



PEDRO GUSTAVO MACHADO

**EFFECTS OF AEROBIC AND RESISTANCE TRAINING ON
BONE AND MUSCLE REMODELING IN AN
OVARIECTOMY-INDUCED OSTEOPENIA MURINE
MODEL**

**LAVRAS – MG
2026**

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Tese apresentada à Universidade Federal de
Lavras, como parte das exigências do Curso de
Pós-Graduação em Ciências Veterinárias, área
de concentração em Fisiologia e Metabolismo
Animal, para a obtenção do título de Doutor.

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**Ficha Catalográfica elaborada pelo Sistema de Geração
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Machado, Pedro.

Effects of Aerobic and Resistance Training on Bone and Muscle Remodeling in
an Ovariectomy-Induced Osteopenia Murine Model / Pedro Machado. - 2026.
104 p. : il.

Orientador: Luciano Pereira

Coorientador: Eric Andrade

Tese (Doutorado) - Universidade Federal de Lavras, 2026.

Bibliografia.

1. Osteoporose . 2. Senescência ovariana. 3. Exercício físico. I. Pereira, Luciano.
II. Andrade, Eric. III. Universidade Federal de Lavras. IV. Título.

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Aprovado em 19 de março de 2026.

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AGRADECIMENTOS

Ao final dessa etapa tão grandiosa em minha vida, entendi que grandes feitos nunca são realizados sozinhos. Por esse motivo, agradeço primeiramente a Deus pela vida e pela oportunidade de seguir realizando sonhos e construindo minha carreira. Sou eternamente grato pelo caminho percorrido na educação, desde a graduação até a conquista do título de doutorado. Agradeço à minha família, especialmente à minha esposa Susan, pelo suporte incondicional durante essa jornada longa e desafiadora. Sem seu apoio, essa caminhada seria muito mais difícil.

Minha gratidão se estende de forma muito especial ao meu orientador, Prof. Luciano José Pereira, e ao coorientador, Prof. Eric Francelino de Andrade, pela confiança em mim, pela orientação, amizade, dedicação e inspiração constante na busca pela excelência na ciência, apesar dos desafios enfrentados. Tenho enorme orgulho de ter aprendido com vocês e guardarei para sempre a lembrança dessa oportunidade. Vocês são inspirações.

Agradeço à UFLA e ao Programa de Pós-Graduação em Ciências Veterinárias pela oportunidade de crescimento acadêmico e profissional. O apoio do programa foi essencial, possibilitando o desenvolvimento dos experimentos e proporcionando uma experiência transformadora com a bolsa de doutorado, além da oportunidade de conseguir uma bolsa sanduíche nos EUA, que expandiu meus horizontes.

Aos professores que contribuíram durante o doutorado sanduíche, em especial ao Prof. Pedro Halal, meu coorientador no exterior, pela recepção em seu laboratório. Agradeço também ao Prof. Rafael Tassitano, Prof. Cecilia Tenorio, Prof. Diego Hernandez e, especialmente, ao Prof. Otavio Leao, cuja amizade levarei para a vida. Sou igualmente grato aos amigos que fiz nesse período: Aline Brandão, Lauana Oliveira, Bruno Zanini, Wanessa Gusmão, Thiago Trafane, Viviane Viana, Julia Campos, Larissa Solano, Hank Huang, Clay Weidenhamer e tantos outros.

Às minhas grandes amigas de doutorado, Luciana Botelho e Juliana Canaan, agradeço pelo apoio, aprendizado compartilhado e pela amizade que construímos. Cada uma, com seu jeito único e carinhoso, tornou essa jornada mais leve e significativa. Tenho certeza de que nos lembraremos de todos os momentos vividos nesse longo período pelo resto de nossas vidas. Vocês são muito especiais para mim.

Agradeço a todos que contribuíram para a realização dos experimentos: aos professores Natalia Bertolini, Soraia Macari, Debora Galdino, Ivam Junior, Rafael Remedio, Heloisa Santos e Rodrigo Moura; aos colegas João Paulo, Clara, Victor de Jesus e Aline Chaves; ao técnico William, cuja sabedoria foi essencial; e ao biotério do Departamento de Nutrição, com agradecimentos especiais às professoras Isabella Castro e Laura Pimenta, bem como ao técnico Geraldo Cândido.

A todos vocês, meu eterno agradecimento por fazerem parte dessa jornada inesquecível.

“A maior recompensa do trabalho não é o que se obtém com ele, mas aquilo em que nos tornamos por meio dele.” (John Ruskin)

RESUMO

A deficiência estrogênica associada à senescência ovariana promove desequilíbrio no remodelamento ósseo, resultando em perda de massa mineral, deterioração da microarquitetura e aumento do risco de fraturas. Nesse contexto, o exercício físico tem sido amplamente investigado como estratégia não farmacológica capaz de modular o metabolismo ósseo e o eixo músculo–osso. O presente estudo foi estruturado em dois capítulos complementares, contemplando uma revisão sistemática com metanálise e um estudo experimental pré-clínico. O Capítulo 2, intitulado “*Resistance training and bone mineral density in rodent models of menopause: review and meta-analysis*”, teve como objetivo sintetizar e quantificar os efeitos do treinamento resistido sobre a densidade mineral óssea em modelos de osteopenia induzida por ovariectomia. O protocolo foi registrado no PROSPERO e conduzido conforme as diretrizes PRISMA. A metanálise, composta por nove estudos, demonstrou aumento significativo da densidade mineral óssea em comparação aos grupos sedentários, com tamanho de efeito padronizado de 3,02 (IC95% 1,81–4,24) para protocolos de escalada e 1,27 (IC95% 0,72–1,82) para protocolos de salto. O Capítulo 3, intitulado “*Aerobic and resistance training in estrogen deficiency: a comparative study of bone remodeling and skeletal muscle adaptations*”, investigou os efeitos do treinamento aeróbio e resistido em camundongos ovariectomizados. Ambos os protocolos reduziram significativamente o número de osteoclastos e aumentaram a atividade osteoblástica ($p < 0,05$), além de diminuir a razão RANKL/OPG ($p < 0,001$). O treinamento resistido apresentou efeitos superiores, incluindo maior densidade de osteócitos ($p < 0,01$), maior expressão de RUNX2 ($p < 0,001$) e aumento da área de fibras musculares, acompanhado de elevação de IGF-1 e FNDC5, indicando maior estímulo osteogênico e intensificação do crosstalk músculo–osso. Em conjunto, os achados demonstram que o exercício físico atenua a deterioração óssea associada à deficiência estrogênica, com destaque para o treinamento resistido como estratégia mais eficaz na promoção integrada da saúde óssea e muscular.

Palavras-chave: Deficiência estrogênica, Ovariectomia, Osteopenia, Remodelamento ósseo, Densidade mineral óssea, Treinamento resistido, Treinamento aeróbio

ABSTRACT

Estrogen deficiency associated with ovarian senescence leads to an imbalance in bone remodeling, resulting in reduced bone mineral density, microarchitectural deterioration, and increased fracture risk. In this context, physical exercise has been widely investigated as a non-pharmacological strategy capable of modulating bone metabolism and the muscle–bone axis. The present study was structured into two complementary chapters, comprising a systematic review with meta-analysis and a preclinical experimental study. Chapter 2, entitled “*Resistance training and bone mineral density in rodent models of menopause: review and meta-analysis*”, aimed to synthesize and quantify the effects of resistance training on bone mineral density in ovariectomy-induced osteopenia models. The protocol was registered in PROSPERO and conducted in accordance with PRISMA guidelines. The meta-analysis, including nine studies, demonstrated a significant increase in bone mineral density compared to sedentary groups, with a standardized effect size of 3.02 (95% CI 1.81–4.24) for climbing protocols and 1.27 (95% CI 0.72–1.82) for jumping protocols. Chapter 2, entitled “*Aerobic and resistance training in estrogen deficiency: a comparative study of bone remodeling and skeletal muscle adaptations*”, investigated the effects of aerobic and resistance training in ovariectomized mice. Both exercise protocols significantly reduced osteoclast number and increased osteoblastic activity ($p < 0.05$), along with a marked reduction in the RANKL/OPG ratio ($p < 0.001$). However, resistance training elicited superior effects, including higher osteocyte density ($p < 0.01$), increased RUNX2 expression ($p < 0.001$), and greater muscle fiber cross-sectional area, accompanied by elevated IGF-1 and FNDC5 expression, indicating enhanced osteogenic stimulus and intensified muscle–bone crosstalk. Taken together, these findings demonstrate that physical exercise attenuates bone deterioration associated with estrogen deficiency, with resistance training emerging as a more effective strategy for promoting integrated bone and muscle health.

Keywords: Estrogen deficiency; Ovariectomy; Osteopenia; Bone remodeling; Bone mineral density; Resistance training; Aerobic training.

INDICADORES DE IMPACTO

O presente estudo apresenta impactos científicos, tecnológicos e sociais ao contribuir para o avanço do conhecimento sobre os mecanismos fisiopatológicos da deficiência estrogênica no metabolismo ósseo e muscular, utilizando modelo experimental de ovariectomia em roedores. O emprego desse modelo permite a reprodução controlada de alterações estruturais, celulares e moleculares características da osteopenia e da osteoporose, constituindo ferramenta relevante para investigações pré-clínicas em biologia óssea, endocrinologia e fisiologia. Sob o ponto de vista científico, o estudo amplia a compreensão dos processos de remodelação óssea associados à senescência ovariana, especialmente quanto à dinâmica entre osteoblastos, osteoclastos e osteócitos, bem como à modulação de vias moleculares osteogênicas e osteoclastogênicas. Além disso, fornece evidências experimentais sobre o papel do estímulo mecânico induzido pelo exercício físico na preservação da microarquitetura óssea e nas adaptações do eixo osteomuscular, contribuindo para a consolidação de estratégias não farmacológicas e de baixo custo em modelos animais. No aspecto tecnológico e metodológico, a pesquisa contribui para a padronização de protocolos de treinamento físico em roedores ovariectomizados, bem como para a aplicação integrada de análises histológicas, histomorfométricas, histoquímicas e de expressão gênica. Esses procedimentos fortalecem o desenvolvimento de metodologias experimentais reprodutíveis e podem subsidiar futuros estudos pré-clínicos nas áreas de ortopedia veterinária, metabolismo ósseo e fisiologia do exercício. Quanto ao impacto social e institucional, os resultados favorecem a geração de conhecimento aplicável à medicina veterinária experimental, ao manejo de condições musculoesqueléticas relacionadas ao envelhecimento animal e ao aprimoramento de práticas de pesquisa envolvendo modelos biológicos. Adicionalmente, a pesquisa contribui para a formação de recursos humanos qualificados, envolvendo estudantes de graduação e pós-graduação em atividades laboratoriais, cirúrgicas e analíticas, promovendo capacitação técnica e científica. O trabalho apresenta ainda caráter extensionista indireto, ao possibilitar a transferência de conhecimento para a comunidade acadêmica e científica por meio de publicações, apresentações em eventos e atividades formativas, fortalecendo a integração entre ensino, pesquisa e extensão.

IMPACT INDICATORS

The present study presents scientific, technological, and social impacts by contributing to the advancement of knowledge regarding the pathophysiological mechanisms of estrogen deficiency on bone and muscle metabolism, using an experimental ovariectomy model in rodents. The use of this model enables the controlled reproduction of structural, cellular, and molecular alterations characteristic of osteopenia and osteoporosis, constituting a relevant tool for preclinical investigations in bone biology, endocrinology, and physiology. From a scientific perspective, the study expands the understanding of bone remodeling processes associated with ovarian senescence, particularly concerning the dynamics among osteoblasts, osteoclasts, and osteocytes, as well as the modulation of osteogenic and osteoclastogenic molecular pathways. Furthermore, it provides experimental evidence on the role of mechanical stimuli induced by physical exercise in preserving bone microarchitecture and promoting adaptations in the bone–muscle axis, contributing to the consolidation of non-pharmacological and low-cost strategies in animal models. From a technological and methodological standpoint, the research contributes to the standardization of physical training protocols in ovariectomized rodents, as well as to the integrated application of histological, histomorphometric, histochemical, and gene expression analyses. These procedures strengthen the development of reproducible experimental methodologies and may support future preclinical studies in veterinary orthopedics, bone metabolism, and exercise physiology. Regarding social and institutional impact, the results promote the generation of knowledge applicable to experimental veterinary medicine, the management of musculoskeletal conditions related to animal aging, and the improvement of research practices involving biological models. Additionally, the study contributes to the training of qualified human resources by engaging undergraduate and graduate students in laboratory, surgical, and analytical activities, fostering technical and scientific capacity building. The work also presents an indirect extension component by enabling knowledge transfer to the academic and scientific community through publications, conference presentations, and educational activities, thereby strengthening the integration of teaching, research, and extension.

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RANKL	Ligante do receptor ativador do fator nuclear kappa-B
OPG	Osteoprotegerina
RUNX2	Fator de transcrição relacionado à Runt 2
IL-6	Interleucina 6
IGF	Fator de crescimento semelhante à insulina
CSF-1	Fator Estimulador de Colônias 1
PDGF	Fator de Crescimento Derivado de Plaquetas
BMP-2	Proteína Morfogenética Óssea 2
FNDC5	Proteína de domínio fibronectina tipo III contendo 5
TRAP	Fosfatase ácida resistente ao tartarato
DMO	Densidade Mineral Óssea
TNF- α	Fator de necrose tumoral alfa
IL-10	Interleucina 10
IL-1 β	Interleucina 1 beta
PTH	Paratormônio (hormônio da paratireoide)
ER α	Receptor de estrogênio alfa
Wnt	Caminho de sinalização Wnt
VO ₂ max	Consumo máximo de oxigênio
ROS	Espécies reativas de oxigênio
MAPK	Quinase ativada por mitógenos
JNK	Quinase N-terminal de c-Jun
p38	Subtipo da família MAPK p38
ERK1/2	Quinases reguladas por sinal extracelular 1 e 2
AMPc	Adenosina monofosfato cíclico
PKA	Proteína quinase A
CREB	Proteína ligadora do elemento de resposta ao AMP cíclico
PGC-1 α	Coativador 1 alfa do receptor gama ativado por proliferador de peroxissoma
TA	Treino Aeróbico
TR	Treino Resistido

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CAPÍTULO 1

1 INTRODUÇÃO

A integridade do tecido ósseo é essencial para a manutenção da mobilidade, da proteção de órgãos vitais e da homeostase mineral do organismo (ŠROMOVÁ; SOBOLA; KASPAR, 2023). Entretanto, alterações hormonais associadas ao envelhecimento, especialmente a deficiência estrogênica decorrente da senescência ovariana, comprometem o equilíbrio entre formação e reabsorção óssea, favorecendo a perda de massa mineral, a deterioração da microarquitetura e o aumento do risco de fraturas (BIJELIC; MILICEVIC; BALABAN, 2017). Esse processo caracteriza a osteoporose, uma condição esquelética sistêmica, que representa importante problema de saúde pública devido ao impacto funcional, econômico e social decorrente das fraturas por fragilidade (HSU; CHEN; CHEN, 2024).

