscular também se difiram entre os sexos em resposta ao RT.

É importante ressaltar que embora grande parte dos indivíduos apresentem adaptações hipertróficas ao RT, indivíduos classificados como não-respondedores não apresentam hipertrofia muscular significativa frente à períodos prolongados de RT (AHTIAINEN *et al.*, 2016; HUBAL *et al.*, 2005; LIXANDRÃO *et al.*, 2024; SWINTON *et al.*, 2018). Um dos mecanismos sugeridos capazes de influenciar a magnitude das adaptações hipertróficas é o conteúdo de AR (ROBERTS *et al.*, 2023). Entretanto, até o presente momento, a literatura acerca dessa temática é escassa e nenhum estudo identificou os indivíduos como não-respondedores à hipertrofia (MOBLEY *et al.*, 2018; MORTON *et al.*, 2018). Enquanto Morton *et al*. (2018) demonstrou que indivíduos treinados classificados como altos-respondedores possuem mais AR total que indivíduos baixos-respondedores, Mobley *et al*. (2018) não mostrou diferenças entre os conteúdos de AR entre os mesmos clusters em indivíduos não-treinados. Entretanto, é possível que essa ausência de diferença esteja relacionada ao fato de que os indivíduos classificados como baixos-respondedores no estudo de Mobley *et al*. (MOBLEY *et al.*, 2018) apresentaram adaptações hipertróficas significativas. Assim, ainda não está claro se indivíduos não-respondentes e respondedores diferem no conteúdo total de AR, bem como se nas respostas agudas e crônicas ao RT. Além disso, nenhum estudo até o momento investigou extensivamente o efeito dos demais marcadores de AR (cAR, nAR e AR-DNA) nas respostas hipertróficas desses indivíduos ao RT.

Visando preencher as lacunas expostas, dois estudos foram conduzidos e são apresentados a seguir em forma de capítulos. O Capítulo 1 teve como objetivo investigar se os conteúdos basais de AR, cAR, nAR e AR-DNA, bem como nas respostas agudas e crônicas de RT estariam associadas à hipertrofia muscular. Além disso, o estudo visou determinar se essas possíveis associações se difeririam entre homens e mulheres. Já o Capítulo 2 teve como objetivo investigar potenciais diferenças entre não-respondedores e respondedores nos conteúdos basais, respostas agudas e crônicas de AR, cAR, nAR e AR-DNA ao RT.

Métodos

O presente estudo foi conduzido a partir da análise de dados coletados em um ensaio controlado randomizado registrado no Registro Brasileiro de Ensaios Clínicos (ReBEC; UTN: U1111-1283-2662; disponível em: https://ensaiosclinicos.gov.br/rg/RBR-57v9mrb). Esse estudo teve delineamento experimental intra-sujeitos onde cada sujeito foi submetido a dois protocolos de treinamento ("REPSprog" ou "LOADprog"). Os resultados do presente estudo se deram a partir da análise de tecido muscular referentes ao protocolo "LOADprog".

Na primeira visita do estudo, foi a ultrassonografia foi utilizada para determinação da área de secção transversa do músculo vasto lateral (mCSA), como descrito em Lixandrão *et al*. (2014). A segunda visita ocorreu após 72 h, onde após a repetição da ultrassonografia, os participantes foram submetidos ao teste de uma repetição máxima (1-RM) na cadeira extensora, para determinação da força máxima – de acordo com Brown e Weir (2001). Após 72 h, o teste de 1-RM foi repetido. Após 96 h, os participantes foram submetidos à biópsia muscular, para determinação dos conteúdos basais dos marcadores de AR, seguida pela primeira sessão de RT. Vinte e quatro horas após a primeira sessão de RT, a biópsia muscular foi repetida, para determinação das respostas agudas ao RT. Assim, um total de 23 sessões de RT foram conduzidas em 10 semanas. Ao final desse período, 96 h após a última sessão de treinamento, a ultrassonografia e as biópsias musculares foram repetidas, seguidas pelo teste de 1-RM – realizado após 96 h.

Após realização de aquecimento geral e específico, as sessões de treinamento de força consistiam em quatro séries de 9-12 repetições máximas na cadeira extensora, com intervalo de 90s. Sempre que os participantes realizavam menos de 9 ou mais de 12 repetições, a carga foi reduzida ou aumentada em cerca de 5-10%.

O tecido muscular coletado por meio das biópsias musculares foi dividido em duas alíquotas para realização de diferentes análises. Cerca de 20 mg de tecido foi armazenada em *optimum cutting temperature* (OCT) para a realização das análises de imuno-histoquímica. Estas análises foram realizadas para determinação da área de secção transversa das fibras musculares (fCSA), bem como os tipos I (fCSA tipo I) e II (fCSA tipo II). O restante do tecido muscular (~80-100mg) foi armazenado como tecido puro em freezer -80ºC. A partir da homogeneização deste tecido muscular, foram extraídas as frações citoplasmática e nuclear. Os conteúdos proteicos dos homogenatos total (AR), citoplasmático (cAR) e nuclear (nAR) foram

determinados através da técnica de *Western blotting.* Por fim, a fração nuclear foi utilizada para a determinação da atividade enzimática de AR ligado ao DNA através de kits comercialmente disponíveis.

Principais resultados

Capítulo 1

Os homens apresentaram maiores valores basais de mCSA ($P = 0.0003$), fCSA tipo I ($P = 0.037$), fCSA tipo II ($P < 0.001$), 1-RM ($P < 0.001$) e nAR comparado às mulheres ($P = 0.005$). Além disso, o conteúdo proteico basal de cAR nos homens se mostrou significativamente associado à hipertrofia de fCSA tipo II ($r = 0.499$; $P = 0.030$). Considerando as respostas agudas, ambos os sexos apresentaram diminuição significante em AR ($P < 0.0001$) e cAR ($P = 0.017$), enquanto os homens demonstraram maiores diminuições no nAR ($P = 0.010$) – significância não suportada pela análise de tamanho do efeito com intervalo de confiança de 95% – e não foram observadas alterações significantes na atividade de AR-DNA ($P > 0.05$) para ambos os sexos. Após 10 semanas de RT, AR e nAR permaneceram inalterados ($P > 0.05$), os homens demonstraram maiores conteúdos de cAR em comparação com as mulheres ($P = 0.021$) – significância não suportada pela análise de tamanho do efeito com intervalo de confiança de 95% – e ambos os sexos apresentaram diminuição na atividade de AR-DNA ($P < 0.0001$). Por fim, mudanças agudas e crônicas em todos os marcadores de AR não se correlacionaram com a hipertrofia muscular (mCSA, fCSA tipo I e fCSA tupo II) em mulheres ou homens ($P > 0.05$).

Capítulo 2

Doze participantes foram identificados como não-respondedores (média ΔmCSA: -1,32%), e os 12 participantes que apresentaram as maiores respostas hipertróficas foram identificados como o grupo de respondedores (média ΔmCSA: 21,35%). Não houve diferenças basais entre os grupos em mCSA, AR, cAR, nAR ou AR-DNA (P > 0,05). Considerando as respostas agudas, houve diferença significativa entre não-respondedores (+19,5%) e respondedores (-14,4%) na atividade de AR-DNA (ES = -1,39; IC 95%: -2,53 a -0,16; P = 0,015). Não foram observadas diferenças agudas entre os grupos em nenhum outro marcador

de AR ($P > 0,05$). Por fim, não foram observadas diferenças significativas entre os grupos nas respostas crônicas em nenhum marcador de AR ($P > 0,05$).

Conclusões gerais

Considerando conjuntamente os achados de ambos os estudos, é possível sugerir que a hipertrofia muscular pode ser influenciada (associação moderada) pelo conteúdo basal de cAR apenas em homens. Em contrapartida, nem as alterações agudas, nem as alterações crônicas induzidas pelo RT nos conteúdos proteicos de AR, cAR e nAR, ou na atividade de AR-DNA, parecem estar associadas à hipertrofia muscular em mulheres ou homens. Por fim, ao passo que não-respondedores e respondedores apresentaram resultados semelhantes nos conteúdos basais, bem como respostas agudas e crônicas ao RT nos marcadores de AR, nossos resultados não suportam a influência dos marcadores de AR na responsividade à hipertrofia muscular em indivíduos não treinados.

CAPÍTULO 1

ACUTE AND CHRONIC CHANGES IN MUSCLE ANDROGEN RECEPTOR MARKERS ARE NOT ASSOCIATED WITH MUSCLE HYPERTROPHY IN WOMEN AND MEN

Running title: Androgen receptors and muscle hypertrophy

ABSTRACT

Purpose: Androgen receptor (AR) expression and signaling has been regarded as a mechanism for regulating muscle hypertrophy. However, little is known about the associations between acute and chronic changes in skeletal muscle total AR, cytoplasmic AR (cAR), nuclear AR (nAR) and AR DNA-binding (AR-DNA) induced by resistance training (RT) and hypertrophy outcomes in women and men. This study aimed to investigate the acute and chronic effects of RT on skeletal muscle total AR, cAR, nAR contents and AR-DNA in women and men. Additionally, we investigated whether these acute and chronic changes in these markers were associated with muscle hypertrophy in both sexes. **Methods:** Nineteen women and 19 men underwent 10 weeks of RT. Muscle biopsies were performed at baseline, 24 h after the first RT session and 96-120 h after the last session. AR, cAR and nAR were analyzed using Western blotting, and AR-DNA using an ELISA-oligonucleotide assay. Fiber cross-sectional area (fCSA) was analyzed through immunohistochemistry and muscle cross-sectional area (mCSA) by ultrasound. **Results:** At baseline, men demonstrated greater nAR than women. Baseline cAR was significantly associated with type II fCSA hypertrophy in men. Acutely, both sexes decreased AR and cAR, whereas men demonstrated greater decreases in nAR. After 10 weeks of RT, AR and nAR remained unchanged, men demonstrated greater cAR compared to women, and both sexes decreased AR-DNA activity. Acute and chronic changes in AR markers did not correlate with muscle hypertrophy (type I/II fCSA and mCSA) in women or men. **Conclusion**: Baseline cAR content may influence hypertrophy in men, while neither RT-induced acute nor chronic changes in AR, cAR, nAR, and AR-DNA are associated with muscle hypertrophy in women or men.

Keywords: Muscle fiber, protein content, resistance training, sex.

INTRODUCTION

Androgenic hormones and receptors play regulatory roles in several tissues of the human body, including in skeletal muscle (e.g. contractile functions and mass maintenance) (DAVEY *et al.*, 2016; JANSSEN *et al.*, 1994; RUIZEVELD DE WINTER *et al.*, 1991). For decades, acute elevations in circulating anabolic hormones and changes in the total content of androgen receptors (AR) following a bout of resistance training (RT) have been suggested to contribute to muscle hypertrophy (BAMMAN *et al.*, 2001; HULMI *et al.*, 2008; KRAEMER, 1988; KRAEMER *et al.*, 2006; RATAMESS *et al.*, 2005; SPIERING *et al.*, 2009; VINGREN *et al.*, 2009; WILLOUGHBY *et al.*, 2004). However, while transient elevations in hormone levels have been shown to be disassociated from anabolic signaling and hypertrophic adaptations (MORTON *et al.*, 2016; MORTON *et al.*, 2018; WEST *et al.*, 2010; WEST *et al.*, 2009) , the association between RT-induced acute changes in AR and muscle hypertrophy remains to be determined.

In contrast, AR baseline values and RT-induced long-term adaptations (i.e., chronic changes) in AR content have been significantly associated with muscle hypertrophy (AHTIAINEN *et al.*, 2011; MITCHELL *et al.*, 2013; MORTON *et al.*, 2018), although these results are not universal (HATT *et al.*, 2024; MOBLEY *et al.*, 2018). While such findings are difficult to reconcile, it has been suggested that analyzing AR fractionally (i.e., cytoplasmic AR [cAR], nuclear AR [nAR] and AR DNA binding [AR-DNA]) may provide more comprehensive understanding of the role of AR in muscle hypertrophy (MORTON *et al.*, 2018; ROBERTS *et al.*, 2023). Considering the canonical AR signaling pathway, after interacting with androgenic hormones, these receptors undergo conformational transformation and translocate from the cytoplasm to the nucleus (CLAESSENS *et al.*, 2001; DAVEY *et al.*, 2016; EDER *et al.*, 2001; EVANS, 1988). Inside the nucleus, AR bind to DNA, specifically to androgen response elements, to promote specific up- or downregulation of genes that may be related to muscle plasticity (CLAESSENS *et al.*, 2001; DAVEY *et al.*, 2016; EDER *et al.*, 2001; EVANS, 1988; WYCE *et al.*, 2010). Thus, separating and analyzing the different fractions of AR could provide a broader perspective on the potential association between changes in AR content and muscle hypertrophy following chronic RT.

The majority of studies that investigated the effects of RT on acute and chronic AR responses in the skeletal muscle of humans included exclusively male participants (AHTIAINEN *et al.*, 2011; CARDACI *et al.*, 2020; HULMI *et al.*, 2008; KRAEMER *et al.*,

2006; MOBLEY *et al.*, 2018; MORTON *et al.*, 2018; RATAMESS *et al.*, 2005; ROBERTS *et al.*, 2009; SPIERING *et al.*, 2009; SPILLANE *et al.*, 2015; WILLOUGHBY *et al.*, 2004). It is well established that women and men possess different androgenic hormone concentrations (NEALE *et al.*, 2013) and AR contents (HATT *et al.*, 2024; VINGREN *et al.*, 2009). Although the between-sexes hormonal differences do not seem to impact the rate of protein synthesis (WEST *et al.*, 2012) and relative muscle hypertrophy (REFALO *et al.*, 2024; ROBERTS *et al.*, 2020), it cannot be extrapolated to the AR. Indeed, Hatt *et al*. (2024) demonstrated a significant association between changes in the nAR and muscle fiber hypertrophy only for men. While these findings provide valuable insights into the differences between sexes in AR induced by RT, it is possible that responses in the other AR fractions (i.e., cAR) or nuclear AR-DNA binding also differ between women and men.