A remodelação óssea é regulada por uma complexa interação entre osteoblastos, osteoclastos e osteócitos, mediada por vias moleculares e fatores sistêmicos, como hormônios, citocinas e estímulos mecânicos (FLORENCIO-SILVA et al., 2015). A deficiência de estrogênio altera esse equilíbrio ao estimular a atividade osteoclástica, reduzir a formação óssea e intensificar processos inflamatórios e oxidativos, resultando em perda progressiva de densidade mineral óssea (DMO) (KHALID; KRUM, 2016; CAULEY, 2015). Modelos experimentais de ovariectomia em roedores têm sido amplamente utilizados para reproduzir essas alterações, permitindo a investigação controlada dos mecanismos celulares e moleculares envolvidos na osteopenia induzida por deficiência hormonal (SOPHOCLEOUS; IDRIS, 2014).

Dentre as estratégias não farmacológicas propostas para a prevenção e o manejo da perda óssea, o exercício físico tem se destacado como uma intervenção capaz de promover adaptações simultâneas no tecido muscular e ósseo (ROWLANDS et al., 2020). Evidências demonstram que estímulos mecânicos gerados pela contração muscular são fundamentais para a manutenção da massa óssea, modulando vias osteogênicas e inibindo a reabsorção (KANG; RATAMESS, 2014). Diferentes modalidades de treinamento, como o resistido e o aeróbico, podem influenciar o metabolismo ósseo por mecanismos distintos, envolvendo desde a ativação de vias mecanossensíveis até a modulação de processos inflamatórios, oxidativos e endócrinos (PEREIRA et al., 2020; HONG; KIM, 2018).

Embora os benefícios do exercício sobre a saúde óssea sejam reconhecidos, ainda existem lacunas quanto à magnitude dos efeitos, aos protocolos mais eficazes e aos mecanismos moleculares específicos responsáveis pelas adaptações observadas, especialmente em

condições de deficiência estrogênica (PEREIRA et al., 2020). A comparação entre modalidades de treinamento e a integração de análises histológicas, histomorfométricas e de expressão gênica tornam-se, portanto, fundamentais para compreender como o estímulo físico pode modular o eixo osteomuscular e atenuar as alterações induzidas pela ovariectomia.

Nesse contexto, o presente estudo investiga os efeitos do treinamento aeróbico e do treinamento resistido sobre o metabolismo ósseo e as adaptações músculo-esqueléticas em modelo experimental de ovariectomia, integrando evidências da literatura e análises experimentais para elucidar os mecanismos envolvidos e contribuir para o avanço do conhecimento sobre intervenções não farmacológicas no manejo da osteoporose.

2 REFERENCIAL TEÓRICO

2.1 Tecido ósseo

O tecido ósseo é um tecido conjuntivo especializado que compõe o esqueleto dos vertebrados, localizando-se nos ossos longos, curtos, planos e irregulares que sustentam o corpo, protegem órgãos vitais, possibilitam o movimento em conjunto com o sistema muscular e atuam como importante reservatório metabólico de minerais, especialmente cálcio e fósforo (CROCKETT et al., 2011; FLORENCIO-SILVA et al., 2015; ŠROMOVÁ; SOBOLA; KASPAR, 2023). Além de sua função mecânica e estrutural, o osso participa ativamente da homeostase mineral, da regulação endócrina e da hematopoiese, abrigando a medula óssea (CROCKETT et al., 2011; FATTORE, ANDREA, 2012).

Durante o desenvolvimento embrionário, o esqueleto é formado por dois principais processos de ossificação: a ossificação intramembranosa, na qual células mesenquimais se diferenciam diretamente em osteoblastos para formar ossos planos, como os do crânio, e a ossificação endocondral, na qual um molde cartilaginoso é progressivamente substituído por tecido ósseo, processo responsável pela formação da maioria dos ossos longos (FLORENCIO-SILVA et al., 2015). Esses mecanismos garantem a organização estrutural inicial do esqueleto e estabelecem a base para sua posterior remodelação ao longo da vida.

Do ponto de vista estrutural, o tecido ósseo é composto por matriz extracelular mineralizada e por diferentes populações celulares especializadas. Macroscopicamente, distinguem-se dois tipos principais: o osso cortical (compacto) e o osso trabecular (esponjoso),

que apresentam a mesma composição química, porém organização arquitetônica e funções biomecânicas distintas (CROCKETT et al., 2011; NAKAHAMA, 2010).

O osso cortical representa aproximadamente 80% da massa esquelética total, sendo denso, compacto e altamente resistente à flexão e torção, formando a camada externa protetora dos ossos. Possui menor porosidade e menor taxa de remodelação, conferindo principalmente suporte mecânico e proteção estrutural (TETI, 2011). Em contraste, o osso trabecular corresponde a cerca de 20% da massa óssea, mas apresenta grande área de superfície, localizando-se nas epífises dos ossos longos e nos corpos vertebrais. Sua estrutura porosa e altamente vascularizada favorece elevada taxa metabólica e rápida remodelação, atuando como importante reservatório mineral e respondendo prontamente a alterações hormonais e mecânicas (HADJIDAKIS; ANDROULAKIS, 2006).

Microscopicamente, o osso maduro organiza-se predominantemente na forma de osso lamelar (ou laminar), caracterizado pela disposição ordenada de lamelas concêntricas de colágeno e minerais. No osso cortical, essas lamelas formam sistemas de Havers (osteóns), enquanto no osso trabecular dispõem-se paralelamente às superfícies das trabéculas (CROCKETT et al., 2011; FLORENCIO-SILVA et al., 2015; ŠROMOVÁ; SOBOLA; KASPAR, 2023).

A matriz óssea constitui a maior parte do tecido e é composta por uma fração orgânica e uma fração inorgânica. A porção orgânica, que representa cerca de 90% da matriz, é formada principalmente por fibras de colágeno tipo I e proteínas não colágenas, responsáveis pela resistência à tração e pela flexibilidade do tecido (ŠROMOVÁ; SOBOLA; KASPAR, 2023). A fração inorgânica é composta predominantemente por cristais de hidroxiapatita $[\text{Ca}_3(\text{PO}_4)_2 \cdot (\text{OH})_2]$, que se depositam ao longo das fibras colágenas e conferem rigidez e resistência à compressão (CROCKETT et al., 2011; FLORENCIO-SILVA et al., 2015; ŠROMOVÁ; SOBOLA; KASPAR, 2023).

Ao nível celular, o tecido ósseo apresenta três tipos celulares principais: osteoblastos, osteócitos e osteoclastos, que atuam de forma coordenada na formação, manutenção e reabsorção da matriz. Os osteoblastos são células formadoras de osso, derivadas de células-tronco mesenquimais multipotentes. Localizam-se na superfície óssea e são responsáveis pela síntese do osteoide e pela regulação da mineralização. Sua diferenciação é controlada por fatores de transcrição como Runx2 e osterix, considerados essenciais para o fenótipo osteoblástico (FLORENCIO-SILVA et al., 2015; NAKAHAMA, 2010; PHAN; XU; ZHENG, 2004).

Morfologicamente, os osteoblastos apresentam formato cúbico ou colunar quando metabolicamente ativos e localizam-se ao longo da superfície óssea, organizando-se em camadas contínuas que revestem áreas de formação de matriz (ROSENBERG; ROSENBERG; SOUDRY, 2012). Sua principal função é a síntese da matriz orgânica do osso, denominada osteoide, além de proteínas não colágenas como osteocalcina, osteopontina e sialoproteína óssea. Após a deposição do osteoide, os osteoblastos também participam da regulação do processo de mineralização, promovendo a deposição de cristais de hidroxapatita (RUTKOVSKIY; STENSLØKKEN; VAAGE, 2016).

Além da função estrutural, os osteoblastos exercem papel regulador na remodelação óssea por meio da secreção de fatores de crescimento e citocinas, como IGF, TGF- β , BMPs e prostaglandinas, que modulam a proliferação celular, a diferenciação osteogênica e a comunicação com osteoclastos (RUTKOVSKIY; STENSLØKKEN; VAAGE, 2016). Essas células também expressam moléculas-chave do eixo RANK/RANKL/OPG, controlando diretamente a osteoclastogênese e, conseqüentemente, a reabsorção óssea (TANAKA; NAKAYAMADA; OKADA, 2005). Ao final do processo de formação, os osteoblastos podem seguir três destinos: sofrer apoptose, permanecer como células de revestimento ósseo quiescentes ou serem aprisionados na matriz mineralizada, diferenciando-se em osteócitos (ROSENBERG; ROSENBERG; SOUDRY, 2012).

Os osteócitos representam a forma celular mais abundante do tecido ósseo e constituem o estágio terminal de diferenciação da linhagem osteoblástica (CHEN; SENDA; KUBO, 2015). Localizam-se em lacunas na matriz mineralizada e estendem longos prolongamentos citoplasmáticos através de canaliculos, formando uma extensa rede intercelular que permite comunicação por junções comunicantes (PRIDEAUX; FINDLAY; ATKINS, 2016).

Essa organização estrutural confere aos osteócitos papel central como mecanossensores do esqueleto. Essas células detectam deformações mecânicas geradas por cargas físicas e alterações no fluxo de fluido intersticial, convertendo esses estímulos em sinais bioquímicos que regulam a atividade de osteoblastos e osteoclastos (PRIDEAUX; FINDLAY; ATKINS, 2016). Dessa forma, os osteócitos coordenam a adaptação do osso às demandas mecânicas (CHEN; SENDA; KUBO, 2015).

No nível molecular, os osteócitos produzem mediadores como esclerostina, RANKL, óxido nítrico e prostaglandinas (DIVIETI PAJEVIC; KRAUSE, 2019). A esclerostina atua como inibidora da via Wnt/ β -catenina, reduzindo a atividade osteoblástica, enquanto o RANKL estimula a diferenciação osteoclástica. Assim, essas células exercem papel regulador tanto da

formação quanto da reabsorção óssea, sendo consideradas as principais orquestradoras da remodelação (DIVIETI PAJEVIC; KRAUSE, 2019).

Os osteoclastos são células gigantes multinucleadas originadas da fusão de precursores hematopoiéticos da linhagem monócito-macrófago (KYLMAOJA; NAKAMURA; TUUKKANEN, 2016). Diferentemente de osteoblastos e osteócitos, derivam do sistema imune e especializam-se na reabsorção óssea. Localizam-se em superfícies ósseas ativas, formando depressões denominadas lacunas de Howship (BELLIDO; PLOTKIN; BRUZZANITI, 2019).

A diferenciação e ativação osteoclástica dependem principalmente da interação entre RANKL, expresso por osteoblastos e osteócitos, e seu receptor RANK, presente nos precursores osteoclásticos. A osteoprotegerina (OPG), por sua vez, atua como receptor “isca”, bloqueando essa ligação e inibindo a osteoclastogênese. Esse equilíbrio molecular constitui o principal mecanismo regulador da reabsorção óssea (HOFBAUER; KÜHNE; VIERECK, 2004).

Portanto, a homeostase do tecido ósseo depende da interação coordenada entre osteoblastos, osteócitos e osteoclastos. Enquanto os osteoblastos promovem a síntese e mineralização da matriz, os osteoclastos realizam sua reabsorção, e os osteócitos atuam como sensores mecânicos e reguladores centrais desse processo. A desregulação desse equilíbrio, seja por alterações hormonais, inflamatórias ou mecânicas, resulta em distúrbios metabólicos como a osteopenia e a osteoporose.

2.2 Remodelação óssea

A remodelação óssea é um processo contínuo e dinâmico que ocorre ao longo de toda a vida, permitindo a substituição de tecido envelhecido ou danificado, a adaptação do esqueleto às demandas mecânicas e a manutenção da homeostase mineral sistêmica, especialmente do cálcio e do fósforo (SCHINI et al., 2023). Esse processo garante a integridade estrutural do esqueleto e o equilíbrio entre resistência mecânica e função metabólica. Este fenômeno envolve duas atividades principais e acopladas: a reabsorção da matriz óssea mineralizada pelos osteoclastos e a subsequente formação de nova matriz óssea pelos osteoblastos (GREENBLATT; TSAI; WEIN, 2020).

Owen & Reilly, (2018) descrevem a remodelação em cinco fases sequenciais. Inicialmente, o tecido encontra-se em estado quiescente, caracterizado pela presença de células de revestimento ósseo. Estímulos como microdanos, sobrecarga mecânica ou alterações no

metabolismo mineral promovem a fase de ativação, com recrutamento de precursores osteoclásticos. Em seguida, ocorre a reabsorção, na qual os osteoclastos degradam a matriz mineralizada e liberam fatores de crescimento previamente armazenados. A fase de reversão envolve a remoção de detritos celulares por macrófagos e o preparo da superfície óssea. Posteriormente, na fase de formação, osteoblastos depositam osteoide para preencher a lacuna de reabsorção, culminando na mineralização progressiva dessa matriz. Parte desses osteoblastos diferencia-se em osteócitos, enquanto outros sofrem apoptose ou permanecem como células de revestimento (Figura 1).

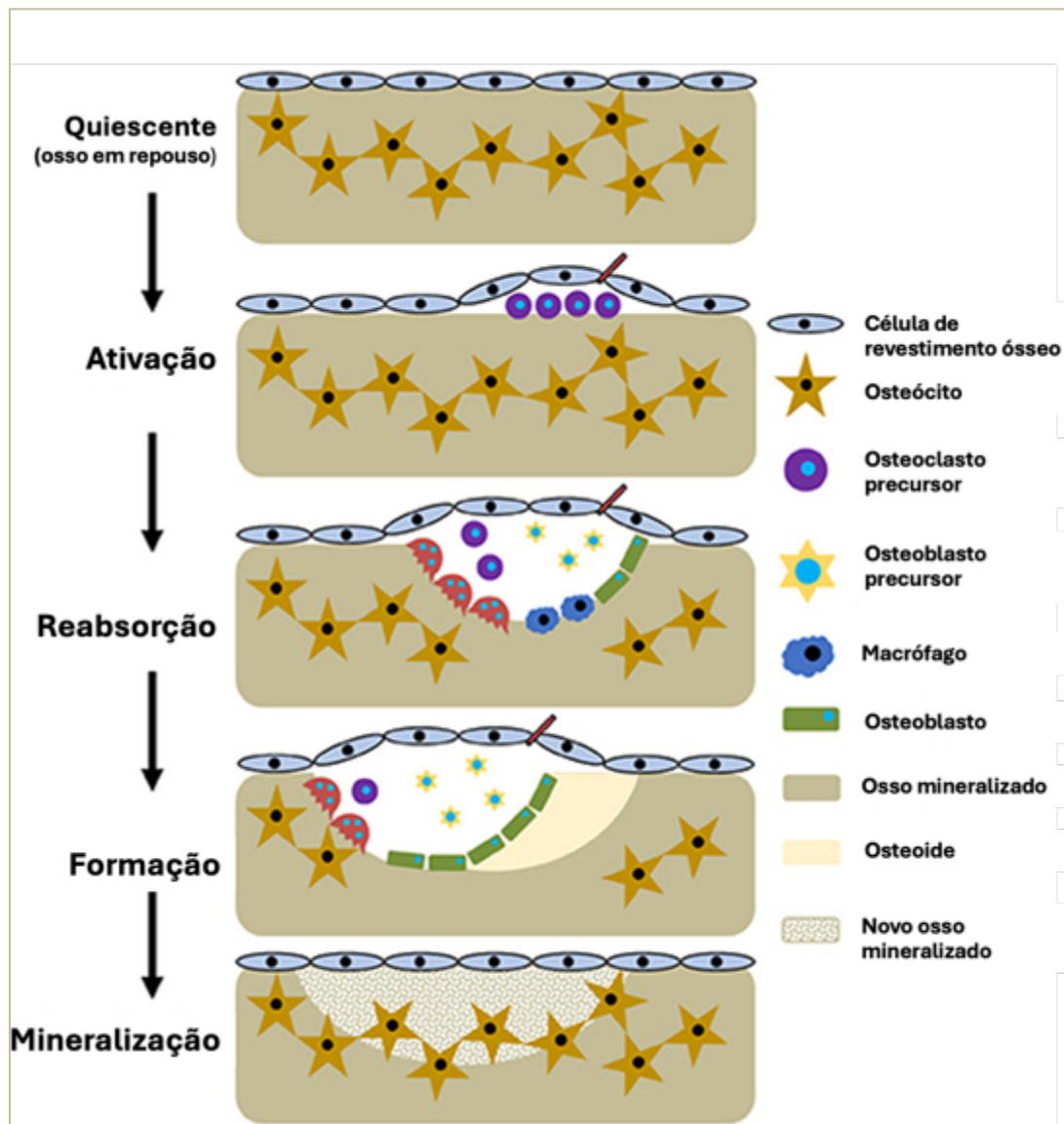


Figura 1. As 5 principais fases da remodelação óssea segundo Owen & Reilly, (2018). **Fonte:** Owen & Reilly, (2018)

Um dos mecanismos mais importantes na regulação da atividade dos osteoclastos é mediado pela interação entre as proteínas RANK, RANKL e OPG. O RANKL (ligante do receptor ativador do fator nuclear κ B) é produzido pelos osteoblastos e se liga ao receptor RANK presente na superfície das células precursoras dos osteoclastos, induzindo sua diferenciação e ativação (HOFBAUER; KÜHNE; VIERECK, 2004). Por outro lado, a OPG, também secretada pelos osteoblastos, atua como um receptor “falso”, ligando-se ao RANKL e impedindo sua interação com o RANK. Esse equilíbrio entre RANKL e OPG regula o número e a atividade dos osteoclastos, promovendo ou inibindo a reabsorção óssea conforme necessário (KHOSLA, 2001).

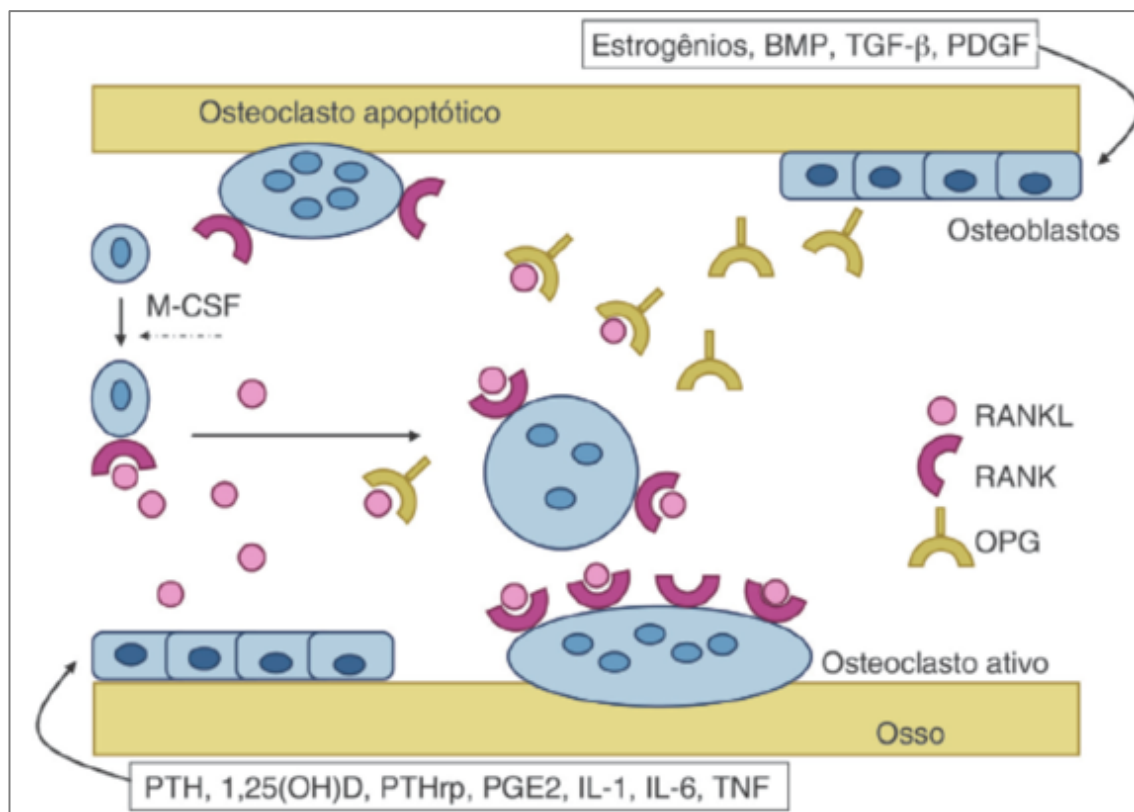


Figura 2. Representação do processo celular de remodelação óssea. Fonte: Aires (2018).

A formação óssea, por sua vez, é iniciada pela diferenciação de pré-osteoblastos em osteoblastos, processo regulado por diversos fatores de crescimento, como IGF, BMP-2 e a via de sinalização Wnt/ β -catenina. A ativação dessa via, especialmente mediada pelo complexo receptor LRP/Frizzled, promove o acúmulo de β -catenina, desencadeando a expressão de genes que estimulam a atividade e a diferenciação osteoblástica, como Runx2 e Osterix (OSX) (ROBINSON et al., 2006). A esclerostina, uma proteína produzida pelos osteócitos, age como um inibidor dessa via, e sua ausência está associada a doenças de alta densidade óssea (GALEA;

LANYON; PRICE, 2017). Durante a formação óssea, os osteoblastos depositam matriz orgânica composta predominantemente por colágeno tipo I, que posteriormente é mineralizada por cristais de hidroxiapatita (SCHINI et al., 2023).

Além do controle local, a remodelação óssea é fortemente modulada por fatores endócrinos e sistêmicos, que ajustam o balanço entre formação e reabsorção de acordo com as necessidades metabólicas do organismo, através de diferentes ações hormonais. O paratormônio (PTH) exerce efeito dependente do padrão de exposição: níveis cronicamente elevados estimulam a reabsorção óssea por aumento da expressão de RANKL, enquanto estímulos intermitentes favorecem a atividade osteoblástica e a formação óssea. A 1,25-dihidroxivitamina D potencializa a absorção intestinal de cálcio e atua sinergicamente com o PTH para manter a calcemia, influenciando indiretamente a remodelação (SCHINI et al., 2023).

Os estrogênios constituem um dos principais reguladores fisiológicos do metabolismo ósseo, exercendo efeitos protetores tanto diretos quanto indiretos sobre o tecido. Esses hormônios reduzem a produção de citocinas pró-inflamatórias (IL-1, IL-6 e TNF- α), diminuem a expressão de RANKL e aumentam a síntese de OPG por osteoblastos e osteócitos, inibindo a diferenciação osteoclástica (KHOSLA; PACIFICI, 2013). Além disso, promovem maior sobrevivência de osteoblastos e osteócitos, ao mesmo tempo em que induzem apoptose de osteoclastos. Como resultado, favorecem o balanço positivo de massa óssea (OKMAN-KILIC, 2015). A deficiência estrogênica, como ocorre no envelhecimento e na menopausa ou em modelos experimentais de ovariectomia, rompe esse equilíbrio, levando ao aumento da osteoclastogênese, intensificação da reabsorção óssea, redução da formação e consequente perda acelerada de densidade mineral e deterioração microestrutural, características típicas da osteopenia e da osteoporose (mais detalhes na seção “2.4 Senescência ovariana”) (KHOSLA; OURSLER; MONROE, 2012). A calcitonina, por sua vez, atua como hormônio anti-reabsortivo ao inibir diretamente a atividade osteoclástica, embora tenha menor relevância fisiológica em comparação aos estrogênios e ao PTH (KHOSLA; PACIFICI, 2013).

A remodelação também responde a estímulos mecânicos. Cargas físicas aumentam o fluxo de fluido nos canaliculos osteocíticos, ativando sinais anabólicos e favorecendo a formação óssea, enquanto a ausência de carga promove perda de massa óssea. Essa capacidade adaptativa fundamenta o efeito osteogênico do exercício físico e da contração muscular (RUBIN; RUBIN; JACOBS, 2006).

Dessa forma, a remodelação óssea resulta da interação entre mecanismos celulares locais, regulação molecular, controle endócrino e estímulos mecânicos, permitindo que o

esqueleto mantenha simultaneamente suas funções estruturais e metabólicas. Alterações nesse equilíbrio, especialmente associadas à deficiência hormonal, comprometem a homeostase óssea e favorecem o desenvolvimento de doenças osteometabólicas.

2.3 Osteoporose

A osteoporose é uma doença esquelética sistêmica caracterizada pela redução da massa óssea e pela deterioração da microarquitetura do tecido, resultando em fragilidade mecânica e aumento do risco de fraturas mesmo após traumas de baixa intensidade (BIJELIC; MILICEVIC; BALABAN, 2017). Essa condição compromete significativamente a mobilidade, a independência funcional e a qualidade de vida, sendo amplamente associada ao envelhecimento, a alterações hormonais, a deficiências nutricionais e a doenças crônicas (TAŃSKI; KOSIOROWSKA; SZYMAMSKA-CHABOWSKA, 2021).

Do ponto de vista fisiopatológico, a osteoporose decorre de um desequilíbrio no processo de remodelação óssea, no qual a reabsorção mediada por osteoclastos excede a formação promovida por osteoblastos, levando à perda progressiva de densidade mineral óssea e deterioração estrutural, especialmente no osso trabecular, que apresenta maior área de superfície e maior taxa de remodelação (LIANG et al., 2022). Esse desacoplamento compromete a resistência óssea e aumenta a susceptibilidade a fraturas em sítios como vértebras, colo do fêmur e extremidades de ossos longos (KOMORI, 2015).

Entre os fatores reguladores do metabolismo ósseo, o estrogênio exerce papel central na manutenção da homeostase esquelética, sendo considerado um dos principais moduladores endócrinos da remodelação (KHALID; KRUM, 2016; MANOLAGAS; O'BRIEN; ALMEIDA, 2013). Seus efeitos são mediados pelos receptores nucleares $ER\alpha$ e $ER\beta$, expressos em osteoblastos, osteócitos e osteoclastos, com predominância funcional do $ER\alpha$ (KHALID; KRUM, 2016). A ativação desses receptores reduz a diferenciação e a atividade osteoclástica, estimula a diferenciação osteoblástica, promove a síntese de matriz extracelular e potencializa vias osteogênicas, como a sinalização Wnt/ β -catenina. Além disso, o estrogênio regula a sobrevivência celular ao induzir apoptose de osteoclastos, parcialmente via Fas/FasL, e ao inibir a apoptose de osteoblastos e osteócitos, contribuindo para a preservação do tecido ósseo (SAXON et al., 2012; ZAMAN et al., 2010). De forma indireta, modula o eixo OPG/RANK/RANKL, reduzindo a expressão de RANKL e aumentando a produção de OPG, o que limita a osteoclastogênese e a reabsorção óssea (CAULEY, 2015; KHALID; KRUM, 2016; HOFBAUER; KÜHNE; VIERECK, 2004).