Therefore, the present study aimed to investigate whether RT induces acute and chronic changes in AR, cAR, nAR and AR-DNA binding. Moreover, we sought to determine if baseline content of AR fractions and AR-DNA binding, and the acute and chronic changes in these markers, were associated with muscle hypertrophy. Finally, we sought to determine if these responses differed between women and men. We hypothesize that baseline values, as well as acute and chronic changes in total AR, cAR, nAR and AR-DNA, would be associated to muscle hypertrophy in men, but not in women.

METHODS

Participants

This study comprises additional analyses using muscle samples obtained from participants of a randomized controlled trial (CHAVES *et al.*, 2024) registered in the Brazilian Registry of Clinical Trials (ReBEC; UTN code: U1111-1283-2662; https://ensaiosclinicos.gov.br/rg/RBR-57v9mrb). In Chaves *et al*. (2024), the sample size was calculated to investigate the effects of overload progression through either increased load (LOADprog) or increased repetitions (REPSprog) on muscle cross-sectional area (mCSA) and one-repetition maximum (1-RM), with the sample size analysis indicating a minimum of 38 participants. However, the present study exclusively analyzed data collected from the "LOADprog" protocol to investigate potential differences in AR changes induced by RT between women and men. Therefore, the sample size calculation was based on findings from

Hatt *et al*. (2024), where significant differences in total AR protein content between women and men were identified. Initially, the effect size was estimated to conduct the Monte Carlo simulation. Subsequently, 10,000 simulations were performed using a significance level of 0.05 and a statistical power of 0.80, which indicated a minimum of 10 women and 10 men.

Initially, 53 previously untrained participants (26 women and 27 men), aged between 18-35 years, non-obese (i.e., BMI < 30 kg/m^2), who self-declared not to have engaged in RT or any other physical training program for at least six months were deemed eligible for participation. Ten subjects discontinued the study due to discomfort reported during the muscle biopsy procedure, and four withdrew due to personal reasons unrelated to the intervention. Of the 39 starting participants, one decided not to perform 24h and Post muscle biopsies and was included only in the mCSA statistical analysis. Thus, 38 participants (age: 24 ± 4 years, body mass: 67.8 ± 12.5 kg, height: 1.70 ± 0.1 m, BMI: 23.4 ± 3.7 kg/m^2), including 19 women (age: 24 ± 5 years, body mass: 62.7 ± 8.5 kg, height: 1.64 ± 0.05 m, BMI: 23.5 ± 3.3 kg/m^2) and 19 men (age: 24 ± 4 years, body mass: 73.0 ± 13.9 kg, height: 1.76 ± 0.07 m, BMI: 23.4 ± 4.2 kg/m^2), completed all the study procedures. Exclusion criteria included pharmacological intake that could impact the endocrine system, hormonal disorders, neuromuscular disorders, injuries or any chronic diseases hindering compliance with the study protocol. Additionally, participants were advised to maintain nutritional habits, not to consume nutritional supplements or engage in structured physical exercise outside the laboratory. Prior to proceeding with the assessments, all the subjects received information regarding all procedures performed during the study and provided written and verbal consent for their participation. The study was approved by the Federal University of São Carlos ethics committee (number: 5.505.441) and was carried out in accordance with the Declaration of Helsinki.

Experimental design

After a preliminary interview, the first visit involved study enrollment with informed consent and mCSA measurement, assessed via ultrasound. After a 72-h interval, the ultrasound measurements were conducted again, followed by the 1-RM test. Subsequently, the 1-RM test was repeated after another 72 h. Both sets of measurements were duplicated to ensure measurement reproducibility. Ninety-six hours after the second 1-RM test, the first muscle biopsy of the vastus lateralis was conducted. Prior to the muscle biopsies, subjects were instructed to abstain from moderate to vigorous physical activity and alcohol consumption for

at least 48 h prior to muscle sample collection and to maintain their habitual diet. After performing baseline biopsies (Pre), the first RT session was performed. Twenty-four hours after the first RT session, participants underwent another muscle biopsy for acute analyses (24 h). For the next 10 weeks, two to three RT sessions were performed per week, totaling 23 sessions. At least 96 h after the 23rd RT session, ultrasound measurements and chronic muscle biopsies were obtained (Post). Ninety-six hours after the last muscle biopsy, 1-RM was performed.

Muscle cross-sectional area

As previously described in Chaves *et al*. (2024), participants were instructed to refrain from engaging in moderate to vigorous physical activity for at least 72 h prior to the assessments. For the procedure, individuals laid in the supine position for 15 minutes for fluid homogenization, after which femur total length was measured using a measuring tape. Following, the distance between the lateral epicondyle and the greater trochanter of the femur was identified. After the determination of 50% of the femur total length, a fixation band was applied to the ankle region to avoid movement during the collection of the images. Next, markings were made every 2 cm in sagittal plane, serving as reference points for sequential B-mode ultrasound (US) imaging using a 7.5 MHz linear probe (MySono U6, Samsung, Sao Paulo, Brazil). Water-soluble water gel was used to ensure appropriate acoustic coupling between the probe and skin without dermal compression. Images of the vastus lateralis muscle were manually reconstructed using PowerPoint software (Microsoft, EUA). After obtaining a panoramic image of the whole muscle, the mCSA was calculated using the "polygonal" tool of the ImageJ software. Connective and bone tissue were properly excluded from the area calculation. The same experienced evaluator performed all procedures involved in the mCSA analysis. The typical error and coefficient of variation, considering two images' acquisitions and mCSA quantifications performed within a 72-h period, were 0.52 cm² and 2.47%, respectively.

Maximal dynamic muscle strength

Maximal dynamic strength was assessed through the unilateral one-maximum repetition test (1-RM) performed on a knee extension equipment (Effort NKR; Nakagym, São Paulo, Brazil), according to Chaves *et al*. (2024). Participants warmed up for 5 minutes in a

cycle ergometer between 20 and 25 km.h-1 (Ergo-Fit®, Pirmasens, Rheinland-Pfalz, Germany). Next, specific warm up included one set of 8 repetitions with approximately 50% of the 1-RM, followed by one set of three repetitions with approximately 70% of the 1-RM, with 2 min of interval between sets. Participants initiated the 1-RM test with their knee flexed at a 90° angle and were instructed to perform the concentric phase of the movement until reaching their maximum comfortable amplitude and to return to the starting position. Participants had up to 5 attempts to reach their 1-RM load five attempts, with 3 minutes of rest attempts. The typical error (TE) and coefficient of variation (CV) between tests was 2.6 kg 5.19%, respectively.

Resistance training protocol

A total of 23 sessions of RT were completed over a 10-week period, with sessions occurring between two and three times per week. A minimum interval of 48 h was maintained between sessions. Prior to each RT session, participants performed five minutes at 20 $km \cdot h^{-1}$ on a cycle ergometer (Ergo-Fit®, Pirmasens, Rheinland-Pfalz, Germany) for general warm-up, followed by a set of 10 repetitions at approximately 50% of training load performed on the unilateral leg extension machine (Effort NKR; Nakagym, São Paulo, Brazil) for specific warm-up. The training protocol consisted of four sets of 9-12 repetitions maximum performed on the unilateral leg extension machine, with 90 s of rest between sets. The exercise selection was based on the participants' training status and specificity in targeting the quadriceps muscles. In the case that subjects performed less than 9 or more than 12 repetitions with a proper form (i.e., to perform a complete range of motion, individually established, with hips and trunk stabilized), loads were adjusted at ~5–10% by an experienced supervisor. This adjustment aimed to ensure that concentric muscle failure occurred within the targeted repetitions maximum zone. All participants accomplished 100% of the training sessions.

Muscle biopsy

Muscle biopsies were performed on the vastus lateralis muscle using percutaneous biopsy needles coupled with a suction apparatus. Prior to the procedure, ~2.5 ml of 1% xylocaine was applied to the biopsy site, and 5-10 min was allowed for the onset of anesthesia. Approximately 100-120 mg of muscle tissue was collected and cleaned from blood and connective tissue before storage. Most of the tissue (~80 mg) was stored in RNA-free cryotubes

for biochemical analyses, while ~20 mg was prepared at the optimum cutting temperature and frozen in nitrogen-cooled isopentane with fibers perpendicularly oriented to the horizontal surface for immunohistochemistry. All samples were subsequently stored at -80°C until analysis.

Tissue homogenization for general cell lysates

Using an analytical scale (Mettler Toledo ME303, Greifensee, Switzerland), approximately 20–25 mg of tissue was homogenized in 1.7 ml tubes using tight-fitting pestles and 400 µL of commercially available 1x cell lysis buffer (Cell Signaling Technology, Cat. No. 9803). Slurries were centrifuged at 500 × G and at 4°C for 10 min. Supernatants were extracted and the protein concentrations were quantified using a commercially available bicinchoninic acid (BCA) assay (Thermo Fisher Scientific, Waltham, MA, USA) in 96-well plates. Plates were colorimetrically read in a microplate reader using Gen5 software (BioTek Instruments, Inc., Winooski, VT, USA). Cell lysates were then prepared for Western blotting detailed in the next section.

Cell localization of androgen receptors proteins

Cytoplasmic and nuclear fractions were separated from cell lysates using a nuclear extraction kit (Abcam, Cat. No.: ab113474) according to the manufacturer's instructions. BCA kits were used to determine the protein concentrations in both the cytoplasmic and nuclear fractions. Cytoplasmic fraction aliquots were stored at -80°C for cAR protein expression analysis and nuclear fractions were separated into two aliquots including one for nuclear AR (nAR) protein expression analysis and the other for AR DNA-binding analyses (AR-DNA).

Western blotting

For Western blotting, general cell lysates were prepared at a concentration of 1 µg/µL with deionized water (diH_2O) and 4x Laemmli buffer and boiled at 100°C for 5 min. A total of 10 µL of Trident Prestained Protein Ladder (GeneTex, Irvine, CA, USA, Cat. No. GTX50875) was loaded into the first well of SDS–polyacrylamide gels (4%–15% Criterion TGX; Bio-Rad Laboratories, Hercules, CA, USA, Cat. No. 5671085), and 15 µL of each sample was added

into the following wells. After electrophoresis at 180 V for 50 min, proteins were subsequently transferred to preactivated (via methanol) polyvinylidene difluoride membranes (Bio-Rad Laboratories, cat. no. 1620177) for two hours at 200 mA. Membranes were Ponceau stained for 10-12 min on a rocking platform, briefly rinsed with diH_2O, dried and imaged using a gel documentation system in colorimetric mode (ChemiDoc Touch; Bio-Rad Laboratories). Next, membranes were reactivated and blocked in nonfat milk for 1 h (5% wt/vol diluted in Tris-buffered saline and 0.1% Tween 20 (TBS-T)). After blocking, membranes were washed three times for five minutes in TBS-T and incubated for 24 h on a rocking platform at 4°C with rabbit anti-human androgen receptor (AR; 1:1,000 in 5% BSA) (D6F11; Cell Signaling Technology, Danvers, MA, United States, Cat. No. 5153). Following incubation with the primary antibody, membranes were washed three times for 5 min each in TBS-T and incubated for 1 h on a rocking platform at room temperature in horseradish peroxidase-conjugated anti-rabbit IgG (Cell Signaling Technology; Cat. No. 7074) which was diluted 1:2,000 in 5% BSA. The membranes were then washed three times for 5 min each in TBS-T, developed with chemiluminescence substrate (Immobilon Forte Western HRP substrate; Millipore; Burlington, MA, USA; Cat. No.: WBLUF0500) and digitally imaged using the chemiluminescence mode (ChemiDoc Touch; Bio-Rad Laboratories). AR (termed total AR throughout), cAR and nAR were identified with molecular weights of ~110 kDa. Using ImageLab software (v6.0.1; Bio-Rad Laboratories), Ponceau density and raw target band density were analyzed using the lane and rectangle tools, respectively. Relative expression of general cell lysate AR protein, cAR and nAR were obtained by the ratio of the raw target band densities and the respective Ponceau lane densities.

Androgen receptors DNA-binding assays

Using the nuclear fraction obtained as previously described, a commercially available ELISA-oligonucleotide kit was used to analyze the AR-DNA binding activity (Aviva Systems Biology Corporation, San Diego, CA, USA; Cat. No.: OKAG00363) according to the manufacturer's instructions. The absorbance was obtained using a microplate-based spectrophotometer with Gen5 software (BioTek Instruments, Inc.). Relative AR DNA-binding activity values were determined by the ratio between the mean of the duplicates and the protein concentration of each sample. The overall intra-assay CV was 13.4%.

Immunohistochemistry

The fiber type I and II cross-sectional areas (fCSA) were obtained through immunohistochemistry, as described in Scarpelli *et al*. (2024). Briefly, samples previously prepared in OCT were transferred from the -80°C freezer to a cryostat (Leica Biosystems, Buffalo Grove, IL, USA) set at -23°C for approximately 30 minutes. The samples were then sectioned at 14 µm, placed on positively charged histology slides and stored at -80°C until batch processing. All timepoints of each subject were placed on the same slide to avoid batch variation. Slides were removed from -80°C and air-dried for two hours. Afterwards, the slides were fixed in acetone at -20°C for five minutes, washed three times for five minutes in 1x phosphate-buffered saline (PBS), and incubated for 10 minutes in 3% H_2O_2. The slides were then washed two times for five minutes in PBS, incubated for one minute with autofluorescence quenching reagent (TrueBlack, Biotium, Fremont, CA, USA; Cat. No. 23007), rewashed three times for five minutes in PBS and blocked with 5% goat serum, 2.5% horse serum and 0.1% Triton X-100 for at least 1 h. After blocking, the slides were incubated overnight at 4°C with a primary antibody cocktail containing 1:100 Dystrophin (GeneTex, No. GTX57970) + 1:100 BA-D5 (Myosin Heavy Chain I, Developmental Studies Hybridoma Bank, Cat. No. BA-D5) + 2.5% horse serum in PBS. The next day, the slides were washed four times for five min in PBS and incubated for 60 min in a secondary antibody cocktail containing 1:250 goat anti-mouse IgG2b Alexa Fluor 647 (Thermo Fisher Scientific, Cat. No. A-21242) + 1:250 goat anti-rabbit IgG DyLight488 (Vector Laboratories, Cat. No. DI-1488) in PBS. After three washes of five min in PBS, coverslips were placed on slides using PBS and glycerol as mounting media.