Durante a senescência ovariana, ocorre esgotamento folicular e consequente redução acentuada dos níveis circulantes de estrogênio, fenômeno que rompe esse equilíbrio protetor. A deficiência estrogênica favorece aumento da atividade osteoclástica, redução da formação óssea, maior inflamação sistêmica e estresse oxidativo, resultando em perda acelerada de massa e qualidade óssea (KHADILKAR, 2019; LI; WANG, 2018). Paralelamente, alterações musculares, como diminuição de massa e força, contribuem para maior risco de quedas e fraturas, evidenciando a interdependência funcional do eixo músculo–osso (BACON, 2017; MINKIN, 2019).

Nesse contexto, a osteoporose primária associada à senescência ovariana em modelos animais, ou à menopausa em humanos, representa uma das formas mais prevalentes da doença (BACON, 2017; MINKIN, 2019). Em estudos experimentais, a ovariectomia é amplamente utilizada para mimetizar a deficiência hormonal pós-menopausa, reproduzindo alterações celulares, estruturais e moleculares características da osteopenia e da osteoporose, constituindo um modelo consolidado para investigação de estratégias terapêuticas e intervenções, como o exercício físico (SOPHOCLEOUS; IDRIS, 2014).

2.4 Ovariectomia

Como consequência direta da deficiência estrogênica associada à senescência ovariana, modelos experimentais que mimetizam a queda hormonal pós-menopausa são amplamente utilizados para investigar os mecanismos fisiopatológicos da osteoporose. Nesse contexto, a ovariectomia (OVX), ou remoção cirúrgica dos ovários, constitui o modelo animal padrão-ouro para reproduzir a deficiência de hormônios gonadais e suas repercussões sistêmicas. Esse procedimento tem sido essencial para entender o papel dos hormônios gonadais em sistemas como o esquelético, metabólico e imunológico, além de eventos associados à menopausa, como osteoporose, distúrbios cardiometabólicos e doenças inflamatórias (SOPHOCLEOUS; IDRIS, 2014).

Em roedores, os ovários estão localizados nos polos posterolaterais dos rins, fixados pelo mesovário à parede dorsal da cavidade abdominal. A remoção dos ovários pode ser realizada por três abordagens principais: incisão única; incisões dorsolaterais duplas e incisão ventral transversal única (SOUZA et al., 2019). As incisões dorsolaterais duplas são vantajosas, pois facilitam o acesso aos ovários e permitem uma recuperação mais rápida após a cirurgia, conforme observado em nossa experiência (SOUZA et al., 2019).

A OVX induz redução abrupta dos níveis circulantes de estrogênio, resultando em aumento da atividade osteoclástica, diminuição da formação óssea, alterações musculares e desbalanço metabólico, reproduzindo características estruturais, celulares e moleculares observadas na osteopenia e na osteoporose pós-menopausa (SOPHOCLEOUS; IDRIS, 2014). Por esse motivo, o modelo tem sido amplamente empregado para avaliar intervenções terapêuticas e estratégias não farmacológicas, como o exercício físico, permitindo investigar os efeitos dessas abordagens sobre o eixo osteomuscular e a remodelação esquelética (GALDINO-ALVES et al., 2022; ZHOU; TAO, 2022).

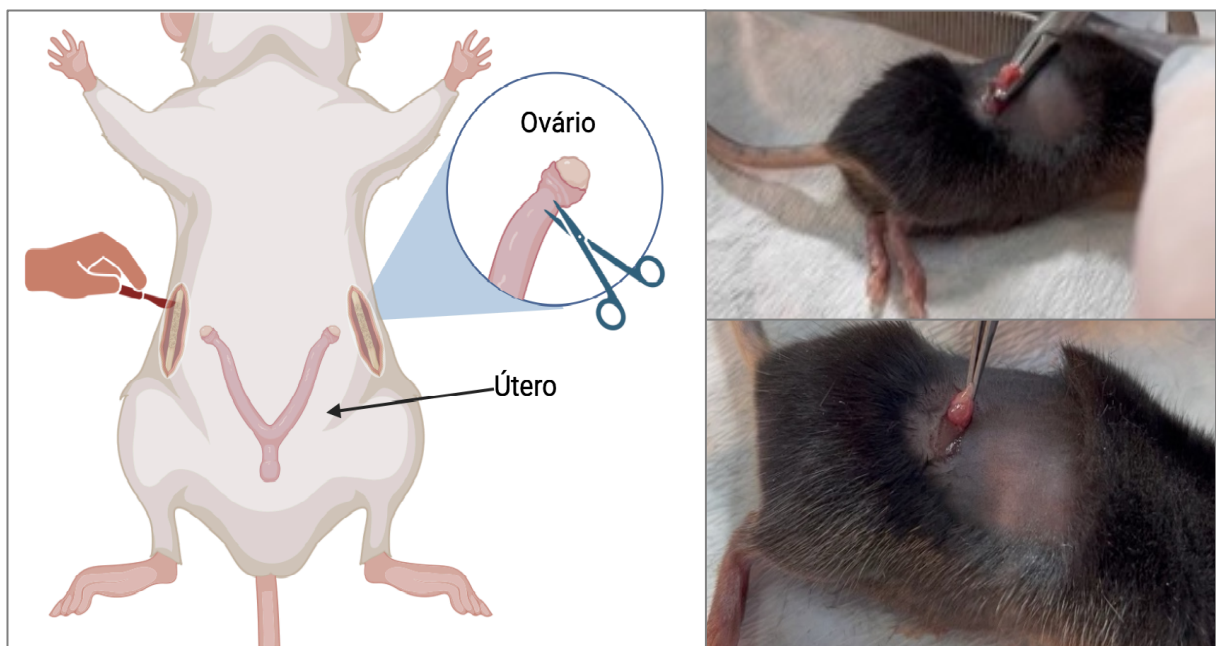


Figura 3. Incisão bilateral da OVX. Remoção dos ovários. Fonte: Do autor (2025).

2.5 Senescência ovariana

Fisiologicamente, a deficiência de hormônios sexuais é mais prejudicial em fêmeas, pelo esgotamento dos folículos ovarianos e consequente interrupção dos ciclos estrais decorrente do envelhecimento natural (BACON, 2017; MINKIN, 2019). A deficiência de estrogênio pode causar efeitos adversos nos ossos, músculos, ligamentos, tendões, colágeno, cartilagem, membrana sinovial e cápsula das articulações, podendo comprometer a saúde musculoesquelética (KHADILKAR, 2019; LI; WANG, 2018). O estrogênio atua direta e indiretamente no músculo e nos ossos, contribuindo para a patogênese da sarcopenia e da osteoporose (JANSSEN; HEYMSFIELD; ROSS, 2002; JIANG et al., 2019; KHADILKAR, 2019). Como resultado, pode ocorrer um quadro de fragilidade muscular e incapacidade

locomotora, podendo levar a aumento da incidência de quedas, e não somente isso, pode comprometer substancialmente a qualidade de vida e contribuir para maior morbimortalidade em idades avançadas (BACON, 2017; MINKIN, 2019).

O estrogênio desempenha papel central na manutenção da densidade mineral óssea (DMO) em camundongos e humanos, atuando como um dos principais reguladores endócrinos da remodelação óssea (CAULEY, 2015). Seus efeitos são mediados principalmente pelos receptores nucleares ER α e ER β , expressos em osteoblastos, osteócitos e osteoclastos, sendo o ER α considerado o principal modulador da fisiologia óssea. A ativação desse receptor promove ações diretas e indiretas que favorecem o balanço positivo de massa óssea, reduzindo a reabsorção e estimulando a formação (KHALID; KRUM, 2016).

No compartimento trabecular, o estrogênio diminui a diferenciação e a atividade osteoclástica, enquanto, nos osteoblastos e seus precursores, estimula a diferenciação celular, a síntese de matriz e a sinalização anabólica, especialmente por meio da via Wnt/ β -catenina, que aumenta a expressão de genes osteogênicos. Além disso, o ER α integra sinais hormonais e mecânicos, potencializando a resposta osteogênica ao carregamento físico (KHALID; KRUM, 2016; MANOLAGAS; O'BRIEN; ALMEIDA, 2013).

Em nível molecular, o estrogênio regula a sobrevivência celular ao induzir apoptose de osteoclastos, parcialmente pela ativação da via Fas/FasL, ao mesmo tempo em que inibe a apoptose de osteoblastos e osteócitos, inclusive por mediadores como a semaforina 3^a (SAXON et al., 2012; ZAMAN et al., 2010). Adicionalmente, exerce controle indireto da osteoclastogênese por meio do eixo OPG/RANK/RANKL, reduzindo a expressão de RANKL e aumentando a produção de OPG, o que limita a formação e a ativação de osteoclastos (HOFBAUER; KÜHNE; VIERECK, 2004; SUN; QIN, 2024).

Consequentemente, a redução dos níveis estrogênicos, como ocorre na menopausa ou em modelos experimentais de ovariectomia, rompe esse equilíbrio protetor, favorecendo aumento da reabsorção, redução da formação óssea e perda progressiva de massa e qualidade do tecido, alterações características da osteopenia e da osteoporose

2.6 Exercício físico e tecido ósseo

Tradicionalmente, a atividade física tem sido definida como qualquer movimento corporal produzido pelos músculos esqueléticos que resulta em gasto energético; entretanto, abordagens contemporâneas ampliam esse conceito ao considerar seu papel na promoção da

saúde e do bem-estar, bem como a influência de fatores sociais, ambientais e contextuais (SALVO et al., 2026). Já o exercício físico é um subgrupo da atividade física, caracterizado por ser estruturado, planejado, repetitivo e com um objetivo específico, como melhorar a aptidão física, desempenho muscular ou saúde geral (MCARDLE; KATCH; KATCH, 2006).

A baixa DMO aumenta significativamente o risco de fraturas e quedas. Assim, estratégias que aumentem a massa óssea e/ou reduzam o risco de quedas são fundamentais para diminuir a morbidade e a mortalidade associadas às fraturas osteoporóticas (KOHRT et al., 2004; ROWLANDS et al., 2020). Embora medicamentos possam ajudar a aumentar a massa óssea, a atividade física é uma intervenção não medicamentosa capaz de melhorar simultaneamente a resistência óssea e reduzir o risco de fraturas (ROWLANDS et al., 2020).

Os benefícios do exercício físico são amplamente reconhecidos, tanto nas modalidades aeróbica e resistida, que apresentam resultados bem documentados. O treinamento aeróbico promove adaptações importantes, como aumento do $VO_2\text{máx}$ (GORMLEY et al., 2008), maior capacidade oxidativa (SHORT et al., 2003) estímulo à biogênese mitocondrial, maior densidade mitocondrial, aumento dos estoques de glicogênio intramuscular e melhora na irrigação muscular (PRIOR et al., 2015). Essas mudanças favorecem o transporte de nutrientes e por consequência a reparação óssea. O exercício aeróbico também reduz mediadores inflamatórios, como $TNF-\alpha$ e $IL-1\beta$ (EDSFELDT et al., 2015), enquanto aumenta a síntese de $IL-10$ (CERVANTES-BARRAGAN et al., 2006), uma citocina anti-inflamatória essencial para o metabolismo ósseo.

Por outro lado, o treinamento resistido, caracterizado por contrações musculares contra uma sobrecarga externa, é geralmente realizado de forma intervalada para evitar fadiga periférica (KANG; RATAMESS, 2014). Essa modalidade é especialmente eficaz no aumento da força, da massa muscular e da densidade óssea, além de contribuir para a melhora da capacidade funcional e qualidade de vida. Ela também desempenha um papel crucial na prevenção de sarcopenia e osteoporose (GARBER et al., 2011; KHADILKAR, 2019; LI; WANG, 2018).

Ambas as modalidades de treinamento promovem adaptações importantes no metabolismo ósseo e são estratégias valiosas no manejo de doenças osteometabólicas, devido as várias vias moleculares envolvidas nos processos de remodelação óssea. No entanto, é fundamental compreender os mecanismos específicos de cada tipo de treinamento sobre os parâmetros ósseos na osteoporose.

2.7 Treinamento resistido

O treinamento resistido, ou treino de resistência (TR), é uma modalidade de treinamento físico amplamente utilizada para melhorar a aptidão, a saúde e o desempenho muscoesquelético (KANG; RATAMESS, 2014). Ele envolve o uso de pesos livres, máquinas de musculação, bolas medicinais, faixas elásticas e variações de velocidade de movimento. Além de promover ganhos de força e massa muscular, o treino resistido é fundamental para a saúde óssea, pois a carga mecânica gerada durante os exercícios induz tensões que excedem os limites necessários para estimular a modelagem óssea (BORDE; HORTOBÁGYI; GRANACHER, 2015). Essa característica o torna uma ferramenta valiosa na prevenção e manejo de condições como osteoporose e sarcopenia, especialmente em populações mais velhas ou em mulheres na menopausa, ao mesmo tempo que contribui para a melhora da capacidade funcional e da qualidade de vida (HONG; KIM, 2018).

Os mecanismos relacionados ao treinamento resistido envolvem estímulos mecânicos aplicados ao osso, que podem promover uma resposta osteogênica. As forças de tensão, compressão e torção geradas durante o treinamento de resistência produzem sinais elétricos que estimulam o metabolismo ósseo e a deposição de minerais, levando ao aumento da DMO e potencialmente inibindo a reabsorção óssea (MOREIRA et al., 2014). A carga mecânica é essencial para a formação e manutenção da massa óssea (STEWART et al., 2020). O osso possui um sistema biológico intrínseco que estimula a formação óssea em resposta a tensões mecânicas elevadas, promovendo seu fortalecimento (KLEIN-NULEND et al., 2013). Esse sistema envolve a interação das células ósseas, especialmente os osteócitos, que detectam as cargas mecânicas e transmitem sinais para osteoblastos e osteoclastos, responsáveis por manter a homeostase esquelética (KLEIN-NULEND et al., 2013; STEWART et al., 2020).

Resumidamente, uma proteína-chave nesse processo é a esclerostina, produzida pelos osteócitos, que regula a formação óssea. A esclerostina atua como um antagonista da via Wnt, bloqueando a sinalização da via Wnt/ β -catenina, crucial para aumentar as populações de células osteoprogenitoras e reduzir a apoptose de osteoblastos maduros. A carga mecânica diminui a expressão de esclerostina nos ossos, promovendo a formação óssea por osteoblastos e inibindo a reabsorção óssea pela supressão da atividade dos osteoclastos (GALEA; LANYON; PRICE, 2017). Além disso, a contração muscular resultante do treinamento resistido está associada ao aumento da expressão do peptídeo irisina, codificado pelo gene FNDC5, que regula o metabolismo dos osteócitos por meio de receptores específicos da classe αV (KAWAO et al.,

2018). Estudos mostraram que o treinamento de resistência pode elevar os níveis de expressão do gene *FNDC5* (PEREIRA et al., 2020) e uma revisão sistemática dos níveis de irisin e treinamento físico, incluindo modalidades de resistência, descobriu que níveis mais altos desse peptídeo estão ligados a melhorias na DMO em camundongos (PEREIRA et al., 2022). A carga mecânica também é capaz de induzir a secreção de fatores osteomusculares como miostatina, TGF- β e PGE2 (BONEWALD, 2019).

O treino resistido aumenta significativamente a DMO em várias regiões de ossos de roedores. Esse efeito pode ser atribuído à capacidade da TR de modular a resposta inflamatória e promover um ambiente mais favorável ao anabolismo ósseo (CALLE; FERNANDEZ, 2010). Evidências sugerem que o TR pode suprimir a expressão de citocinas pró-inflamatórias e promover a liberação de fatores de crescimento, como o IGF-1 e proteínas morfogenéticas ósseas (BMPs), como a TGF- β (BORDUKALO-NIKŠIĆ; KUFNER; VUKIČEVIĆ, 2022; CALLE; FERNANDEZ, 2010) que são essenciais para a formação óssea. Além disso, a contração muscular parece influenciar positivamente a remodelação óssea, equilibrando a atividade de osteoclastos e osteoblastos (BORDUKALO-NIKŠIĆ; KUFNER; VUKIČEVIĆ, 2022). Esse equilíbrio é crucial para manter a integridade estrutural do osso, especialmente em condições de deficiência de estrogênio, onde a atividade osteoclástica é exacerbada (BORDUKALO-NIKŠIĆ; KUFNER; VUKIČEVIĆ, 2022). O TR pode aumentar a expressão da osteoprotegerina (OPG) (PEREIRA et al., 2020) um inibidor da osteoclastogênese, e reduzir a expressão do ativador do receptor do ligante do fator nuclear kappa-B (RANKL), que promove a osteoclastogênese (HOFBAUER; KÜHNE; VIERECK, 2004; SATTLER et al., 2004).

2.8 Treinamento aeróbico

O treinamento aeróbico (TA) é uma prática amplamente realizada de forma contínua ou intervalada, principalmente por meio de caminhadas, corridas (na esteira ou solo) ou bicicleta, e é caracterizado pela ausência de carga externa, com ênfase no consumo de oxigênio (KANG; RATAMESS, 2014). Os principais benefícios do TA incluem melhorias no $VO_{2\text{máx}}$, aumento da capacidade oxidativa, biogênese e densidade mitocondrial, além de promover um aumento na irrigação muscular e no estoque de glicogênio intramuscular (GORMLEY et al., 2008; PRIOR et al., 2015). Embora o TA não envolva a sustentação de peso, como o treinamento resistido (TR), ele também contribui para a melhora da densidade mineral óssea (DMO), especialmente em ossos diretamente relacionados ao exercício, como o colo do fêmur (MA;

WU; HE, 2013), no entanto, de forma menos significativa do que o TR (MOREIRA et al., 2014).

Este efeito do TA sobre a DMO é mediado por alterações no metabolismo ósseo, que é regulado pela interação entre osteoclastos, osteoblastos e sinais de remodelação óssea, como RANK, RANKL e OPG (HADJIDAKIS; ANDROULAKIS, 2006; HOFBAUER; KÜHNE; VIERECK, 2004; LIANG et al., 2022). Na osteoporose, o desequilíbrio entre a reabsorção óssea e a formação óssea é acentuado, com um aumento na expressão de RANKL e uma redução na OPG, o que favorece a osteoclastogênese e acelera a reabsorção óssea (SUN; QIN, 2024). Além disso, a inflamação mediada por citocinas, como IL-6 e TNF- α , está envolvida nesse processo, promovendo a ativação dos osteoclastos e prejudicando a atividade dos osteoblastos (BRYLKA; SCHINKE, 2019).

Nesse contexto, o exercício aeróbico pode exercer um impacto importante na modulação do sistema imunológico. O TA pode aumentar a expressão de IL-6, que, estimulada por vias musculares em resultado ao exercício físico, atua de maneira anti-inflamatória, promovendo a liberação de mediadores anti-inflamatórios, como IL-10, IL-4 e TGF- β (SMITH, 1999). Esses efeitos anti-inflamatórios ajudam a criar um ambiente favorável para a preservação da saúde óssea, principalmente através da redução das citocinas pró-inflamatórias e do aumento de fatores que promovem a regeneração e a saúde celular, como a liberação de miohormônios como a irisina (VITALE et al., 2018).

Pesquisas indicam que o exercício aeróbico pode influenciar o sistema imunológico por meio de duas vias distintas. O primeiro envolve uma resposta local, caracterizada pelo aumento da expressão de IL-6 no músculo ativo, o que subsequentemente leva à elevação sistêmica dos níveis de IL-10 e à redução dos níveis de IL-1 β (NEVES et al., 2014). A segunda via é sistêmica, ocorrendo via redistribuição celular mediada por fatores neuroendócrinos (NEVES et al., 2014). Classicamente, a produção de IL-6 é intensificada em resposta ao intenso esforço físico (NEVES et al., 2014). O exercício aeróbico modula o sistema imunológico, causando um aumento sistêmico agudo de mediadores pró-inflamatórios, seguido por um aumento de mediadores anti-inflamatórios, reduzindo assim a inflamação (DAGDEVIREN et al., 2017).

O efeito anti-inflamatório do TA também pode ser atribuído à modulação da produção de óxido nítrico (NO) e espécies reativas de oxigênio (ROS), bem como à ativação de suas vias sinalizadoras subsequentes. O exercício físico estimula a síntese de NO e ROS, os quais desempenham papéis cruciais na indução de mecanismos de defesa anti-inflamatórios, especialmente ao regular a expressão gênica no tecido muscular (SCHEELE; NIELSEN;

PEDERSEN, 2009). No caso das ROS, o aumento na produção induzido pelo treinamento cíclico desencadeia uma resposta adaptativa. Essa resposta protege o músculo esquelético da exposição excessiva às ROS geradas pelo próprio exercício. Além disso, esse fenômeno está associado à redução da expressão de TNF- α , uma citocina que contribui para processos inflamatórios (SCHEELE; NIELSEN; PEDERSEN, 2009).

Embora a produção excessiva de ROS possa contribuir para o desequilíbrio na homeostase óssea, a regulação adaptativa promovida pelo exercício pode mitigar esses efeitos adversos. ROS desempenham papéis importantes na modulação de vias de sinalização intracelulares em células ósseas, influenciando tanto a reabsorção quanto a formação óssea (DZUBANOVA et al., 2024). Eles promovem a osteoclastogênese ao ativar as vias de sinalização MAPK, como JNK, p38 e ERK1/2, que regulam a proliferação, diferenciação e sobrevivência dos osteoclastos. Além disso, ROS aumentam indiretamente a ativação dos osteoclastos ao estimular a expressão de RANKL nos osteoblastos, por meio da via de sinalização de AMPc, envolvendo PKA e CREB. A fosforilação de CREB permite sua ligação ao promotor de RANKL, aumentando sua transcrição e destacando o papel central dos osteoblastos nesse processo (DZUBANOVA et al., 2024).

O TA estimula a expressão de PGC-1 α , aumentando a biogênese mitocondrial e a atividade antioxidante, o que contribui para a redução de ROS. A via PGC-1 α auxilia na regulação da osteoclastogênese ao controlar os níveis de ROS, que são importantes mediadores da ativação de osteoclastos (DALLE CARBONARE et al., 2023). Por meio da interação com SIRT3 e a sinalização PGC-1 α a via reduz o acúmulo de ROS, limitando a ativação de vias como AMPK e a expressão de RANKL, que promovem a diferenciação de osteoclastos (DING et al., 2017; JING et al., 2011). Dessa forma, o TA potencializa a capacidade da via PGC-1 α de mitigar os efeitos pró-osteoclásticos de ROS, promovendo a homeostase óssea e reduzindo o risco de reabsorção óssea exacerbada (DALLE CARBONARE et al., 2023).

A Figura 4 ilustra os mecanismos relacionados as diferentes respostas fisiológicas dos mecanismos celulares relacionados ao TR e TA.

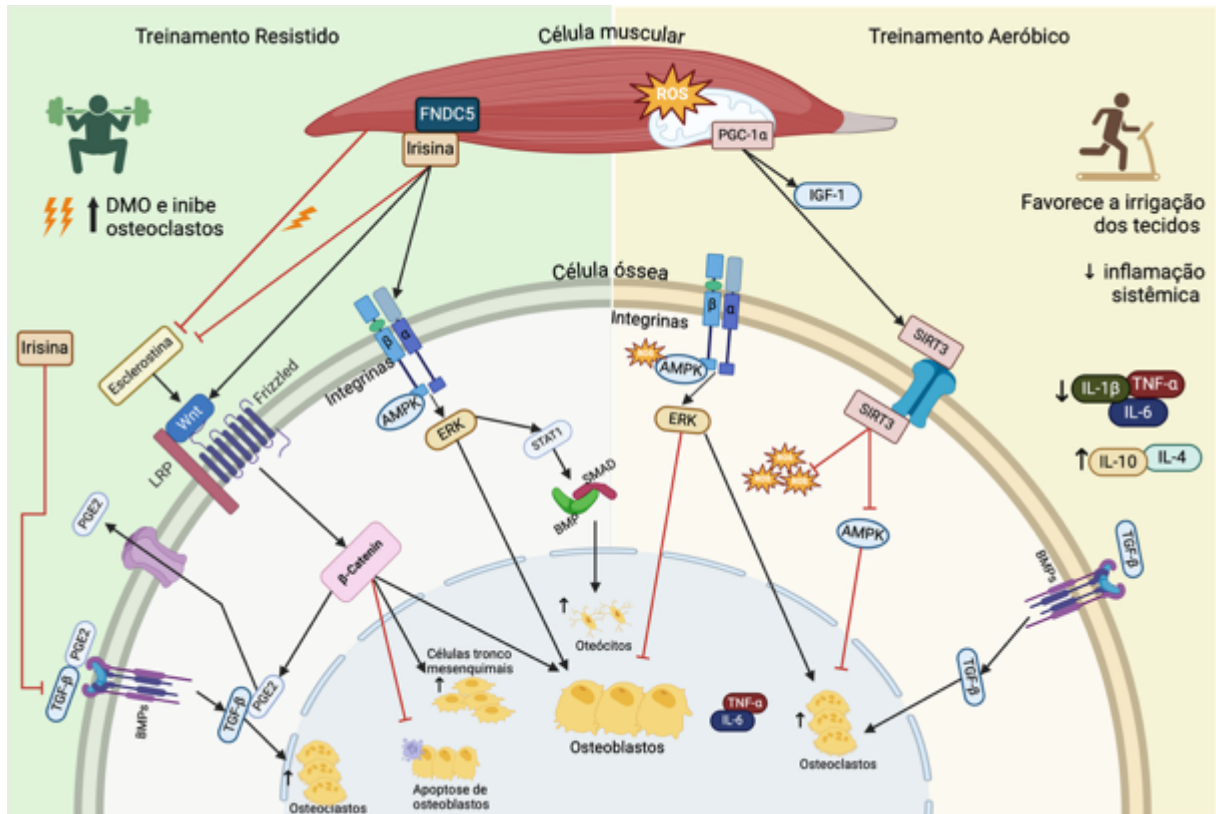


Figura 4. O treinamento resistido aumenta a densidade mineral óssea (DMO) e inibe os osteoclastos por meio da liberação de irisina, que ativa a via Wnt/ β -catenina e inibe a esclerostina, favorecendo a formação óssea. Já o treinamento aeróbico melhora a irrigação dos tecidos e reduz a inflamação sistêmica ao modular citocinas pró e anti-inflamatórias, além de ativar a via PGC-1 α /SIRT3, que regula a produção de espécies reativas de oxigênio (ROS) e mantém a função mitocondrial. Ambos os tipos de exercício influenciam diretamente as células musculares e ósseas, promovendo adaptações como a proliferação de osteoblastos, a regulação da atividade osteoclástica e a preservação da matriz extracelular, contribuindo para a saúde óssea e muscular. Fonte: Do autor (2025). Criado com BioRender.com

3 OBJETIVOS

3.1 Objetivo geral

Investigar os efeitos do treinamento físico aeróbico e resistido sobre o metabolismo ósseo e as adaptações músculo-esqueléticas em condições de deficiência estrogênica, integrando evidências da literatura e resultados experimentais em modelo animal de ovariectomia, com ênfase na comparação entre treinamento aeróbico e treinamento resistido.

3.2 Objetivos específicos

- Comparar os efeitos do treinamento aeróbico e do treinamento resistido sobre a remodelação óssea femoral e muscular em camundongos ovariectomizados, avaliando parâmetros histológicos, histoquímicos e a expressão gênica de marcadores ósseos e musculares, através de diferentes análises:
 - Verificar os efeitos da ovariectomia sobre o metabolismo ósseo e muscular em camundongos fêmeas, caracterizando um modelo experimental de deficiência estrogênica.
 - Comparar os efeitos do treinamento aeróbico e do treinamento resistido sobre a remodelação óssea femoral em animais ovariectomizados e SHAM, por meio da quantificação de osteoblastos, osteoclastos e osteócitos.
 - Avaliar o impacto das diferentes modalidades de treinamento físico sobre a expressão gênica de marcadores osteogênicos e osteoclastogênicos no osso femoral, incluindo RUNX2, OPG, RANKL e a razão RANKL/OPG.
 - Investigar as adaptações morfológicas do músculo esquelético induzidas pelo treinamento aeróbico e resistido, por meio da análise histomorfométrica da área de secção transversal das fibras musculares.
 - Analisar a expressão gênica de fatores relacionados ao eixo músculo–osso no músculo esquelético, especificamente IGF-1 e FNDC5, em resposta às diferentes modalidades de treinamento físico.