Using a fluorescence microscope (Nikon Instruments; Melville, NY, USA), an investigator blinded for sexes captured 10 digital images per sample using a 20x objective, aiming for a minimum of 50 fibers per sample (MACKEY *et al.*, 2009). Myovision software was used for automated fCSA measurements and type I and type II fiber counting (WEN *et al.*, 2018). After software analysis, measurement-related outline errors (e.g., quantification of empty spaces or damaged fibers) were rectified through visual inspection and excluded from the analysis.

Statistical analyses

The statistical analyses were carried out with SAS 9.3 software (SAS Institute, Inc., Cary, NC, USA) and GraphPad Prism 9.5 (GraphPad Software, San Diego, CA, USA), in which

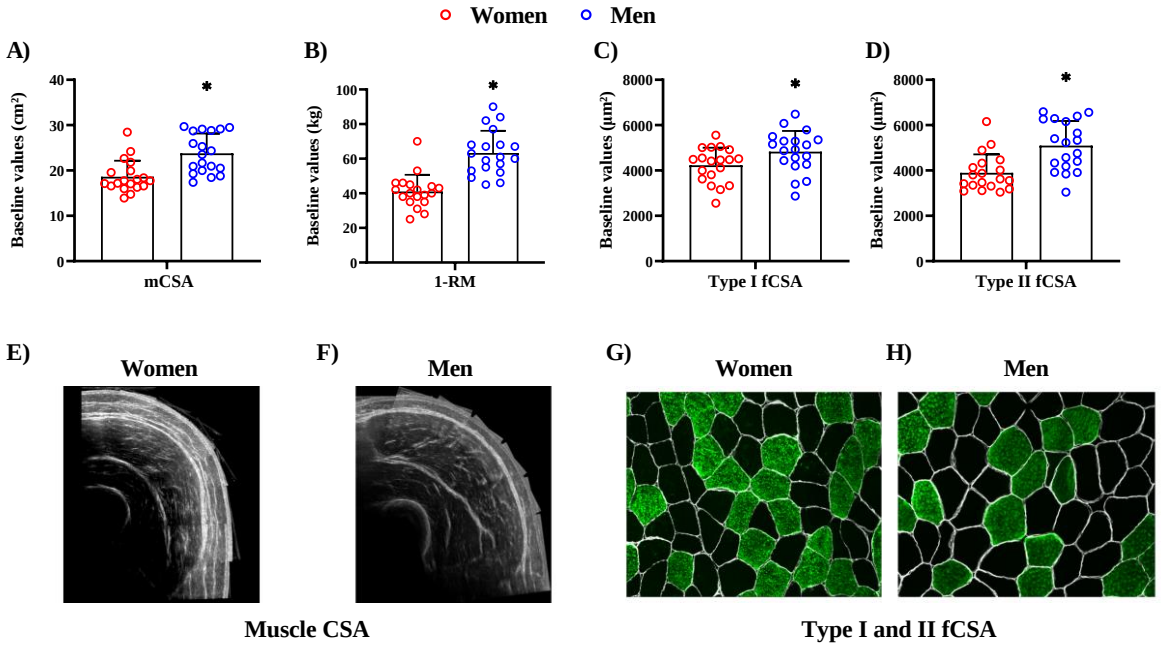
all the graphs were also designed. Paired t tests were used to analyze the general adaptations induced by RT (i.e., mCSA, type I fCSA, type II fCSA and 1-RM) across the entire sample. Unpaired t-tests were used to compare baseline values between women and men for all outcomes (mCSA, type I fCSA and type II fCSA, 1-RM, AR, cAR, nAR, AR-DNA). When significant between-sex baseline differences were observed (i.e., mCSA, type I fCSA and type II fCSA, 1-RM and nAR), analysis of covariance (ANCOVA) was performed with sex as a fixed factor, baseline values as a covariate, and subjects as a random factor. Tukey's post hoc test was used when F was significant. For acute AR, cAR and AR-DNA (Pre vs. 24 h) and chronic changes (Pre vs. Post), mixed model analyses were adopted considering sex and time as fixed factors and participants as random factors. In the case of significant F values, Tukey's adjustment was used for multiple comparisons. Additionally, we compared the acute and chronic changes in AR, cAR, nAR and AR-DNA between women and men using effect sizes (ES) with 95% confidence intervals (CI) (HEDGES *et al.*, 1985). Positive and negative CIs not crossing zero (0) were considered significant (NAKAGAWA *et al.*, 2007). Associations between baseline, acute and chronic (relative and absolute) changes in AR, cAR, nAR and AR-DNA and changes in mCSA, type I fCSA and type II fCSA were determined using Pearson's correlations for women and men separately. The correlation magnitudes were classified as very weak (< 0.20), weak (0.20-0.39), moderate (0.40-0.59), strong (0.60-0.79) or very strong (>0.80) (EVANS, 1996). Significance was established as $P < 0.05$.

RESULTS

Baseline sex-based comparisons

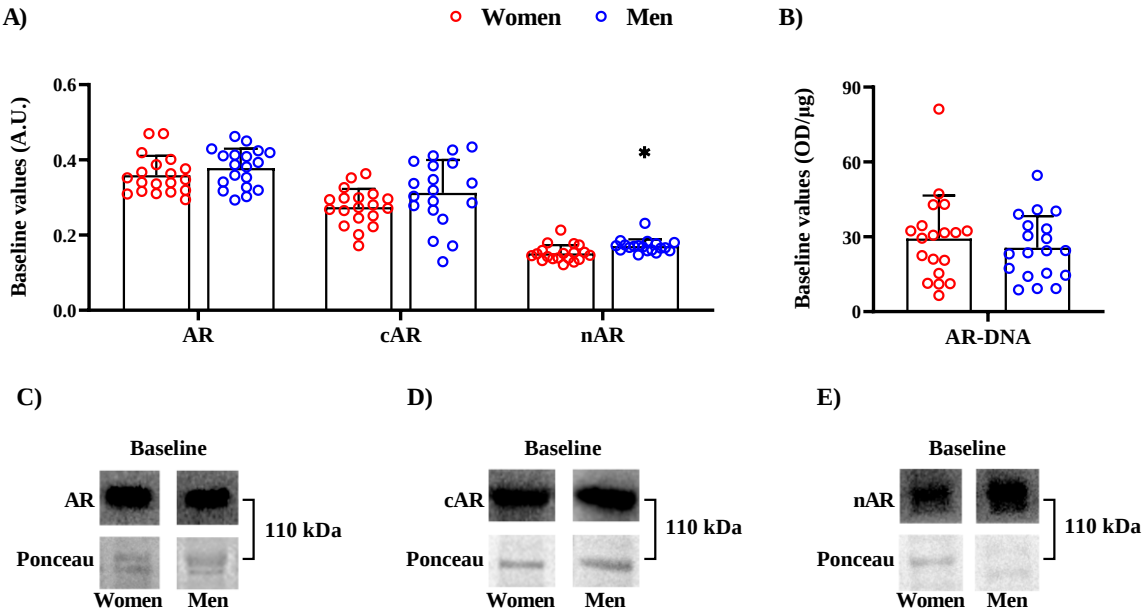
Baseline sex-based comparisons for mCSA, type I fCSA, type II fCSA and 1-RM are displayed in Figure 1, and AR, cAR, nAR and AR-DNA are illustrated in Figure 2. Unpaired t-tests demonstrated significantly greater baseline values of mCSA ($P = 0.0003$), type I fCSA ($P = 0.037$), type II fCSA ($P < 0.001$), 1-RM ($P < 0.001$) and nAR ($P = 0.005$) for men compared to women. There were no significant differences in the baseline values of total AR, cAR and AR-DNA ($P > 0.05$ for all) between women and men.

Figure 1. Baseline sex-based comparisons



Legend: Absolute baseline values of women and men of general lysate androgen receptor (AR, panel A), cytoplasmic AR (cAR, panel A), nuclear AR (nAR, panel A) and AR DNA-binding (AR-DNA, panel B). Individual data from women are represented by red circles and individual data from men are represented by blue circles. Representative western blot protein content and respective Ponceaus are displayed for AR (panel C), cAR (panel D) and nAR (panel E) for women and men at baseline. A.U.: arbitrary units; OD/µg: optical density per microgram; kDa: kilodalton. *Significantly different from women ($P < 0.05$).

Figure 2. General adaptations to resistance training



Legend: Absolute baseline values of women and men of muscle cross-sectional area (mCSA, panel A), one-repetition maximum test (1-RM, panel B), type I fiber cross-sectional area (Type I fCSA, panel C) and type II fiber cross-sectional area (Type II fCSA, panel D). Individual data from women are represented by red circles and individual data from men are represented by blue circles. Ultrasound images of vastus lateralis muscles of women (panel E) and men (panel F). Immuno-histochemistry staining images of muscles of women (panel G) and men (panel H), with dystrophin labeled in white, type I fCSA stained in green and type II fCSA unstained in black. cm2: centimeter square; kg: kilograms; µm2: micrometer square. *Significantly different from women ($P < 0.05$).

General adaptations to resistance training

The general adaptations to RT results can be found in Chaves *et al*. (2024) and Scarpelli *et al*., (2024). Briefly, there were significant increases in mCSA, type II fCSA, and 1RM from pre- to post-training, without significant changes in type I fCSA. Additionally, no significant differences were observed between sexes for any of these variables.

Acute AR marker responses

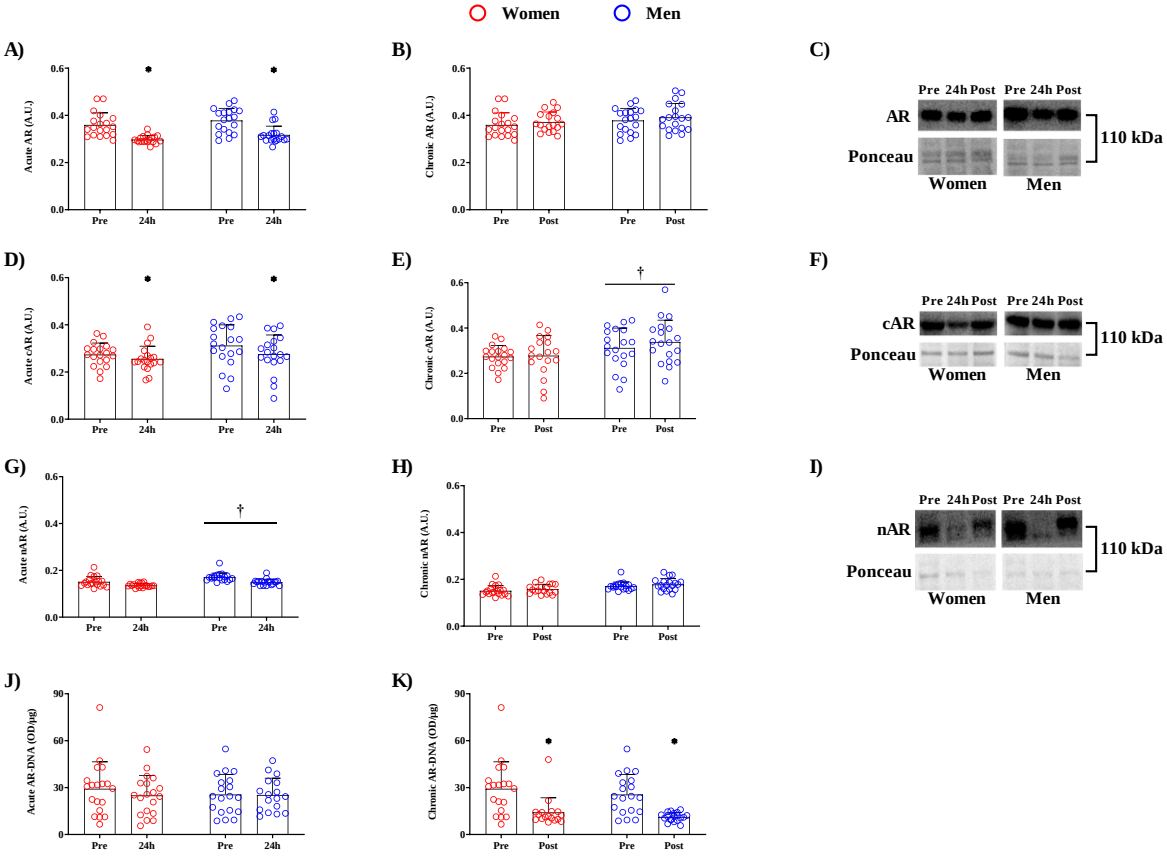
The following data are related to acute (i.e., first bout) effects on AR markers. Mixed model analysis revealed only a main time effect for total AR ($P < 0.0001$) and cAR ($P = 0.017$), in which the 24 h values were lower than Pre values. There were no significant main effects or

interaction for AR-DNA ($P > 0.05$). Additionally, the ANCOVA demonstrated a significant difference in the nAR between sexes ($P = 0.010$), where men presented greater acute decreases compared to women. The 95% CI of ES indicated no significant differences between women (W) and men (M) for the differences in delta changes in AR (ΔW vs. ΔM: ES (95% CI) = 0.02 (-0.59 to 0.63)), cAR (ΔW vs. ΔM: ES (95% CI) = -0.27 (-0.93 to 0.41)), nAR (ΔW vs. ΔM: ES (95% CI) = -0.32 (-0.75 to 0.13)) and AR-DNA (ΔW vs. ΔM: ES (95% CI) = 0.16 (-0.26 to 0.58)). The acute results are displayed in Figure 3 and are also detailed in Table 1 of supplemental digital content (SDC).

Chronic AR marker responses

The following data are related to 10-week training effects on AR markers. The mixed model analysis showed only a main time effect for AR-DNA ($P < 0.0001$), where Post values were lower than Pre values. Additionally, there was only a main sex effect on the cAR ($P = 0.021$), in which men presented greater protein content than women. There were no significant main effects or interaction for AR ($P > 0.05$). The ANCOVA showed no significant between-sex differences in the nAR ($P = 0.520$). The 95% CI of ES analysis revealed no significant differences between sexes for changes in AR (ΔW vs. ΔM: ES (95% CI) = -0.09 (-0.63 to 0.46)), cAR (ΔW vs. ΔM: ES (95% CI) = 0.27 (-0.41 to 0. 94)), nAR (ΔW vs. ΔM: ES (95% CI) = -0.13 (-0.67 to 0. 41)) and AR-DNA (ΔW vs. ΔM: ES (95% CI) = 0.06 (-0.40 to 0.52)). The chronic results are displayed in Figure 3 and are also detailed in Table 2 of SDC.

Figure 3. Sex-based comparisons in acute and chronic changes in AR markers



Legend: Absolute baseline (Pre) and acute 24 h values of women and men of general lysate androgen receptor (AR, panel A), cytoplasmic AR (cAR, panel D), nuclear AR (nAR, panel G) and AR DNA-binding (AR-DNA, panel J); absolute Pre and chronic post-training (Post) values of women and men of general lysate androgen receptor (AR, panel B), cytoplasmic AR (cAR, panel E), nuclear AR (nAR, panel H) and AR DNA-binding (AR-DNA, panel K) are represented in panels. Representative western blot protein content and respective Ponceaus are displayed for AR (panel C), cAR (panel F) and nAR (panel I) for women and men at Pre, 24 h and Post. Individual data from women are represented by red circles and individual data from men are represented by blue circles. A.U.: arbitrary units; OD/µg: optical density per microgram; kDa: kilodalton. *Significantly different from Pre (main time effect, P < 0.05); †Significantly different from women (main sex effect; P < 0.05); †Significantly different from women (ANCOVA; P < 0.05).

Correlations

For men, there was a significant and moderate correlation between baseline cAR protein content and changes in type II fCSA ($r = 0.499$; $P = 0.030$) but not in mCSA and type I fCSA ($P > 0.05$). However, there were no significant correlations between baseline total AR, nAR or AR-DNA and changes in mCSA, type I or type II fCSA ($P > 0.05$). For women, there

were no significant correlations between baseline AR-related outcomes and changes in mCSA, type I and type II fCSA ($P > 0.05$). Correlations between baseline AR, cAR, nAR and AR-DNA and changes in mCSA, type I and type II fCSA for women and men are shown in Table 1.

Table 1. Correlations between baseline absolute values of AR, cAR, nAR and AR-DNA and absolute changes in mCSA, type I fCSA and type II fCSA.

Variables	WOMEN						MEN					
	mCSA (Δcm^2)		fCSA I ($\Delta \mu m^2$)		fCSA II ($\Delta \mu m^2$)		mCSA (Δcm^2)		fCSA I ($\Delta \mu m^2$)		fCSA II ($\Delta \mu m^2$)	
	r	***P***	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***
Pre AR (A.U.)	0.102	0.678	-0.050	0.849	0.005	0.984	0.061	0.803	0.369	0.120	0.139	0.570
Pre cAR (A.U.)	-0.067	0.784	-0.099	0.706	-0.086	0.742	-0.080	0.744	0.445	0.056	0.499	0.029*
Pre nAR (A.U.)	0.028	0.911	0.120	0.646	0.259	0.316	0.231	0.341	-0.198	0.416	-0.234	0.335
Pre AR-DNA (OD/µg)	-0.016	0.949	0.241	0.351	0.067	0.796	0.326	0.173	0.195	0.425	0.196	0.421

Pre: baseline time point; AR: androgen receptor protein content in the whole tissue lysate; cAR: androgen receptor protein content in the cytoplasmic fraction; nAR: androgen receptor protein content in the myonuclear fraction; AR-DNA: androgen receptor DNA-binding activity; mCSA: muscle cross-sectional area; fCSA I: type I fiber cross-sectional area; fCSA II: type II fiber cross-sectional area; A.U.: arbitrary expression units; OD/ µg: Optical density per microgram; Δcm^2: absolute changes in mCSA; $\Delta \mu m^2$: absolute changes in fCSA. *Statistically significant ($P < 0.05$).

There were no significant correlations when considering relative acute and chronic changes in total AR, cAR, nAR or AR-DNA with changes in mCSA, type I and type II fCSA for both women and men ($P > 0.05$) (Table 2). Similar results were observed considering correlations between absolute acute and chronic changes in these molecular markers with changes in mCSA, type I and type II fCSA for both women and men. Correlations considering absolute values are detailed in Tables 3 and 4 of SDC.

Table 2. Correlations between acute and chronic relative changes in AR, cAR, nAR and AR-DNA and relative changes in mCSA, type I fCSA and type II fCSA.

Variables	WOMEN						MEN					
	mCSA (Δ%)		fCSA I (Δ%)		fCSA II (Δ%)		mCSA (Δ%)		fCSA I (Δ%)		fCSA II (Δ%)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Acute AR (Δ%)	-0.103	0.676	-0.154	0.553	-0.082	0.754	-0.127	0.605	-0.089	0.717	0.159	0.516
Acute cAR (Δ%)	0.340	0.155	0.211	0.416	0.238	0.358	-0.434	0.063	-0.014	0.954	0.209	0.390
Acute nAR (Δ%)	0.044	0.858	-0.132	0.614	-0.165	0.527	-0.165	0.501	-0.019	0.938	-0.023	0.924
Acute AR-DNA (Δ%)	-0.455	0.050	0.025	0.923	-0.026	0.922	-0.182	0.484	0.163	0.530	0.249	0.334
Chronic AR (Δ%)	-0.324	0.190	-0.071	0.787	-0.077	0.769	-0.352	0.140	0.066	0.787	0.190	0.436
Chronic cAR (Δ%)	0.190	0.450	0.219	0.397	0.354	0.163	-0.312	0.193	-0.132	0.589	-0.017	0.946
Chronic nAR (Δ%)	-0.138	0.584	-0.311	0.224	-0.362	0.153	-0.443	0.057	0.059	0.810	0.134	0.584
Chronic AR-DNA (Δ%)	-0.118	0.651	-0.187	0.472	-0.188	0.469	-0.070	0.776	-0.208	0.393	-0.122	0.620

Acute (24 h – Pre); Chronic: Post-intervention - Pre; AR: androgen receptor protein content in the whole tissue lysate; cAR: androgen receptor protein content in the cytoplasmic fraction; nAR: androgen receptor protein content in the myonuclear fraction; AR-DNA: androgen receptor DNA-binding activity; mCSA: muscle cross-sectional area; fCSA I: type I fiber cross-sectional area; fCSA II: type II fiber cross-sectional area; Δ%: relative changes.

DISCUSSION

As previously mentioned, several investigations have sought to determine how muscle total AR protein content is affected by RT, and some (though not all) studies suggest that this marker may be predictive of hypertrophic outcomes. However, given that prior investigations have primarily assayed total AR content in muscle, and that canonical AR signaling involves receptor translocation to the nucleus and AR-DNA interactions to affect downstream gene expression, insight into AR actions in different muscle cell fractions is limited. Hence, this is the first study to extensively investigate how AR markers in skeletal muscle are acutely and chronically affected by RT in women and men. Several observations were notable. First, while baseline nAR values were greater in men versus women, women did present similar total AR, cAR and AR-DNA values relative to men which contrasts robust sex differences in circulating androgens reported in the scientific literature (NEALE *et al.*, 2013). Second, decreases in total AR and cAR were observed for both sexes 24 h following the first naïve training bout, whereas nAR was significantly decreased only in men. Third, AR-DNA binding significantly decreased for both sexes following 10 weeks of RT. Finally, although there was a significant association

between baseline cAR content and type II fCSA hypertrophy in men, there were no significant associations between acute or chronic AR, cAR, nAR, and AR-DNA responses with myofiber hypertrophy outcomes in either sex. Details of each outcome are discussed sequentially in the following paragraphs.

Baseline sex-based comparisons

No significant between-sex differences were observed in total AR values prior to the intervention. This contrasts the findings from Vingren *et al*. (2009) who reported greater baseline total AR protein content in men, while Hatt *et al*. (2024) reported greater baseline total AR protein content in women. Although these mixed findings are difficult to reconcile, the two prior reports along with the current data suggest that sex differences in muscle tissue AR protein content are likely minimal. Indeed, this contrasts a plethora of evidence indicating men present ~20-fold greater total and free blood testosterone concentrations and suggests that AR signaling in the skeletal muscle of women is intact. Extracting cAR and nAR from tissue along with analyzing AR-DNA binding activity provided additional insights. In this regard, greater nAR protein content in men versus women and no differences in cAR and AR-DNA were observed. In the absence of ligands, inactive AR are localized to the cytoplasm of cells and are associated with heat shock proteins. After binding to androgens, the AR undergo conformational changes, dissociate from these chaperones, translocate to the nucleus, and act as a transcriptional regulator through binding to androgen response elements (DAVEY *et al.*, 2016; EDER *et al.*, 2001). Speculatively, greater baseline nAR protein content in men may be attributed to the higher levels of circulating androgen hormones (NEALE *et al.*, 2013), (NEALE *et al.*, 2013), leading to increased exposure of AR to activating hormones and thus higher rates of translocation to the nucleus of the cells. However, we did not collect blood at the respective time points, so this complex interaction between sexes requires further investigation.

Our results demonstrated no significant correlation between baseline total AR protein content and muscle hypertrophy in women and men. In contrast, Morton *et al*. (2018) reported a significant correlation between preintervention total AR protein content and muscle hypertrophy in trained young men. Although our total AR association data differed from this prior report, cAR was moderately correlated with type II fCSA in men ($r = 0.499$; $P = 0.0298$). Given that total AR protein is a summation of the cAR and nAR, analyzing only the total AR fraction provides limited insights. Although we interpret our association findings with caution,

it appears that baseline cAR content partially explains the hypertrophic adaptations in previously untrained men. However, as there were no significant associations between any AR-related outcomes with hypertrophy for women, and there were also no between-sex differences in mCSA, type I and II fCSA, other mechanisms that were not investigated in the present study (e.g., mechanosensitive signaling mechanisms) are likely responsible for the hypertrophic effects observed in women.

Acute responses

The acute effects of RT on AR protein expression have been extensively investigated during the last two decades although no study has sought to investigate how training acutely affects all the AR markers assayed herein. Our results revealed that both women and men presented acute decreases in total AR and cAR protein levels 24 h after RT. Additionally, although ANCOVA revealed a statistically significant difference, with more pronounced decreases in nAR for men compared to women, this finding was not replicated when considering the analysis of uncertainty surrounding the ES (ΔW vs. ΔM: ES (95% CI) = -0.32 (-0.75 to 0.13)). This discrepancy may be attributed to the influence of confounding covariates (e.g., baseline differences), which are likely to have affected the calculated effect size (NAKAGAWA *et al.*, 2007).

To date, only two studies have compared sex-based differences in acute AR protein responses to RT. Vingren *et al*. (2009) demonstrated that both women and men presented a significant reduction in AR protein content shortly after RT (i.e., 10 and 70 min; six sets of squats at 80% of the 1RM). Conversely, immunohistochemical experiments by Hatt *et al*. (2024) demonstrated acute increases in AR and nAR for both sexes, while the immunoblotting results showed increases in total AR protein content only for men 48 h following RT (300 eccentric repetitions). It seems tempting to assume that RT induces AR protein content decreases at earlier timepoints followed by increases at later timepoints without noticeable influences of sex, albeit it is important to consider RT protocol differences between studies. In contrast to Vingren *et al*. (2009) and our study, Hatt *et al*. (2024) adopted a protocol involving a high volume of eccentric actions to induce muscle damage. Thus, future studies should adopt the same RT protocol to verify the influence of the timepoint on the response of the canonical AR signaling markers.

Contrary to all other assayed AR variables, acute AR-DNA binding affinity was unaltered following the first naïve bout of training. Given that all the other AR fractions decreased 24 h after RT, it is surprising that the AR-DNA results did not follow the same pattern. Despite not reaching significance, however, both women and men demonstrated numerical decreases in this marker (-14.5% and -1.2%, respectively), which may have been masked by a high intra-assay variability. Nonetheless, our AR-DNA results agree with the results reported by Cardaci *et al*. (2020), who also demonstrated no significant changes in AR-DNA binding 24 h after RT.

RT-induced acute responses in AR were first investigated due to its interaction with endogenous androgen hormones, which are acutely elevated after an RT session and were believed to play a fundamental role in muscle hypertrophy (KRAEMER *et al.*, 2005). While acute elevations androgen hormones have been reported to be disassociated from RT-induced skeletal muscle hypertrophy (WEST *et al.*, 2010), it has been suggested a possible impact of acute changes in AR protein content on muscle adaptations (AHTIAINEN *et al.*, 2011). Nonetheless, the present study demonstrates for the first time that acute responses in the assayed AR markers are not associated with RT-induced skeletal muscle hypertrophy in women or men.