4 CONSIDERAÇÕES GERAIS

A deficiência estrogênica decorrente da senescência ovariana promove profundo desequilíbrio na remodelação óssea, caracterizado pelo predomínio da reabsorção sobre a formação, aumento da osteoclastogênese mediado pelo eixo RANKL/OPG, supressão de vias osteogênicas como Wnt/ β -catenina, maior apoptose de osteoblastos e osteócitos e intensificação de processos inflamatórios e oxidativos. Paralelamente, alterações musculares reduzem a produção de fatores anabólicos e o estímulo mecânico sobre o esqueleto, comprometendo o eixo osteomuscular e agravando a fragilidade esquelética. Em conjunto, esses mecanismos contribuem para a deterioração progressiva da microarquitetura e da qualidade do tecido ósseo observada em condições de senescência ovariana.

Os achados desta tese demonstram que o exercício físico atua diretamente sobre esses mecanismos fisiopatológicos. O estímulo mecânico induzido pelo treinamento promove mecanotransdução osteocitária, ativação de vias osteogênicas, aumento da diferenciação osteoblástica (RUNX2), redução da atividade osteoclástica e modulação favorável do eixo RANKL/OPG, restabelecendo o balanço da remodelação óssea. Além disso, as adaptações musculares observadas, incluindo aumento da área de fibras e maior expressão de IGF-1 e FNDC5, reforçam a comunicação endócrina e parácrina entre músculo e osso, potencializando os efeitos anabólicos sobre o tecido esquelético.

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CAPÍTULO 2 – ARTIGO 1

Resistance training and bone mineral density in rodent models of menopause: review and meta-analysis

Published version: <https://doi.org/10.1080/13697137.2025.2530434>

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Effect of resistance training on bone mineral density in rodent models of ovariectomy-induced osteopenia: a systematic review and meta-analysis

Abstract: The decline in bone mineral density (BMD) is common among older adults, especially due to reduced sex hormone production in women. Understanding the impact of resistance training on bone tissue is essential for developing effective strategies for the prevention and treatment of this condition. This review investigated the effects of resistance training on BMD in murine models of osteopenia induced by ovariectomy (OVX). The protocol was registered on PROSPERO (CRD42024549460). Inclusion criteria focused on OVX female rodents engaged in resistance training, with no restrictions on duration, frequency, or intensity. A comprehensive search across six databases identified 314 references, of which 18 were selected for qualitative evaluation. The results indicated that resistance training, compared to control groups, significantly increased BMD, particularly in the femur and tibia. The included studies employed various training protocols, such as climbing, jumping, and squatting, with variations in training duration and intensity. The meta-analysis showed a standardized mean difference (SMD) of 3.02 (95% CI: 1.81–4.24) for climbing protocols and 1.27 (95% CI: 0.72–1.82) for jumping, indicating significant improvements in BMD, particularly in the femur and tibia. These findings suggest that resistance training positively impacts bone remodeling and may help restore bone microarchitecture in postmenopausal conditions.

Keywords: menopause; resistance training; animal models; systematic review

Introduction

Bone diseases such as osteopenia and osteoporosis are marked by reduced bone mineral content, leading to fragility and increased fracture risk [1–3]. Aging is a major contributor to these conditions, particularly during menopause, when estrogen levels—crucial for maintaining bone metabolism in women—decline significantly [4,5].

To better understand and mitigate the effects of menopause on bone health, experimental models have been developed including female rodents undergoing ovariectomy (OVX) [6]. In these models, exercise-based interventions have been studied in relation to bone health, evaluating frequently Bone Mineral Density (BMD) [7–12] or Bone Mineral Content (BMC) [13,14]. Besides, different exercise modalities have been studied evaluating osteoclastic and osteoblastic activity [11,15]. Various exercise protocols, including resistance and aerobic training, have been explored with differing intensities and durations [7,8,16–18], including the combination of exercise with supplements like vitamins [19], prebiotics [20], or medications [9] to assess potential synergistic effects. These studies also examine different bone tissues and employ varied tools and measurement methods, making it challenging to standardize the analyses. Consequently, the findings are still inconclusive regarding the best exercise modality, intensity or duration needed for bone microarchitecture maintenance [21].

Studies involving rodents are conducted under controlled environmental conditions, free from confounding factors such as varied dietary habits, tobacco use, and alcohol consumption, which are commonly present in studies with humans [22]. Moreover, systematic reviews and meta-analyses of preclinical studies serve as essential tools for synthesizing evidence on the effects of resistance training on bone mineral density (BMD) in ovariectomy (OVX)-induced osteopenia models. These reviews consolidate findings from diverse experimental settings, highlight gaps in the existing literature, and offer valuable insights to guide future research [23]. Therefore, this study aims to perform a systematic review and meta-analysis to evaluate the impact of resistance training on BMD in rodent models of OVX-induced osteopenia.

Methods

Registration protocol and study design

This systematic review was registered at PROSPERO under protocol number CRD42024549460 and followed PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) adapted guidelines [24].

Eligibility Criteria, Search Strategy, and Data Extraction

Three independent reviewers (P.G.M, J.C.R.C, and L.B.R.) searched for animal studies published up to September 20, 2024, across six online databases and gray literature: Lilacs (<https://lilacs.bvsalud.org/en/>), PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Scopus (<https://www.scopus.com/>), Web of Science (<https://www.webofscience.com/>) Embase (<https://www.embase.com/>), and Science Direct (<https://www.sciencedirect.com/>). A combination of searches with controlled predefined MeSH (Medical Subject Headings) used combinations of the following keywords: ("rodent models" OR "rats" OR "mice") AND ("ovariectomy-induced osteopenia" OR "ovariectomy" OR "ovariectomized") AND ("resistance training" OR "strength training" OR "weightlifting" AND "bone mineral density" OR "BMD"). No filters were applied to any of the available databases. Articles written in English with no time restriction were included. The PICO question aimed to investigate if in ovariectomized female rodents (Population), does resistance training (Intervention) influences bone mineral density (Outcome) compared to sedentary control rodents (Comparison)?

The exclusion criteria included studies whose titles and abstracts did not meet the eligibility requirements, such as opinion or technical reports, review articles, guidelines, and editor letters. We also excluded studies involving male animals, those that did not induce osteopenia via ovariectomy, or those that used exercise protocols other than resistance training, such as aerobic treadmill running, aerobic swimming, or other forms of muscle stimulation.

A reference manager (Mendeley® (www.mendeley.com)) was employed to collect references from all databases and to remove duplicates. Study selection involved a 2-phase process conducted by 3 independent reviewers (add reviewers). The first phase consisted of titles and abstracts screening

utilizing Rayyan® (www.rayyan.ai) for the independent study selection and the evaluation of agreement between reviewers. The second phase involved reading the articles' full texts. Disagreements were solved through discussion, with consensus reached in sessions led by the coordinator (add coordinator).

The searches were further supplemented by the database ProQuest and Google Scholar ("gray literature") and related terms. Manual searches were also performed on the references of the selected studies.

Data extraction

The designated articles underwent data extraction, encompassing authors, year of publication, study design, animal characteristics (source and sample size), average age, type of bone, type and period of resistance training, and bone mineral density levels. In instances where a lack of information affected data extraction or posed a risk of bias, efforts were made to contact the authors via email for up to four consecutive weeks. Three independent authors determined the quality classification criteria and risk of bias.

Risk of Bias assessment

We assessed the risk of bias using the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) Risk of Bias tool. This tool includes 10 criteria that assess 6 types of bias: selection, performance, detection, attrition, reporting, and other biases [25].

Quality criteria assessment

The selected studies were evaluated for quality based on the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines [26,27]. This evaluation involved rating the studies on 20 predefined categories using a scoring system. Each criterion was graded, and the total score ranged from zero to 36 points. The maximum score for each category was also calculated as described by other studies [22,28].

A Quality Score/Maximum Score ratio was determined, with scores falling in the range of 0.8–1 considered "excellent," 0.5–0.8 considered "average," and scores below 0.5 considered "poor" [22,28].

Statistical analysis

Meta-analyses were conducted using Review Manager software (version 5.3; The Cochrane Collaboration) [29]. To reduce methodological heterogeneity, only studies that assessed bone mineral density (BMD) via X-ray absorptiometry were included in each forest plot. We analyzed OVX sedentary against OVX trained animals separately, focusing solely on studies that involved resistance training. The mean difference (MD) served as the measure of effect, calculated as the difference between the mean BMD values in the OVX sedentary groups and those in the OVX training group both control and trained animals.

The Der Simonian–Laird method and inverse variance were utilized to estimate between-study variance (τ^2). All analyses were performed using random effects models, incorporating the mean values, standard deviations, and sample sizes from each study for both experimental and control groups. Since only a small number of studies were included, publication bias could not be quantitatively assessed using the Egger test or a funnel plot [30].

Results

Study selection and characteristics

The searches in all databases and gray literature resulted in 314 references. After excluding duplicates and reviewing titles and abstracts, thirteen studies were selected from the databases and six from the gray literature for full-text appraisal, totaling nineteen studies. However, one article was excluded after reading the full text and thus, eighteen articles were eligible for qualitative assessment (Figure 1).

Results for individual studies

All studies investigated the effects of some resistance training on bone parameters. Different training protocols were identified. For instance, eight studies involved climbing training for 3 [31], 8 [19,20], 10 [12], 12 [14,15,32], and 17 weeks [9], while five assessed jump training for 8 [7,8,33] and 10 [34] weeks, considering that Stunes et al., (2020) [10] evaluated the effects of jump training at two different time points within (4 and 12 weeks). Additionally, four studies assessed the impact of external weight application on water for 8[13], 9[35], and 10 weeks [36], and also one evaluated the use of external weight application on a treadmill for 10 weeks [11]. Finally, one study evaluated the effect of squat training for 8 weeks [16].

Across the studies, different instruments were utilized to assess bone density (BD). Twelve studies utilized Dual X-ray absorptiometry for BD, BMD, or BMC measurements [7,8,35,36,9,12,16,19,31–34], one employed Micro CT (μ CT) for BMD assessments [15], and three used both Dual X-ray absorptiometry and Micro CT (μ CT) [10,11,20]. Two used Archimedes' principle to measure bone density and mineral content (BMC) [13,14]. Seven studies used the rat lineage Wistar [7–9,13,14,16,34], ten used the lineage Sprague-Dawley [10–12,15,19,31–33,35,36], and one used the mice lineage C57BL/6J [20].

The majority of the studies focused on evaluating the BMD on the femur [7,9,11,15,16,20,31,33,34,36], and tibia [8,12–14,32,36], while one of them evaluated the trunk, upper limbs, and the whole body [35]. The studies that evaluated the femur indicated beneficial effects of exercise on BMD compared to the sedentary OVX groups. Honda et al., (2003) [7], Notomi et al., (2003) [31], Kiuchi et al., (2006) [36], Stringhetta-Garcia et al., (2016) [9], Mosti et al., (2016) [33], Okubo et al., (2017) [34], Gomes et al., (2018) [16], Tang et al., (2022) [11], Galdino-Alves et al., (2022) [20], and Wang et al., (2023) [15] reported significant increases in BMD in the trained groups. The BMD values in the femur across these studies ranged from 87.2 mg/cm² to 650 mg/cm² in the experimental groups.

For the studies investigating the tibia, Kiuchi et al., (2006) [36], Renno et al., (2007) [13], Umemura et al., (2008) [8], Shiguemoto et al., (2012) [14], Souza et al., (2017) [32], and Deriggi-Pisani et al., (2023) [12] also reported beneficial effects of resistance training. In all cases, the OVX animals that underwent training showed significant increases in bone density compared to the sedentary OVX

groups. The impact of physical exercise on BMD has been consistently observed, with variations depending on the bone region assessed.

Risk of bias assessment and quality criteria assessment

Data extraction and risk of bias assessment showed that most studies had a low risk of bias in "selective outcome reporting" (100%) and "baseline characteristics" (100%). The "sequence generation" was considered adequate for 83.33% of the studies. However, all studies showed a high risk of bias in the "allocation concealment," "blinding of participants and personnel," "random outcome assessment," "blinding of outcome assessment," "incomplete outcome data," and "other bias" domains, with 0% of the studies demonstrating low risk in these areas. Most studies did not provide sufficient information or left unclear regarding these domains (see Table 2).

The scores derived from the ARRIVE guidelines ranged between 27 and 32 points (mean score 28.92 ± 1.50) out of a possible 36. Ten categories achieved an "excellent" rating (scoring between 0.8-1.0), including areas such as the title, abstract, background, objectives, ethical statement, experimental animals, housing and husbandry, outcomes, statistics, and overall applicability/relevance. Six categories fell into the "average" range (scoring between 0.5-0.8), covering aspects like study design, experimental procedure, sample size, number analyzed, adverse events, and discussion-interpretation/scientific implications. The category for adverse events was the only one rated as "poor" (below 0.5), with a score of 0.1 (Table 4).

Quantitative analysis of the studies (meta-analyses)

Out of the studies included in the systematic review, nine provided data on bone mineral density (BMD) and were incorporated into the meta-analyses and forest plots (Figure 2). The studies displayed moderate heterogeneity ($I^2 = 60\%$ for climbing training and 42% for jumping training, respectively). Random-effect models were used. Compared to the control groups, BMD outcomes for the groups on climbing training showed a standardized mean difference (SMD) of 3.02 (95% CI 1.81, 4.4.24). For

animals in the jumping training groups, the SMD was 1.27 (95% CI 0.72, 1.82) when compared to control groups (indicated by a diamond on the right side of the midline without crossing it) (Figure 2).

Discussion

The findings of this study indicate that resistance training (RT) plays a significant role in mitigating bone loss in ovariectomized (OVX) rodent models. OVX is a well-established model for simulating postmenopausal osteopenic conditions in rodents due to the induction of estrogen deficiency, which leads to alterations in bone metabolism similar to those observed in human osteoporosis [37]. The loss of estrogen, the primary consequence of OVX, is associated with increased production of pro-inflammatory cytokines such as IL-6, IL-1 β , and TNF- α [38]. These inflammatory mechanisms contribute to osteoclastogenesis increasing the expression of RANKL (receptor activator of nuclear factor kappa-B ligand) while reducing the expression of OPG (osteoprotegerin), a decoy inhibitor of osteoclastogenesis [39], enhancing bone resorption and decreasing bone mineral density (BMD) levels. RT can contribute positively to the modulation of the inflammatory system reducing the grade of inflammation that comes from chronic conditions such as osteopenia and osteoporosis [40].