Chronic responses

Although RT-induced total AR protein responses have been examined in men (AHTIAINEN *et al.*, 2011; MITCHELL *et al.*, 2013; MOBLEY *et al.*, 2018; MORTON *et al.*, 2018), our study contributes novel insights into how chronic training affects total AR protein, cAR protein, nAR protein, and AR-DNA binding in both sexes. RT did not affect total AR content for either sex, albeit cAR protein content was greater in men across the intervention versus women. It is crucial to note that this latter difference was not supported by the analysis of uncertainty surrounding the ES (ΔW vs. ΔM: ES (95% CI) = 0.27 (-0.41 to 0.94)). This discrepancy suggests that the significance observed in mixed model may have been influenced by random variations (NAKAGAWA *et al.*, 2007), underscoring the importance of careful interpretation of the results. Additionally, there were no significant between-sex differences in nAR. Chronic training diminished AR-DNA binding in both sexes, which while novel, is difficult to reconcile. However, this finding resonates with the report by Mobley *et al*. (2018) who showed 12 weeks of RT in previously untrained men downregulated total AR protein content in lower, moderate, and higher hypertrophic responders. Findings from both studies

indicate that chronic RT may result in a negative feedback loop on AR signaling markers, and future studies determining whether this potential feedback affects AR-dependent gene transcription or signifies a refinement in this mechanism are warranted (DAMAS *et al.*, 2018).

Several studies have investigated the associations between chronic changes in total AR content and muscle hypertrophy. For instance, despite nonsignificant increases in total AR content, a positive association has been observed between changes in AR content and muscle hypertrophy in untrained men (AHTIAINEN *et al.*, 2011; MITCHELL *et al.*, 2013). In contrast, Hatt *et al*. (2024) observed significant increases in total AR content, without significant correlation with muscle hypertrophy in women and men. In the present study, we demonstrated no significant associations existed between chronic changes in total AR, cAR, nAR and AR-DNA binding with muscle hypertrophy in women, men, or all 38 participants (data not shown). In contrast to our findings, Hatt *et al*. (2024) found an association between fCSA and nAR. However, it is noteworthy that the authors only used high responders to investigate this association, which may explain the differences between our results. Taken together, the absence of associations questions the potential importance of chronic AR marker adaptations to the hypertrophic response to RT.

Limitations

This study is not without limitations. First, despite acute and chronic timepoints and 10 weeks of RT with a sample composed by men and women (n = 38), we were limited to a three biopsy timepoints, which diminished our capacity to map the translocation of AR. Future studies aiming to examine the translocation of AR after RT should consider analyzing additional muscle biopsy timepoints following exercise. Second, we did not collect blood at the biopsy time points for hormonal analysis. Nevertheless, it has been shown that systemic and local concentrations of androgenic hormones, as well as sex-based differences do not impact protein synthesis (WEST *et al.*, 2009) and muscle hypertrophy (MORTON *et al.*, 2016; MORTON *et al.*, 2018). Furthermore, individuals with greater AR content at baseline did not demonstrate higher androgenic hormone concentrations (MORTON *et al.*, 2018). Third, the intra-assay for AR-DNA binding activity was relatively high, and due to logistical constraints, we were unable to replicate this assay. Therefore, caution is advised in interpreting this finding. Fourth, only the muscle hypertrophy of the vastus lateralis was investigated in this study. Therefore, these findings cannot be extrapolated for other muscle groups. Furthermore, given

the potential for non-uniformity within the vastus lateralis, additional data points would be necessary to capture a comprehensive representation of the entire muscle, which is lacking in the present investigation. It is important to note that non-uniform responses are more frequently encountered when different exercises are performed (FONSECA *et al.*, 2014), which was not observed in the current study. Fifth, it was not feasible to control for menstrual cycle phase and hormonal contraceptive phase. However, existing literature suggests that the menstrual cycle phase does not significantly impact muscle mass (THOMPSON *et al.*, 2021) or AR content (EKENROS *et al.*, 2017). Furthermore, the hormonal contraceptive use appears to have no effect on muscle mass (THOMPSON *et al.*, 2021), although its impact on AR remains unexamined. Finally, it is important to highlight that our study included healthy untrained young women and men; thus, our results cannot be extrapolated to other populations in different training states (i.e., trained), ages (i.e., middle-aged and older populations), or anabolic-androgenic steroid users which likely present robust differences in the assayed markers.

CONCLUSION

Pre-training cytoplasmic androgen receptor protein content may influence muscle hypertrophy in men, while neither RT-induced acute nor chronic changes in total androgen receptor protein content, nuclear androgen receptor content, or androgen receptor DNA binding affinity are associated with muscle hypertrophy in women and men.

SUPPLEMENTAL MATERIAL

Baseline and post-training absolute values in androgen receptor markers and correlations between absolute acute and chronic changes in androgen receptor markers and muscle hypertrophy considering absolute values

Table 1. Muscle protein content for total AR, cAR, nAR and AR-DNA activity following one bout of training.

Variables	WOMEN			MEN			ES (95% CI)
	Pre	24 h	Δ	Pre	24 h	Δ	ΔW vs. ΔM
AR (A.U.)	0.36 ± 0.05	0.30 ± 0.02*	-0.06	0.38 ± 0.05	0.32 ± 0.04*	-0.06	0.02 (-0.59 to 0.63)
cAR (A.U.)	0.27 ± 0.05	0.26 ± 0.05*	-0.01	0.31 ± 0.09	0.28 ± 0.08*	-0.03	-0.27 (-0.93 to 0.41)
nAR (A.U.)	0.15 ± 0.02	0.14 ± 0.01	-0.01	0.17 ± 0.02	0.15 ± 0.01†	-0.02	-0.32 (-0.75 to 0.13)
AR-DNA (OD/µg)	29.29 ± 17.21	25.04 ± 12.68	-4.25	25.62 ± 12.70	25.30 ± 10.72	-0.32	0.16 (-0.26 to 0.58)

Pre: baseline time point; 24 h: 24 hours following first resistance training bout; total AR: androgen receptor protein content in the whole tissue lysate; cAR: androgen receptor protein content in the cytoplasmic fraction; nAR: androgen receptor protein content in the myonuclear fraction; AR-DNA: androgen receptor DNA-binding activity; A.U.: arbitrary expression units; OD/µg: optical density per microgram; ES (95% CI): effect sizes with 95% confidence intervals; **Δ**: absolute values (not percentages) changes from Pre to 24 h; *Significantly different from Pre (main time effect, $P < 0.05$). †Significantly greater decreases compared to women (ANCOVA; $P < 0.05$). Values presented as mean ± SD.

Table 2. Muscle protein content for total AR, cAR, nAR and AR-DNA activity following 10 weeks of resistance training.

Variables	WOMEN			MEN			ES (95% CI)
	Pre	Post	Δ	Pre	Post	Δ	ΔW vs. ΔM
AR (A.U.)	0.36 ± 0.05	0.37 ± 0.04	0.01	0.38 ± 0.05	0.39 ± 0.06	0.01	-0.09 (-0.63 to 0.46)
cAR (A.U.)	0.27 ± 0.05	0.28 ± 0.09	0.01	0.31 ± 0.09#	0.34 ± 0.10#	0.03	0.27 (-0.41 to 0. 94)
nAR (A.U.)	0.15 ± 0.02	0.16 ± 0.02	0.01	0.17 ± 0.02	0.18 ± 0.03	0.01	-0.13 (-0.67 to 0. 41)
AR-DNA (OD/µg)	29.29 ± 17.21	14.16 ± 9.32*	-15.13	25.62 ± 12.70	11.25 ± 2.77*	-14.37	0.06 (-0.40 to 0.52)

Pre: baseline time point; Post: post-intervention time point (following 10 weeks of resistance training); total AR: androgen receptor protein content in the whole tissue lysate; cAR: androgen receptor protein content in the cytoplasmic fraction; nAR: androgen receptor protein content in the myonuclear fraction; AR-DNA: androgen receptor DNA-binding activity; A.U.: arbitrary expression units; OD/µg: optical density per microgram; ES (95% CI): effect sizes with 95% confidence intervals; **Δ**: absolute values (not percentages) changes from Pre to Post; *Significantly different from Pre (main time effect, $P < 0.05$). #Significantly different from women (main sex effect, $P < 0.05$). Values presented as mean ± SD.

Table 3. Correlations between acute absolute changes in AR, cAR, nAR and AR-DNA and type I fCSA, type II fCSA and mCSA absolute changes.

Variables	WOMEN						MEN					
	fCSA I		fCSA II		mCSA		fCSA I		fCSA II		mCSA	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
AR (A.U.)	-0.092	0.690	-0.104	0.726	-0.017	0.944	-0.156	0.524	0.032	0.896	-0.151	0.536
cAR (A.U.)	0.137	0.591	0.140	0.599	0.391	0.098	-0.157	0.521	0.003	0.990	-0.295	0.221
nAR (A.U.)	-0.099	0.461	-0.192	0.706	0.014	0.955	-0.017	0.946	0.048	0.844	-0.190	0.437
AR-DNA (OD/µg)	-0.147	0.556	-0.154	0.573	-0.356	0.134	0.091	0.727	0.115	0.661	-0.158	0.545

AR: androgen receptors; cAR: cytoplasmic androgen receptors; nAR: nuclear androgen receptors, AR-DNA: androgen receptors DNA-binding, fCSA I: muscle fiber cross muscle area type I; fCSA II: muscle fiber cross-sectional area type II; mCSA: vastus lateralis muscle cross-sectional area; A.U.: arbitrary expression units; OD/µg: optical density per microgram.

Table 4. Correlations between chronic absolute changes in AR, cAR, nAR and AR-DNA and type I fCSA, type II fCSA and mCSA absolute changes.

Variables	WOMEN						MEN					
	fCSA I		fCSA II		mCSA		fCSA I		fCSA II		mCSA	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
AR (A.U.)	-0.033	0.899	-0.092	0.725	-0.253	0.312	0.084	0.732	0.204	0.402	-0.342	0.151
cAR (A.U.)	0.164	0.529	0.308	0.229	0.201	0.423	-0.152	0.534	-0.010	0.968	-0.404	0.086
nAR (A.U.)	-0.289	0.261	-0.364	0.151	-0.104	0.682	0.061	0.804	0.164	0.502	-0.392	0.097
AR-DNA (OD/µg)	-0.281	0.275	-0.182	0.483	-0.169	0.518	0.060	0.820	0.040	0.878	-0.207	0.402

AR: androgen receptors; cAR: cytoplasmic androgen receptors; nAR: nuclear androgen receptors, AR-DNA: androgen receptors DNA-binding, fCSA I: muscle fiber cross muscle area type I; fCSA II: muscle fiber cross-sectional area type II; mCSA: vastus lateralis muscle cross-sectional area; A.U.: arbitrary expression units; OD/µg: optical density per microgram.

CAPÍTULO 2

ANDROGEN RECEPTOR MARKERS DO NOT DIFFER BETWEEN NONRESPONDERS AND RESPONDERS TO RESISTANCE TRAINING-INDUCED MUSCLE HYPERTROPHY

Running title: Androgen receptors and responsiveness

ABSTRACT

The aim of this study was to investigate whether baseline values and acute and chronic changes in androgen receptors (AR) markers, including total AR, cytoplasmic (cAR) and nuclear (nAR) fractions, as well as DNA-binding activity (AR-DNA), are involved in muscle hypertrophy responsiveness by comparing young nonresponder and responder individuals. After 10 weeks of resistance training (RT), participants were identified as nonresponders using two typical errors (TE) obtained through two muscle cross-sectional area (mCSA) ultrasound measurements (2×TE; 4.94%), and the highest responders within our sample were numerically matched. Muscle biopsies were performed at baseline, 24h after the first RT session (acute responses) and 96h after the last session (chronic responses). AR, cAR and nAR were analyzed using Western blotting, and AR-DNA using an ELISA-oligonucleotide assay. Twelve participants were identified as nonresponders (ΔmCSA: -1.32%), and twelve as responders (ΔmCSA: 21.35%). There were no baseline differences between groups in mCSA, AR, cAR, nAR or AR-DNA ($P > 0.05$). For acute responses, there was a significant difference between nonresponders (+19.5%) and responders (-14.4%) in AR-DNA (ES = -1.39; 95% CI: -2.53 to -0.16; $P = 0.015$). There were no acute between-group differences in any other AR markers ($P > 0.05$). No significant differences between groups were observed in chronic responses across any AR markers ($P > 0.05$). Nonresponders and responders presented similar baseline, acute and chronic results for the majority of the AR markers. Thus, our findings do not support the influence of AR markers on muscle hypertrophy responsiveness to RT in untrained individuals.

NEW & NOTEWORTHY

We explored, for the first time, the influence of AR through the separation of cytoplasmic and nuclear cell fractions (i.e., cAR, nAR and AR-DNA) on muscle hypertrophy responsiveness to resistance training. The absence of muscle hypertrophy in naïve individuals does not seem to be explained by baseline values, and acute or chronic changes in AR markers.

Keywords: Skeletal muscle, responsiveness, resistance exercise, hormonal receptors, molecular mechanisms.

INTRODUCTION

Resistance training (RT) is widely recommended as the primary exercise modality to promote muscle hypertrophy (ACSM, 2009). Nevertheless, even following RT programs, some individuals do not present meaningful muscle hypertrophy (i.e., nonresponders) (AHTIAINEN *et al.*, 2016; HUBAL *et al.*, 2005; LIXANDRÃO *et al.*, 2024; SWINTON *et al.*, 2018). RT-induced muscle growth arises from molecular responses within a complex network of signaling mechanisms triggered by external loading stimuli (ROBERTS *et al.*, 2023). Among the mechanisms potentially involved in individual responsiveness, androgen receptors (AR) have been suggested to modulate the magnitude of muscle hypertrophy (ROBERTS *et al.*, 2023). To date, research on the role of AR on mechanical overload-induced hypertrophy in individuals categorized according to hypertrophy responsiveness has been limited, and none of these studies have classified individuals as nonresponders (MOBLEY *et al.*, 2018; MORTON *et al.*, 2018). Morton *et al*. (2018) demonstrated that resistance-trained individuals clustered as higher hypertrophic responders had greater AR content at baseline (i.e., prior to a RT period) compared to low responders. In contrast, Mobley *et al*. (2018) showed no differences between untrained individuals clustered as high, modest, and low hypertrophic responders in baseline values and chronic changes in total AR protein content. Although these findings are difficult to reconcile, Mobley *et al*. (2018) reported significant increases in type I and type II fiber cross-sectional area and vastus lateralis thickness in lower responders, which may have impacted the absence of significant differences in total AR content between clusters. Thus, it is still unclear whether nonresponders and responders differ in total AR content.

AR are nuclear transcription factors located within different tissues, including skeletal muscle (JANSSEN *et al.*, 1994; RUIZEVELD DE WINTER *et al.*, 1991). Without the presence of ligands, cytoplasmic AR remain inactively attached to heat-shock proteins. After being activated through androgen binding, AR detach from these proteins, undergo conformational changes, and translocate to the nucleus (nAR) of the cell, where they bind to DNA (AR-DNA) to promote gene transcription (CLAESSENS *et al.*, 2001; DAVEY *et al.*, 2016; EDER *et al.*, 2001; EVANS, 1988). In this sense, analyzing AR markers other than total AR in crude tissue lysate may unveil a potential role of AR in responsiveness to muscle hypertrophy. However, no study to date has extensively investigated whether different AR markers play different roles in nonresponders and responders to muscle hypertrophy.

Therefore, the aim of this study was to investigate whether AR markers, including protein content of general lysates (i.e., total AR), cytoplasmic and nuclear fractions, and DNA-

binding activity, i) at baseline; ii) after a single session of RT (i.e., acute responses); and iii) after 10 weeks (i.e., chronic responses), are related to the muscle hypertrophy responsiveness of young individuals. We hypothesized that nonresponders would present significantly lower baseline and RT-induced responses in AR markers compared to responders.

METHODS

Experimental design

Participants provided informed consent and underwent two muscle cross-sectional area (mCSA) assessments split by 72 h to calculate the measurement reproductivity (i.e., typical error). Participants were subsequently instructed to refrain from vigorous activity and alcohol ingestion, in addition to habitual diet maintenance, for at least 48 h before muscle biopsies on the vastus lateralis muscle. Baseline biopsies were followed by the first RT session, and muscle biopsies were repeated after 24 h (acute responses). Within the following 10 weeks, participants performed 23 sessions of RT 2-3 times per week. Ninety-six hours after the last RT session (post), mCSA and muscle biopsies (chronic responses) were performed.

Participants

This study presents an ancillary analysis of data and muscle samples derived from a randomized controlled trial registered in the Brazilian Registry of Clinical Trials (RBR-57v9mrb). Thirty-nine participants who had not practiced RT, aerobic training or any other physical training program for at least 6 months started the study protocol. One participant withdrew consent for the following biopsies, and 38 participants (age: 24 ± 4 years, body mass: 67.8 ± 12.5 kg, height: 1.70 ± 0.1 m, BMI: 23.4 ± 3.7 kg/m^2), including 19 women (age: 24 ± 5 years, body mass: 62.7 ± 8.5 kg, height: 1.64 ± 0.05 m, BMI: 23.5 ± 3.3 kg/m^2) and 19 men (age: 24 ± 4 years, body mass: 73.0 ± 13.9 kg, height: 1.76 ± 0.07 m, BMI: 23.4 ± 4.2 kg/m^2), were included in the analyses. The exclusion criteria included neuromuscular disorders, injuries, or any chronic conditions that impeded participants' adherence to the study protocol. Furthermore, participants were counseled against engaging in any structured physical activities in addition to the training in the laboratory. This study was approved by the Federal University of São Carlos ethics committee (number: 5.505.441) and carried out in accordance with the Declaration of Helsinki.

Resistance training protocol

A total of 23 sessions of RT were performed two to three times per week over 10 weeks. Briefly, participants underwent a five-minutes warm-up on a cycle ergometer (Ergo-Fit®, Pirmasens, Rheinland-Pfalz, Germany) at a speed of 20 $km \cdot h^{-1}$ for general warm-up. This was followed by a specific warm-up set comprising 10 repetitions at 50% of the training load performed unilaterally on the leg extension machine (Effort NKR; Nakagym, São Paulo, Brazil). The training protocol consisted in four sets of 9-12 repetitions maximum performed on the leg extension machine, with 90-second rest intervals between sets. Whenever participants were unable to complete 9 repetitions or performed more than 12 repetitions with proper form, load was adjusted to maintain participants within the repetition range.

Muscle cross-sectional area

Prior to the vastus lateralis mCSA assessment, participants were instructed to refrain from engaging in moderate to vigorous physical activity for a minimum of 72 h. The total length of the femur was measured. Markings were then made at 2 cm intervals along the sagittal plane, starting from 50% of the femur's total length, serving as reference points for the positioning of the B-mode linear ultrasound probe (MySono U6, Samsung, Sao Paulo, Brazil). Subsequently, participants rested in a supine position for 15 minutes to allow for the homogenization of body fluids, after which sequential images were acquired from the previously established markings. During acquisition, to ensure appropriate acoustic coupling between the probe and the skin without causing dermal compression, a water-soluble gel was applied. After acquisition, the sequential images vastus lateralis muscle were manually reconstructed using PowerPoint software (Microsoft, USA). Following the acquisition of images corresponding to the entire muscle, the mCSA was calculated using the "polygonal" tool in ImageJ software, with connective and bone tissue appropriately excluded from the calculation area. The typical error and coefficient of variation, based on two image acquisitions and mCSA quantifications conducted within a 72-hour period, were 0.52 cm^2 and 2.47%, respectively.

Muscle biopsy

Prior to the procedure, ~2.5 ml of 1% xylocaine was applied to the previously

identified biopsy spot. Approximately 100-120 mg were collected from the vastus lateralis muscle of each participant using percutaneous biopsy needles coupled with a suction apparatus. Blood and connective tissue samples were cleaned before storage. The samples were stored at -80°C until the biochemical analyses.

Tissue homogenization and fraction extraction

Using an analytical scale (Mettler Toledo ME303, Greifensee, Switzerland), approximately 20–25 mg of tissue was homogenized in 1.7 ml tubes using tight-fitting pestles and 400 µl of commercially available 1x cell lysis buffer (Cell Signaling Technology, Cat. No. 9803). The slurries were centrifuged at 500 × G and at 4°C for 10 min. Supernatants containing general tissue lysates were extracted. Cytoplasmic and nuclear fractions were extracted from these lysates using a nuclear extraction kit (Abcam, Cat. No.: ab113474) following the manufacturer's instructions. Total, cytoplasmic and nuclear protein concentrations were quantified using 96-well plates and a commercially available bicinchoninic acid assay (Thermo Fisher Scientific, Waltham, MA, USA). The plates were read in a microplate reader using Gen5 software (BioTek Instruments, Inc., Winooski, VT, USA). Importantly, significantly different proportion between the nuclear and cytoplasmic at baseline, 24 h and post (all $P < 0.0001$), in which nuclear/cytoplasmic ratios were 0.602 at baseline, 0.597 at 24 h and 0.618 post.

Western blotting

General cell lysates and cytoplasmic and nuclear fractions were prepared at a concentration of 1 µg/µL with deionized water (diH_2O) and 4x Laemmli buffer and boiled at 100°C for 5 min for Western blotting analysis. Ten µl of Trident Prestained Protein Ladder (GeneTex, Irvine, CA, USA, Cat. No. GTX50875) and 15 µl of each sample were loaded onto SDS–polyacrylamide gels (4%–15% Criterion TGX; Bio-Rad Laboratories, Hercules, CA, USA, Cat. No. 5671085). After electrophoresis at 180 V for 50 min, the proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Cat. No. 1620177) for two hours at 200 mA. Ponceau staining was performed for 10-12 min, then membranes were briefly rinsed with diH_2O, dried and imaged using a gel documentation system (ChemiDoc Touch; Bio-Rad Laboratories). Membranes were then blocked in nonfat milk for 1 h (5% wt/vol diluted in Tris-buffered saline and 0.1% Tween 20). After blocking, membranes were washed and incubated for 24 h on a rocking platform at 4°C with the following primary antibody: rabbit

anti-human androgen receptor (AR; 1:1,000 in 5% BSA) (D6F11; Cell Signaling Technology, Danvers, MA, United States, Cat. No. 5153). On the next day, the membranes were incubated in horseradish peroxidase-conjugated anti-rabbit IgG (1:2,000 in 5% BSA) (Cell Signaling Technology; Cat. No. 7074 for 1 h on a rocking platform at room temperature) and developed with chemiluminescence substrate (Immobilon Forte Western HRP substrate; Millipore; Burlington, MA, USA; Cat. No.: WBLUF0500) for digital imaging. Exposure times for all membranes were standardized at 5 seconds. Total AR, as well as cytoplasmic (cAR) and nuclear (nAR) fractions, were identified with molecular weights of ~110 kDa. Ponceau density and raw target band density were analyzed using ImageLab software (v6.0.1; Bio-Rad Laboratories). Total AR, cAR and nAR protein contents were obtained though the ratio between the raw target band densities and the respective Ponceau lane densities. Notably, the AR antibody used has been validated using LNCap lysate as described by Haun et al. (HAUN *et al.*, 2019). Likewise, each membrane contained 8 participants (all time points [baseline, 24 h and post-training]), and there were 5 total membranes. To minimize gel-to-gel variability, the averages of raw AR signal/Ponceau values were compared between membranes, and signals across membranes were adjusted with a coefficient to correct gels possessing abnormally lower or higher values due to variability in transfers. Representative Western blot and Ponceau images are available in supplemental material.

Androgen receptor DNA-binding assay

AR-DNA binding activity was determined using part of the nuclear fraction with a commercially available ELISA-oligonucleotide kit (Aviva Systems Biology Corporation, San Diego, CA, USA; Cat. No.: OKAG00363) according to the manufacturer's instructions and as previously performed by our group (MUMFORD *et al.*, 2018). A microplate-based spectrophotometer with Gen5 software (BioTek Instruments, Inc.) was used to obtain the assay absorbance. The relative AR DNA-binding activity values were calculated as the ratio of the mean values obtained from duplicate measurements to the protein concentration of each sample. The intra-assay coefficient of variation was 13.4% on average.

Statistical analyses

All analyses were performed, and graphs were generated using GraphPad Prism software (version 9.5, GraphPad Software, San Diego, CA, USA). Two mCSA typical errors

(2 × TE) were used to identify individuals as nonresponders (≤ 2 × TE) (BONAFIGLIA *et al.*, 2016; BOUCHARD *et al.*, 2012; DAMAS; BARCELOS*; et al.*, 2019; HOPKINS, 2000; LIXANDRÃO *et al.*, 2024; SWINTON *et al.*, 2018), and the highest responders were numerically matched. The TE observed within our sample was 0.52 cm^2 (2 × TE = 1.04 cm^2). However, we standardized this measurement as a percentage of the mean baseline mCSA measurements to compare it with the changes from before to after the intervention in the mCSA. Consequently, our clustering criterion was 4.94% (2 × 2.47%), in which greater percentual changes increase the likelihood of representing a genuine adaptation, surpassing random error sources (i.e., measurement technique and biological variation) (HOPKINS, 2011). The sample size calculation was based on the findings from Morton et al. (MORTON *et al.*, 2018). Initially, the effect size was determined using the significant differences in total AR protein content at baseline between high and low responders. Subsequently, 10,000 Monte Carlo simulations were conducted with a significance level of 0.05 and a statistical power of 0.80, which indicated a minimum requirement of 10 participants per group. After visual inspection of the data, an analysis was conducted to identify outliers. While a few outliers were detected, their presence did not impact the results significantly, and as such, they were maintained in the analyses. The distribution of the data was assessed using Shapiro–Wilk tests. Unpaired t tests (for normally distributed data) or Mann–Whitney tests (for non-normally distributed data) were used to compare baseline values. For acute (baseline vs. 24 h) and chronic (baseline vs. post) analyses, mixed model was applied considering groups (nonresponders vs. responders) and time as fixed factors and participants as random factors for all dependent variables. Tukey's adjustment was used for multiple comparisons in case of significant *F* values. Baseline absolute values, as well as acute and chronic percentual changes in AR, cAR, nAR, and AR-DNA, were used to compare nonresponders and responders using effect sizes (ES) with 95% confidence intervals (CI) (HEDGES *et al.*, 1985). Positive and negative CIs not crossing zero (0) were considered significant (NAKAGAWA *et al.*, 2007). Pearson's correlation analysis was used to determine potential associations between baseline, acute and chronic (relative and absolute) changes in AR markers (total AR, cAR, nAR and AR-DNA) and changes in mCSA for both groups separately. Correlation magnitudes were classified as very weak (< 0.20), weak (0.20 – 0.39), moderate (0.40 – 0.59), strong (0.60 – 0.79) or very strong (> 0.80) (EVANS, 1996). Significance was established as $P < 0.05$.