Physical training interventions have been shown to positively impact bone health in animals subjected to ovariectomy (OVX) in various studies. However, it is necessary to assess the magnitude of these effects to better understand the mechanisms contributing to the effective attenuation of bone loss as a benefit of physical exercise [21]. This study demonstrates that RT significantly increases BMD in several regions of rodent bones. This effect can be attributed to RT's ability to modulate the inflammatory response and promote a more favorable environment for bone anabolism [40]. Evidence suggests that RT may suppress the expression of pro-inflammatory cytokines and promote the release of growth factors and bone morphogenetic

proteins (BMPs) [40,41], which are essential for bone formation. Additionally, muscle contraction appears to positively influence bone remodeling by balancing the activity of osteoclasts and osteoblasts [41]. This balance is crucial for maintaining the structural integrity of bone, especially under estrogen-deficient conditions, where osteoclastic activity is exacerbated [41]. RT may increase the expression of osteoprotegerin (OPG) [42], an inhibitor of osteoclastogenesis, and reduce the expression of receptor activator of nuclear factor kappa-B ligand (RANKL), which promotes osteoclastogenesis [39,43].

Other mechanisms related to RT involve the mechanical stimuli applied to bone, which can promote an osteogenic response. The tension, compression, and torsion forces generated during resistance training produce electrical signals that stimulate bone metabolism and mineral deposition, leading to increased BMD and potentially inhibiting bone resorption [21]. Additionally, muscle contraction resulting from resistance training is associated with increased expression of the peptide Irisin, encoded by the FNDC5 gene, which regulates osteocyte metabolism through specific αV class receptors [44]. Previous studies have shown that resistance training can elevate FNDC5 gene expression levels [42], and a systematic review of irisin levels and physical training, including resistance modalities found that higher levels of this peptide are linked to improvements in BMD in mice [22]. Although our study did not specifically investigate changes in muscle cross-sectional area, this is a commonly observed mechanism in studies that indirectly support bone health [20].

The acquisition of images and the evaluation of bone outcomes varied among the studies analyzed. The inclusion of all selected studies in the metanalysis was limited due to variations in exercise types, frequency, intensity, and duration of resistance training. Additionally, the variability in the bone regions assessed and types of parameters evaluated (DEXA or MicroCT) made it difficult to generalize the findings. Nevertheless, we included only studies that utilized

dual-energy X-ray absorptiometry (DEXA), the most prevalent technique, to balance the analyses and minimize heterogeneity in the meta-analysis. Additionally, some studies focused on bone mineral density (BMD) in specific areas, such as the lumbar spine. In contrast, others evaluated total BMD or areas less prone to osteoporotic fractures. To improve the consistency of comparisons, the studies were subdivided into two groups based on similarities in the types of resistance training evaluated. The observed heterogeneity was moderate (60% in the stair climbing group and 42% in the jump training group). In meta-analyses involving animal studies, it is common to find heterogeneity above 60%. Rather than abandoning the design of the meta-analysis, we deemed it more appropriate to use a random-effects model, which better accommodates the variations present in animal studies [5,28].

While evaluating quality criteria based on the ARRIVE guidelines, we observed that the categories related to 'experimental procedure,' 'sample size,' and 'baseline data' were the most deficient. Previous studies that assessed the quality of research involving animal models of osteopenia in rodents reported similar issues, with limited evidence of sample size calculations, proper randomization, or blinding of assessors [22]. This lack of essential information compromises the interpretation of results, as inadequate sample sizes can lead to incorrect conclusions, such as the failure to detect significant effects. Although the lack of detailed information may be a result of space limitations (especially in abstracts), the growing adherence to the ARRIVE guidelines by scientific journals highlights the need to improve the quality of publications involving animal models [26,27].

Additionally, in our research, we used the SYRCLE tool to assess the risk of bias in the reviewed studies [25]. The results were consistent with previous reviews of preclinical studies, which also pointed to a recurring lack of information on randomization and blinding, critical aspects for minimizing subjective measurements and preventing methodological bias [22]. The

methodological challenges encountered, including insufficient details on randomization, sample size calculation, and blinding, were significant limitations of this study, defaulting direct comparisons between articles. Despite this, overall adherence to the ARRIVE guidelines was around 80%, with this score classified as average to excellent. Nevertheless, most studies reported a beneficial effect of resistance training on bone mineral density (BMD) in the OVX-trained group compared with its control (OVX sedentary group), as shown in the quantitative analyses.

In conclusion, this study suggests that resistance training (RT) is a relevant intervention for mitigating bone loss in conditions of estrogen deficiency, such as osteoporosis. The clinical implications are particularly significant for understanding bone pathophysiology, as osteoporosis can exacerbate generalized bone loss, compromising individual stability and increasing the risk of fractures. Future studies should focus on standardizing RT protocols.

Sources of Funding

This study was supported and funded by the National Council for Scientific and Technological Development (CNPq) <https://www.gov.br/cnpq/pt-br>; the Coordination for the Improvement of Higher Education Personnel (CAPES) <https://www.capes.gov.br>. and the Foundation for Research Support of the State of Minas Gerais (FAPEMIG) <http://www.fapemig.br/pt/#>.

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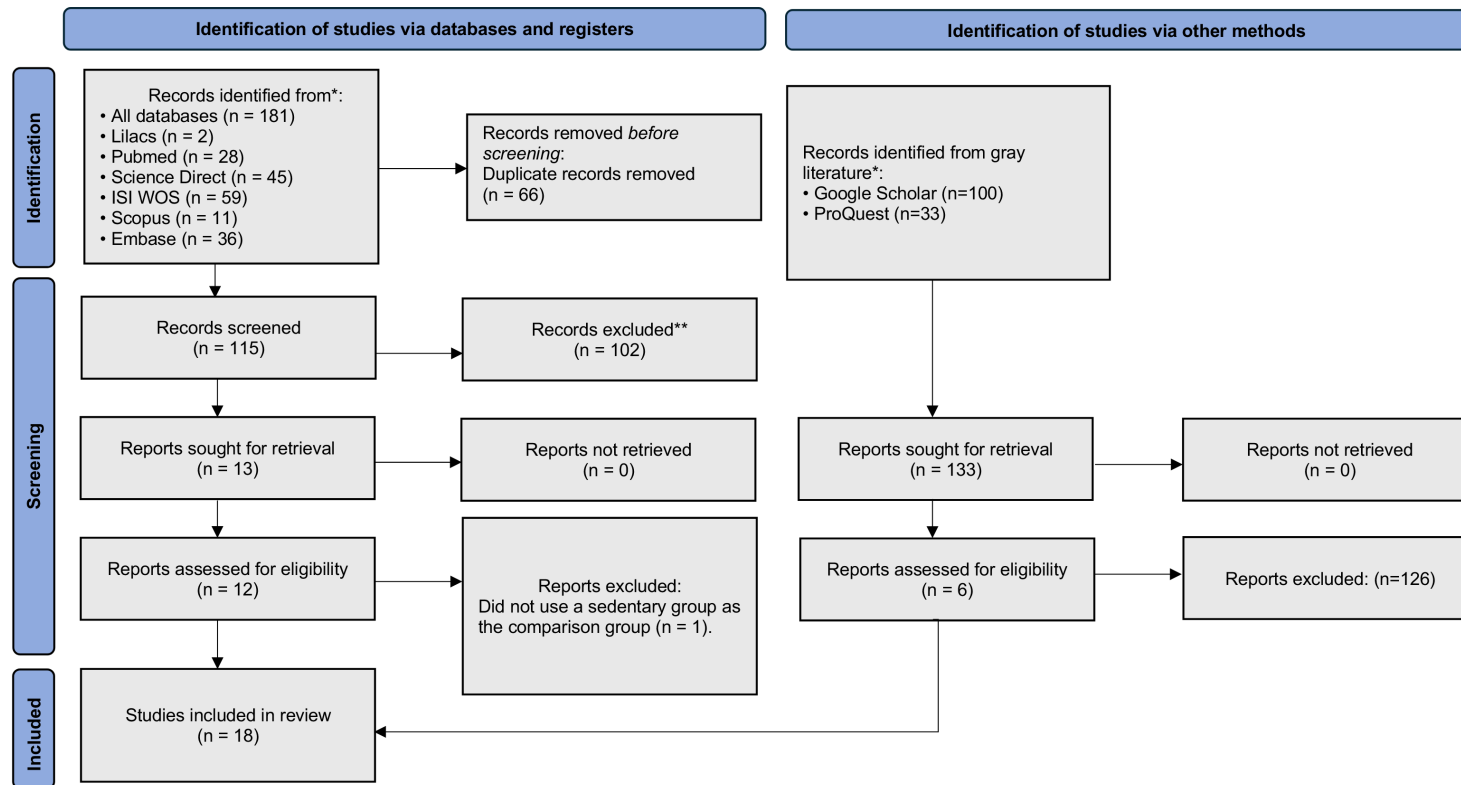


Figure 1. Flow diagram of the screened articles adapted from the PRISMA statement.

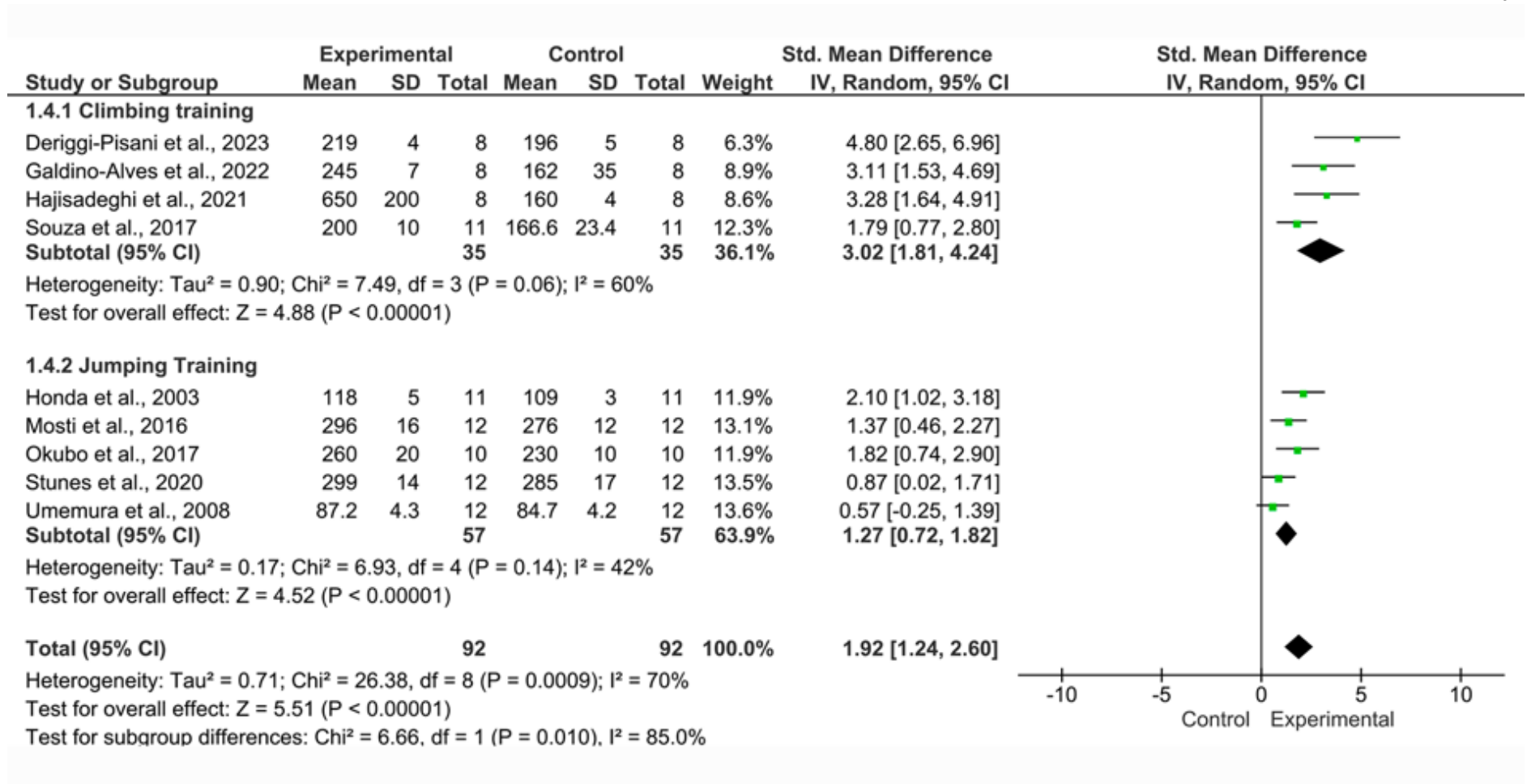


Figure 2. Forest plot and meta-analysis of bone mineral density (BMD) in OVX sedentary and OVX resistance training

Tabela 1. Data extraction of the selected studies.

Authors and year of publication	Animal model, sample, and age	Groups	Exercise protocol	Tissue and evaluation	Statistical analysis	Main results
Honda <i>et al.</i> , (2003) [7]	42 Wistar rats 10 weeks	SHAM sedentary (n=9) SHAM exercise (n=12) OVX sedentary (n=10) OVX exercise (n=11)	Rats in the training groups jumped 10x per day, 5 days a week, for 8 weeks.	Dual-energy X-ray absorptiometry Femur	ANOVA	OVX significantly decreased BMD in all segments of the femur, while training significantly increased BMD in the proximal, distal, and whole femur
Notomi <i>et al.</i> , (2003) [31]	60 Sprague-Dawley rats 12 months	Baseline Control (n=15) Sham Sedentary (n=15) OVX Sedentary and (n=15) OVX Exercise (n=15)	The rats were housed in cages with a wire mesh tower, with two water bottles at the top. Initially, the bottles were set at a height of 20 cm, gradually raised to 200 cm over a week. Climbing activity was monitored 24 hours a day, and in 10-20% of the climbs, the rats ascended without drinking water over three weeks.	Dual-energy X-ray absorptiometry Femur and lumbar	ANOVA	The results showed that the OVX exercise group had significantly higher bone strength compared to the OVX sedentary group. The breaking load/BMD was greater in the OVX exercise group, even surpassing the healthy control in some cases. However, the cortical bone area was lower in the OVX exercise group compared to the group. These findings suggest that exercise improves bone strength in OVX rats, despite a reduction in cortical bone area.