RESULTS

Identification of nonresponders and responders for muscle hypertrophy

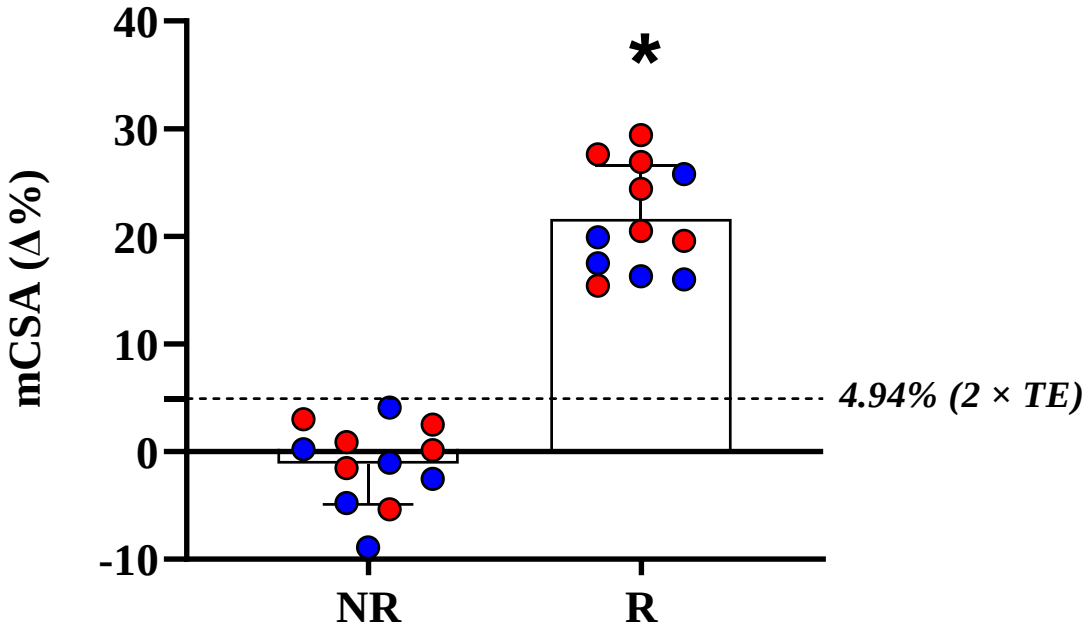
Among the 38 participants, twelve (6 women and 6 men) did not exhibit increases in mCSA greater than the 2 × TE threshold and were classified as nonresponders (ΔmCSA: -1.32%). The twelve participants (7 women, 5 men) presenting the highest responses were classified as responders (ΔmCSA: 21.35%). There was a significant difference between responders and nonresponders in mCSA increases (ES = 5.13; 95% CI: 2.76 to 7.14; $P < 0.0001$) (Figure 1). Table 1 illustrates the characteristics of nonresponders and responders at baseline.

Table 1. Anthropometrics and baseline values for nonresponders and responders.

Variables	Nonresponders	Responders
Age (years)	24 ± 5	24 ± 4
Height (m)	1.72 ± 0.1	1.67 ± 0.1
Body mass (kg)	70.6 ± 10.7	66.7 ± 11.3
BMI (kg/m²)	23.9 ± 2.7	24.0 ± 4.3
mCSA (cm²)	22.3 ± 5.2	21.5 ± 4.6
1-RM (kg)	53.4 ± 19.5	50.3 ± 13.7
AR (A.U.)	0.352 ± 0.05	0.356 ± 0.04
cAR (A.U.)	0.303 ± 0.07	0.287 ± 0.10
nAR (A.U.)	0.158 ± 0.02	0.161 ± 0.03
AR-DNA (OD/µg)	21.4 ± 9.4	26.6 ± 12.2

BMI: body mass index; mCSA: muscle cross-sectional area; 1-RM: one-repetition maximum; AR: androgen receptors; cAR: androgen receptor protein content in the cytoplasmic fraction; nAR: androgen receptor protein content in the myonuclear fraction; AR-DNA: androgen receptor DNA-binding activity; m: meters; kg: kilograms; kg/m²: kilograms per meter squared; A.U.: arbitrary expression units; OD/µg: optical density per microgram.

Figure 1. Comparison of changes in muscle cross-sectional area between nonresponders and responders

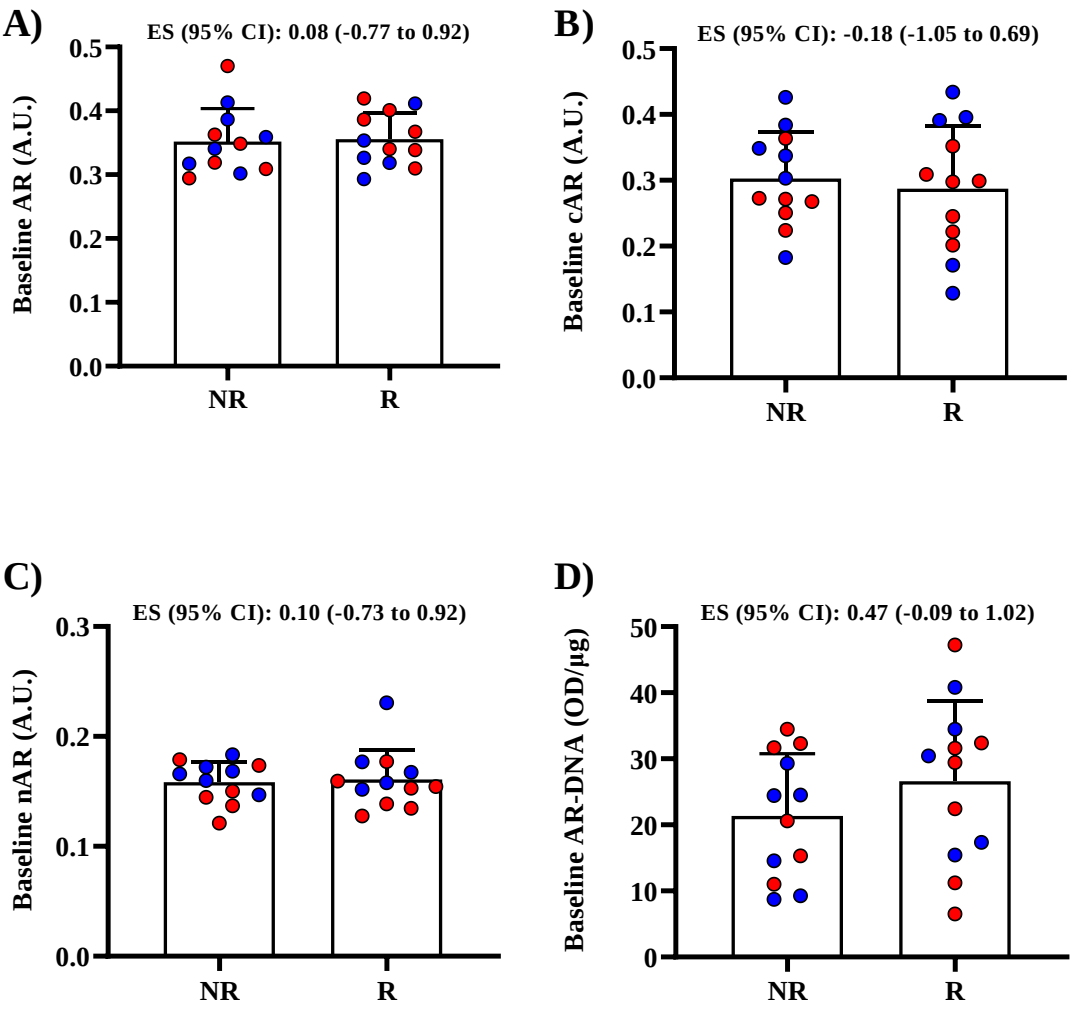


Legend: Relative changes (Δ%) in muscle cross-sectional area (mCSA) between nonresponders (i.e., individuals that did not meet the hypertrophic threshold of 4.94%) and responders (R). Women and men identified as NR and R are represented by red circles and blue circles, respectively. TE: typical error. *Significant differences between NR and R ($P < 0.05$).

Baseline comparisons between nonresponders and responders

There were no significant differences between nonresponders and responders for mCSA (nonresponders: 22.28 ± 5.19 cm^2; responders: 21.47 ± 4.62 cm^2; ES = -0.17; 95% CI: -0.92 to 0.60; $P = 0.688$), total AR (nonresponders: 0.35 ± 0.05 A.U.; responders: 0.36 ± 0.04 A.U.; ES = 0.08; 95% CI: -0.77 to 0.92; $P = 0.850$), cAR (nonresponders: 0.30 ± 0.07 A.U.; responders: 0.29 ± 0.10 A.U.; ES = -0.18; 95% CI: -1.05 to 0.69; $P = 0.658$), nAR (nonresponders: 0.16 ± 0.02 A.U.; responders: 0.16 ± 0.03 A.U.; ES = 0.10; 95% CI: -0.73 to 0.92; $P = 0.809$) or AR-DNA (nonresponders: 21.36 ± 9.43 OD/µg; responders: 26.61 ± 12.20 OD/µg; ES = 0.48; 95% CI: -0.09 to 1.02; $P = 0.251$) baseline values (Figure 2). Finally, there were no significant associations between baseline AR markers and mCSA for any groups, and details are available in Table 1 of the supplemental material.

Figure 2. Comparison of baseline values of androgen receptor markers between nonresponders and responders



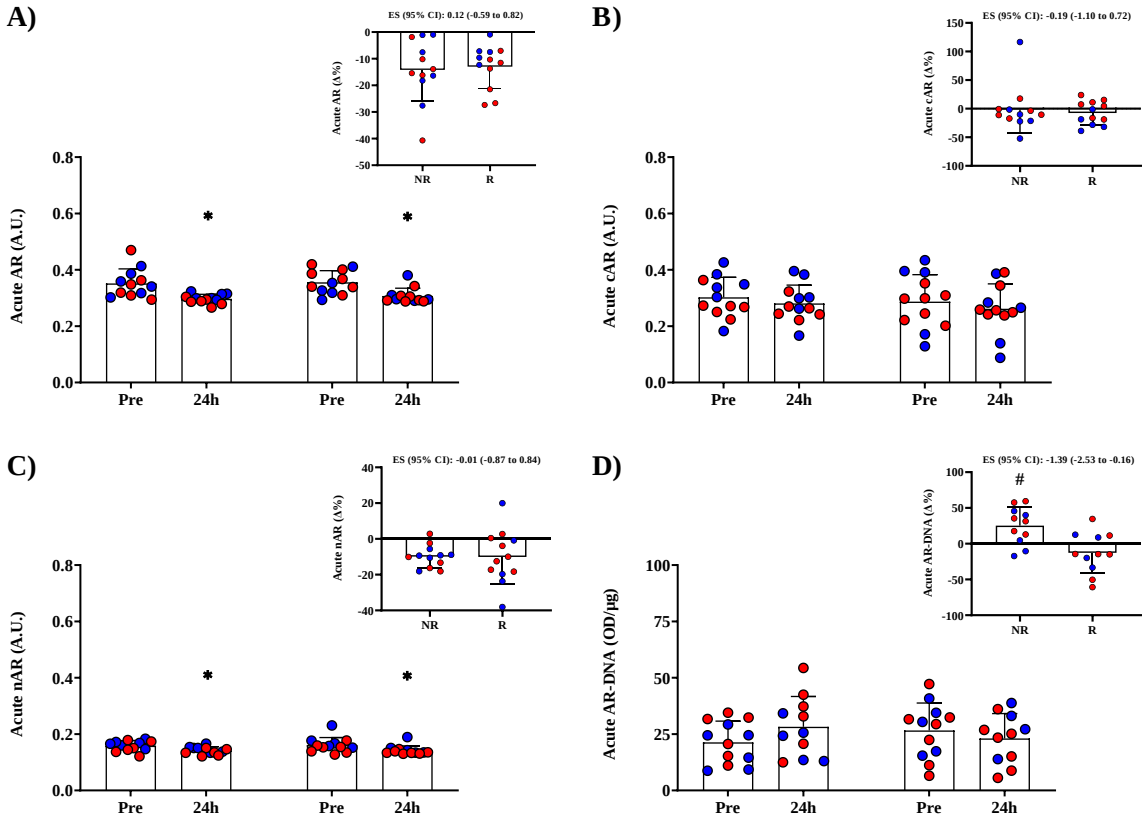
Legend: Crude lysate androgen receptor (AR, panel A), cytoplasmic AR (cAR, panel B), nuclear AR (nAR, panel C) and AR DNA-binding (AR-DNA, panel D) and respective effect sizes (ES) with 95% confidence intervals (CI). Women and men identified as nonresponders (NR) and responders (R) are represented by red circles and blue circles, respectively. A.U.: arbitrary units; OD/µg: optical density per microgram.

Comparisons of acute androgen receptor marker responses between nonresponders and responders

Mixed model analysis revealed only a main time effect for total AR ($P < 0.0001$) and nAR ($P = 0.0007$), in which the 24 h values were lower than baseline values for both nonresponders and responders. For AR-DNA activity, no main effects were observed, but a group vs. time interaction at 24 hours was significant ($P < 0.007$). However, post hoc analysis did not reveal any within-group or between-group differences ($P > 0.05$). Finaly, there were no

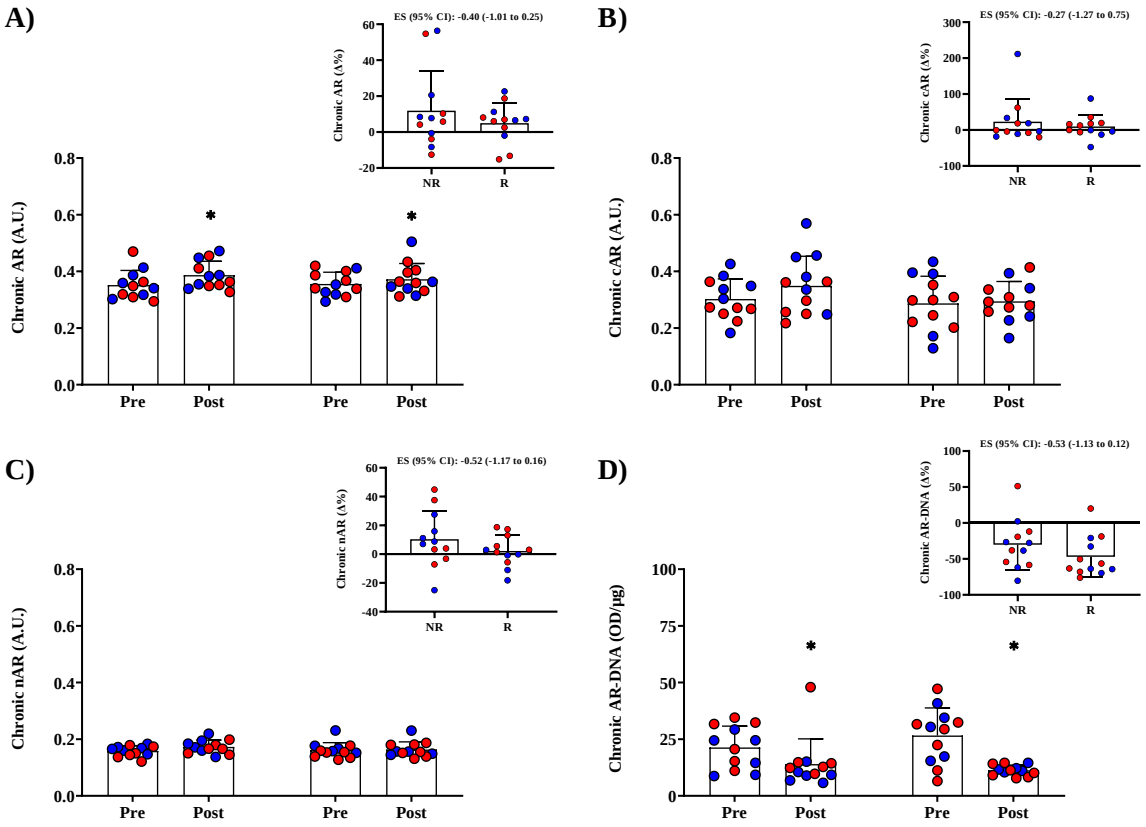
significant main effects or interaction for cAR ($P > 0.05$). The CI do ES did not indicate significant differences in acute changes in total AR (ES: 0.12; 95% CI: -0.59 to 0.82) (Figure 3A), cAR (ES: -0.19; 95% CI: -1.10 to 0.72) (Figure 3B) or nAR (ES: -0.01; 95% CI: -0.87 to 0.84) (Figure 3C) between nonresponders and responders. However, a significant difference in AR-DNA (ES = -1.39; 95% CI: -2.53 to -0.16) was detected. There were no significant associations between acute changes in AR markers and mCSA for any groups, and details are available in Table 1 of the supplemental material.

Figure 3. Comparison of acute changes in androgen receptor markers between nonresponders and responders



Legend: Acute absolute changes in crude lysate androgen receptor (AR, panel A), cytoplasmic AR (cAR, panel B), nuclear AR (nAR, panel C) and AR DNA-binding (AR-DNA, panel D). Inset: Comparison of delta percentual changes (Δ%) in AR markers between nonresponders (NR) and responders (R) and respective effect sizes (ES) with 95% confidence intervals (CI). Women and men identified as nonresponders and responders are represented by red circles and blue circles, respectively. Pre: baseline biopsy time point; 24 h: biopsy time point 24 h after the first RT session; A.U.: arbitrary units; OD/µg: optical density per microgram. *Significantly different from Pre (main time effect; $P < 0.05$). #Significant difference between NR and R (95%CI of ES; $P < 0.05$).

Figure 4. Comparison of chronic changes in androgen receptor markers between nonresponders and responders



Legend: Chronic absolute changes in crude lysate androgen receptor (AR, panel A), cytoplasmic AR (cAR, panel B), nuclear AR (nAR, panel C) and AR DNA-binding (AR-DNA, panel D). Inset: Comparison of delta percentual changes (Δ%) in AR markers between nonresponders (NR) and responders (R) and respective effect sizes (ES) with 95% confidence intervals (CI) Women and men identified as nonresponders and responders are represented by red circles and blue circles, respectively. Pre: baseline biopsy time point; Post: biopsy time point after the last RT session; A.U.: arbitrary units; OD/µg: optical density per microgram. *Significantly different from Pre (main time effect; $P < 0.05$).

DISCUSSION

This study aimed to investigate whether AR, cAR, nAR and AR-DNA at baseline, as well as acute and chronic responses to RT differ between nonresponders and responders to training-induced muscle hypertrophy in younger individuals. Our analysis revealed no significant differences in baseline values, nor in acute and chronic changes between nonresponders and responders, with the exception of acute changes in AR-DNA.

It is well established that muscle hypertrophy is affected by intrinsic mechanisms that vary significantly among individuals, thus impacting the magnitude of individual responses to the same RT stimulus (AHTIAINEN *et al.*, 2016; ANGLERI *et al.*, 2022; ANGLERI *et al.*, 2021; DAMAS; ANGLERI*; et al.*, 2019; ISLAM *et al.*, 2019; ROBERTS *et al.*, 2023). Among these mechanisms, ribosome biogenesis and satellite cell-mediated myonuclear accretion have been extensively investigated and found to be associated with skeletal muscle hypertrophy (ROBERTS *et al.*, 2018). However, other mechanisms, such as acute alterations in AR localization and DNA binding affinity, require further investigation (AHTIAINEN *et al.*, 2011; AHTIAINEN *et al.*, 2016; MORTON *et al.*, 2018). We demonstrated no significant differences in total tissue (i.e., crude lysate) AR expression (baseline, acute and chronic results) between nonresponders and responders. This is the first study in which clusters of nonresponders and responders included both women and men, as unpublished findings from our group showed minimal effects of sex on RT-induced changes in AR content. Our findings align with those of Mobley et al. (MOBLEY *et al.*, 2018), who also showed no differences at baseline or acute and chronic changes in total AR protein content between previously untrained male individuals identified as high, modest, and low hypertrophic responders. In contrast, Morton et al. (MORTON *et al.*, 2018) demonstrated that RT-trained male individuals classified as high responders presented significantly greater total AR content at baseline than low responders. The discrepancy between their data and the current study may be attributed not only to methodological differences in classifying responsiveness but also to the fact that Morton and colleagues investigated previously trained individuals. To this end, muscle AR content may increase over years of RT, and hypertrophy may be impaired in those who do not experience this adaptation. This hypothesis is partly supported by Kadi et al. (KADI *et al.*, 2000) who demonstrated in a cross-sectional study that highly trained powerlifters had greater AR content in the trapezius muscle compared to untrained individuals.

A unique aspect of the current study is the examination of AR subcellular localization and AR-DNA binding, as the majority of investigations have examined the effects of RT on total AR in crude tissue lysates (AHTIAINEN *et al.*, 2011; BAMMAN *et al.*, 2001; CARDACI *et al.*, 2020; HULMI *et al.*, 2008; KADI *et al.*, 2000; KRAEMER *et al.*, 2006; MITCHELL *et al.*, 2013; MOBLEY *et al.*, 2018; MORTON *et al.*, 2018; RATAMESS *et al.*, 2005; ROBERTS *et al.*, 2009; SPIERING *et al.*, 2009; SPILLANE *et al.*, 2015; VINGREN *et al.*, 2009; WILLOUGHBY *et al.*, 2004). Our results demonstrated no differences between nonresponders and responders to RT-induced muscle hypertrophy in baseline cAR, nAR and AR-DNA; acute cAR and nAR; or chronic cAR, nAR and AR-DNA. Recently, Hatt et al. (HATT *et al.*, 2024)

investigated sex-based differences in total AR and nAR (assessed using immunohistochemistry) following acute and chronic RT and explored potential differences in changes in total AR and nAR between high and low responders to RT-induced myofiber hypertrophy. Although the authors demonstrated some differences between sexes, no differences were observed between high and low responders in the changes in total AR and nAR induced by RT. Taken together, these findings suggest that even examining AR subcellular localization, this mechanism does not seem to explain responsiveness to RT-induced hypertrophy, at least in untrained individuals. A final observation worthy of discussion was the between-group difference in acute AR-DNA binding, in which nonresponders presented increased AR-DNA binding activity and responders presented decreased acute responses. Since increases in AR-DNA binding activity would represent a proxy for androgen-dependent gene transcription, it is surprising that responders presented diminished responses compared to nonresponders. We have no plausible explanation for this observation aside from this either being a spurious finding or a potential compensatory mechanism in nonresponders. Hence, this topic requires further investigation.

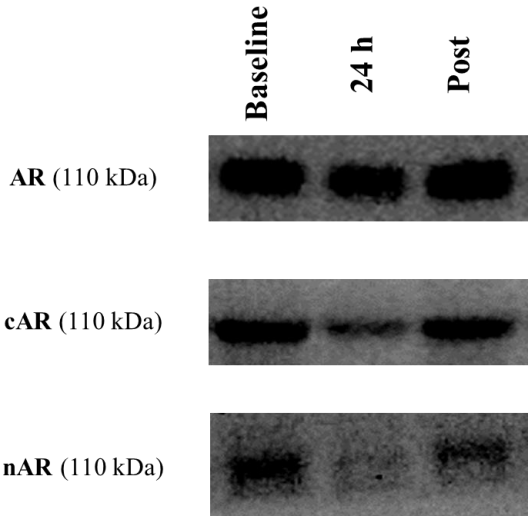
This study is not free of limitations. First, this study included only untrained and healthy women and men, and the results should not be extrapolated to other populations, such as in different training levels (RT-trained), ages (middle-aged and elderly people), or androgen anabolic steroid users. Second, considering the method adopted to identify nonresponder individuals (i.e., 2 × typical error obtained from two different data collections), it was not possible to determine responsiveness using the muscle fiber cross-sectional area (fCSA). Although it is plausible to suggest that the AR would be better related to the fCSA since both derive from the same muscle biopsy, Van Vossel et al. (VAN VOSSEL *et al.*, 2024) showed no significant correlation between baseline or post-training changes between fCSA and AR content among untrained individuals. Third, the muscle biopsies were taken from the vastus lateralis muscle, and results should not be extrapolated to other muscles, which are likely to present different AR concentrations (KADI *et al.*, 2000). Finally, while 24 h has been shown to allow for the detection of acute changes in AR markers (SPILLANE *et al.*, 2015), a single biopsy time point may have limited to detect a potential RT-induced translocation of AR to the nucleus. In this sense, future studies should consider performing a muscle biopsy time course, since the optimal time point to investigate this phenomenon is still to be determined.

In conclusion, nonresponders and responders presented similar results for the majority of the AR markers considering baseline values, acute responses and chronic changes. However, only acute AR-DNA binding activity differed between groups. Future studies should consider

exploring the downstream effects of AR-DNA binding to determine whether there are differences in outcomes between nonresponders and responders. Aside from these future directions, the current findings do not support skeletal muscle AR markers influencing hypertrophy responsiveness in untrained individuals.

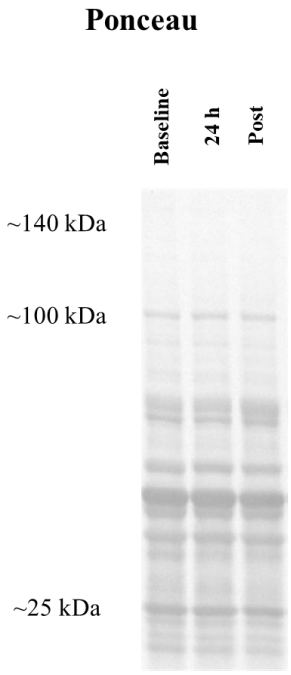
SUPPLEMENTAL MATERIAL

Figure 1. Representative Western blot images



Legend: Representative Western blot images for androgen receptors (AR) whole-tissue lysates, AR protein content in the cytoplasmic fraction and AR protein content in the myonuclear fraction, at baseline, 24 hours (24 h) after the first training bout and after 10 weeks of resistance training (Post). kDa: kilodaltons.

Figure 2. Representative Ponceau image



Legend: Representative Ponceau images to the corresponding Western blot analysis. Lateral labels indicate approximate molecular weights to the representative image. Time points included baseline, 24 hours (24 h) after the first training bout and after 10 weeks of resistance training (Post). kDa: kilodaltons.

Table 1. Correlations between absolute baseline values, and acute and chronic relative changes in AR, cAR, nAR and AR-DNA and relative changes in mCSA.

Variables	mCSA (Δ%)			
	Nonresponders		Responders	
	r	***P***	***r***	***P***
Baseline AR (A.U.)	0.330	0.295	-0.399	0.199
Baseline cAR (A.U.)	0.339	0.281	-0.246	0.441
Baseline nAR (A.U.)	-0.037	0.910	-0.059	0.856
Baseline AR-DNA (OD/µg)	-0.174	0.588	0.133	0.680
Acute AR (Δ%)	-0.463	0.130	-0.220	0.491
Acute cAR (Δ%)	-0.569	0.054	0.322	0.308
Acute nAR (Δ%)	-0.155	0.631	-0.080	0.805
Acute AR-DNA (Δ%)	0.486	0.130	-0.142	0.676
Chronic AR (Δ%)	-0.403	0.193	-0.529	0.077
Chronic cAR (Δ%)	-0.450	0.142	0.483	0.112
Chronic nAR (Δ%)	-0.339	0.281	0.225	0.483
Chronic AR-DNA (Δ%)	0.190	0.554	0.529	0.077

Acute (24 h – Pre); Chronic: Post-intervention - Pre; AR: androgen receptor protein content in the whole tissue lysate; cAR: androgen receptor protein content in the cytoplasmic fraction; nAR: androgen receptor protein content in the myonuclear fraction; AR-DNA: androgen receptor DNA-binding activity; mCSA: muscle cross-sectional area; Δ%: relative changes; A.U.: arbitrary expression units; OD/ µg: Optical density per microgram.

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