Kiuchi et al., (2006) [36]	30 Wistar rats	SHAM sedentary (n=6)	The training protocol used a weight-lifting exercise with a touch stimulus. Rats were divided into LOW, MID, and HIGH groups based on weight load. They completed 2 sets of 10 repetitions, three times a week for ten weeks.	Dual-energy X-ray absorptiometry	ANOVA	The study showed that OVX caused a significant loss of femoral bone mass over 20 weeks. Exercise, depending on intensity, restored bone mass in the proximal and distal femur but not in the shaft. In the tibia, bone loss was only observed at the proximal site, and higher-intensity exercise prevented marrow area enlargement. OVX and low-intensity groups had significantly higher body weight compared to the control.
	1 weeks	OVX sedentary (n=6)				
		OVX LOW weight (n=6)				
		OVX MID weight (n=6)				
		OVX HIGH weight (n=6)				
Renno et al., (2007) [13]	40 Wistar rats	SHAM sedentary (n=10)	Rats in the training group were subjected to a progressive loading exercise program 3x/week for 8 weeks in a water-filled container, using a specially designed vest to secure additional weight	Archimedes' principle	ANOVA and Duncan's tests	Exercised animals had significantly higher bone density and mineral density values than SHAM and OVX sedentary animals, indicating the positive effect of exercise on tibial physical properties
	12 weeks	SHAM exercise (n=10)				
		OVX sedentary (n=10)				
		OVX exercise (n=10)				

Umemura et al., (2008) [8]	48 Wistar rats	SHAM sedentary (n=12)	Rats in the two exercise groups performed jumping exercises 10/day, 5x/week for 8 weeks	Dual-energy X-ray absorptiometry	ANOVA	Exercise significantly increased BMC in the diaphysis and center-distal regions of the tibia, while ovariectomy decreased BMC in the proximal region but increased it in the center-proximal region
	10 weeks	SHAM exercise (n=12)		Tibia		
		OVX sedentary (n=12)				
		OVX exercise (n=12)				
Shiguemoto et al., (2012) [14]	48 Wistar rats	SHAM acute exercise (n=8)	Rats in acute and chronic groups were involved in climbing training sessions on an inclined vertical ladder with progressively heavier weights attached to their tails. The chronic group performed the protocol once every 3 days over 12 weeks. The acute training involved only one climbing session, coinciding with the last session of the chronic training	Archimedes' principle	ANOVA	In the chronically trained groups, both SHAM and OVX had higher mineral and bone density compared to the sedentary and acutely trained groups
	12 weeks	SHAM chronic exercise (n=8)		Tibia		
		SHAM sedentary (n=8)				
		OVX acute exercise (n=8)				
		OVX chronic exercise (n=8)				
		OVX sedentary (n=8)				

Jung et al., (2014) [35]	60 Sprague- Dawley rats	SHAM sedentary (n=10)	The exercise protocol consisted of resistance and endurance training. The resistance exercise involved placing rats in a 2-meter-high cylindrical cage with water bottles at the top, gradually increasing the height, encouraging the rats to climb up and down for 8 weeks. The endurance exercise began with 10 minutes per day at 10 m/min, progressively increasing after a 2-week adaptation period to 60 minutes per day at 20 m/min, five times per week.	Dual-energy X-ray absorptiometry Total, trunk, and upper limb	ANOVA	The 8 week-long low intensity treadmill running did not influence the body weight, bone density and bone mass. However, the 8 week-long climbing exercise significantly influence the body weight, bone density and bone mass.
	12 weeks	OVX sedentary (n=10)				
		OVX soy protein (n=10)				
		OVX alendronate (n=10)				
		OVX endurance training (n=10)				
Stringhetta- Garcia et al., (2016) [9]	40 Wistar rats	OVX sedentary vehicle (n=10)	Rats in the exercise group performed a climbing protocol training 3x/week over 17 weeks	Dual-energy X-ray absorptiometry Femur	ANOVA	The BMD of the femur in the trained animals' group (without administration of raloxifene) was significantly higher than OVX sedentary group
	14 weeks	OVX exercise vehicle (n=10)				
		OVX sedentary + raloxifene (n=10)				
		OVX exercise vehicle + raloxifene (n=10)				

Mosti et al., (2016)[33]	60 Sprague- Dawley rats 15 weeks	SHAM sedentary (n=12)	The protocol involved 8 weeks of high intensity jump training, 3 times a week. Each session lasted 15 minutes, with 3 sets of 6 jumps, and the jump height increased according to the rats' capacity, with 2-3 minutes of rest between sets.	Dual-energy X- ray absorptiometry Femur, spine and the whole body	ANOVA	The results showed that femoral BMD was lower in all OVX groups compared to SHAM. After the intervention, exercise (OVX-Ex and OVX-FenEx) improved femoral BMD, normalizing it compared to SHAM. The group with both exercise and supplementation (OVX-FenEx) had the greatest increase in BMD, while sedentary groups (OVX-Ctr and OVX-Fen) saw no significant improvement.
		OVX sedentary (n=12)				
		OVX training (n=12)				
		OVX fenofibrate (n=12)				
		OVX fenofibrate + training (n=12)				
Souza et al., (2017) [32]	66 Sprague- Dawley rats	SHAM sedentary (n=11)	The 12-week resistance training involved rats climbing a vertical ladder with weights attached to their tails every 3 days.	Dual-energy X- ray absorptiometry Tibia	ANOVA	The OVX training group had significantly higher bone density and bone mineral content than the OVX sedentary group. Hormone replacement (HR) increased BD, BMC, and bone area in both trained (OVX RT- HR) and sedentary (OVX Sed- HR) groups, with the combination of training and HR in the OVX RT-HR group showing the greatest improvements.
		OVX sedentary (n=11)				
		OVX resistance training (n=11)				
		SHAM resistance training (n=11)				
		OVX hormone treatment sedentary (n=11)				

		OVX hormone treatment + resistance training (n=11)				
Okubo et al., (2017) [34]	80 Wistar rats	SHAM sedentary (n=20)	The rats were divided into control and ovariectomized groups, with some subjected to a 12-week jumping protocol, five days per week. The protocol includes 20 jumps per session, with a height of 40 cm. They started at different points in time. 3 days after OVX and 60 days after OVX.	Dual-energy X-ray absorptiometry	ANOVA and ANCOVA	The OVX trained group had a higher BMD compared to the OVX sedentary group on femur and L5 lumbar on both time points experiment (3 days after OVX and 60 after OVX showing both preventive and treatment of bone loss.
	10 weeks	OVX sedentary (n=20)		Femur and lumbar		
		OVX jumping training (n=20)				
		SHAM jumping training (n=20)				
Gomes et al., (2018) [16]	40 Wistar rats	SHAM sedentary (n=10)	Rats in the exercise group were submitted to a simulating squat training. The protocol was performed 3x/week over 60 days	Dual-energy X-ray absorptiometry	ANOVA	The trained group without hormone repositioning-maintained spine and femur BMD in OVX animals at levels similar to Sham animals
	8 weeks	OVX sedentary (n=10)		Spine and femur		
		OVX treated with 17β-estradiol (n=10)				
		OVX exercise (n=10)				

Stunes et al., (2020) [10]	60 Sprague-Dawley rats	SHAM sedentary (n=12)	Rats in the training group performed a high intensity jump program 3/week over 8 weeks	Dual-energy X-ray absorptiometry and Micro-CT	ANOVA	The trained group without metformin administration showed an X-ray BMD result worse than the SHAM group in the whole body, spine, and femur. Still, compared to the other OVX groups, the exercise improved the BMD levels in the whole body and femur. However, the spine result was lower than that of the training + metformin group and higher than that of OVX + metformin and OVX sedentary.
		OVX sedentary (n=12)				
		OVX metformin (n=12)				
		OVX metformin + exercise (n=12)				
Hajisadeghi et al., (2021) [19]	72 Sprague-Dawley rats	OVX exercise (n=12)	Rats in the training group performed ladder and climbing sessions. The training protocol consisted of 3 sets of 5 repetitions over 8 weeks.	Whole body, spine, femur and tibia	ANOVA	
		Control group (n=8)				
		OVX + Ca ⁺ (n=8)				
		OVX + vit D (n=8)				
		OVX + Ca ⁺ + Vit D (n=8)				
	8 months	OVX exercise (n=8)		Whole body, tail, hips, lumbar, and femur	ANOVA	Without any administration, the trained group significantly improved the BMD in all the tissues evaluated compared to the OVX control group and the SHAM placebo group. Moreover, the values were higher than the other trained group with some vitamin administration
		OVX exercise + Ca ⁺ (n=8)				

		OVX exercise + vit D (n=8)				
		OVX exercise + Ca ⁺ + Vit D (n=8)				
		SHAM placebo (n=8)				
Tang et al., (2022) [11]	30 Sprague-Dawley rats 3 months	SHAM sedentary (n=10) OVX sedentary (n = 10) OVX exercise (n = 10).	Rats in the training group performed a treadmill running with a weight bearing for 20 min per session, 6/week over 10 weeks	Dual-energy X-ray absorptiometry and Micro-CT Femur	ANOVA	The femur BMD and was improved in the trained group compared with the OVX group. Compared with the SHAM group, the BMD of the total and distal cancellous femur in the OVX group decreased but in the trained group, it got higher than that in the OVX group
Galdino-Alves et al., (2022) [20]	48 C57BL/6J 8 weeks	SHAM sedentary (n=8) OVX saline (n=8) OVX + alendronate (n=8) OVX + β glucan (n=8)	Rats in the trained group performed climbing sessions 3/week over 60 days	Dual-energy X-ray absorptiometry and Micro-CT Femur	ANOVA	The BMD results showed a higher for the RT groups compared to the other OVX groups but are lower than the SHAM group. However, the trabecular femoral BMD was higher in both trained and trained + BG groups than other OVX groups and lower than the SHAM group.0020

		OVX exercise (n=8)				
		OVX exercise + β glucan (n=8)				
Deriggi- Pisani et al., (2023) [12]	36 Sprague- Dawley rats 7 weeks	SHAM sedentary (n=8) SHAM exercise (n=8) OVX sedentary (n=10) OVX exercise (n = 10)	Rats in the trained group climbed a ladder carrying increasing weights 3x/week over 10 weeks	Dual-energy X-ray densitometry Tibia	ANOVA	The results showed a significant improvement by the BMD on the OVX trained group compared to the OVX sedentary but no difference among the SHAM groups
Wang et al., (2023) [15]	18 Sprague- Dawley rats 9 weeks	SHAM sedentary (n=6) OVX sedentary (n=6) OVX exercise (n = 6)	Rats in the training group performed ladder and climbing sessions. The training protocol consisted of 4 sets of 3 repetitions, 5x/week over 12 weeks.	Micro-CT Femur	ANOVA	The BMD of the femoral cancellous bone was significantly higher in the OVX exercise group compared to the OVX sedentary group but no difference when compared to the SHAM group

Table 1. Evaluation of the risk of bias in included studies.

Studies	A	B	C	D	E	F	G	H	I	J
Honda <i>et al.</i> , (2003) [6]	+	+	-	-	-	?	-	?	+	?
Notomi <i>et al.</i> , (2003) [30]	+	+	-	+	-	?	-	?	+	?
Kiuchi <i>et al.</i> , (2006) [35]	-	+	-	-	-	?	-	?	+	?
Renno <i>et al.</i> , (2007) [12]	+	+	-	+	-	?	-	?	+	?
Umemura <i>et al.</i> , (2008) [7]	-	+	-	-	-	?	-	?	+	?
Shiguemoto <i>et al.</i> , (2012) [13]	+	+	-	+	-	?	-	?	+	?
Jung <i>et al.</i> , (2014) [34]	-	+	-	-	-	?	-	?	+	?
Stringhetta-Garcia <i>et al.</i> , (2016) [8]	+	+	-	+	-	?	-	?	+	?
Mosti <i>et al.</i> , (2016)[32]	+	+	-	+	-	?	-	?	+	?
Souza <i>et al.</i> , (2017) [31]	+	+	-	+	-	?	-	?	+	?
Okubo <i>et al.</i> , (2017) [33]	+	+	-	+	-	?	-	?	+	?
Gomes <i>et al.</i> , (2018) [15]	+	+	-	+	-	?	-	?	+	?
Stunes <i>et al.</i> , (2020) [9]	+	+	-	+	-	?	-	?	+	?
Hajisadeghi <i>et al.</i> , (2021) [18]	+	+	-	+	-	?	-	?	+	?
Tang <i>et al.</i> , (2022) [10]	-	+	-	+	-	?	-	?	+	?
Galdino-Alves <i>et al.</i> , (2022) [19]	+	+	-	+	-	?	-	?	+	?
Deriggi-Pisani <i>et al.</i> , (2023) [11]	+	+	-	+	-	?	-	?	+	?
Wang <i>et al.</i> , (2023) [14]	+	+	-	+	-	?	-	?	+	?

A: sequence generation; B: baseline characteristics; C: allocation concealment; D: random housing; E: blinding of participants and personnel; F: random outcome assessment; G: blinding of outcome assessment; H: incomplete outcome data; I: selective outcome reporting; J: other bias; +: Yes (Low risk of bias); ?: unclear; -: no (high risk of bias).

Tabela 2. Quality assessment scores, including animal models, according to the study's ARRIVE guideline

Studies	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	Total
Honda <i>et al.</i> , (2003) [6]	1	2	2	1	2	1	1	2	1	1	0	2	2	1	2	2	0	2	2	0	27
Notomi <i>et al.</i> , (2003) [30]	1	2	2	1	2	1	1	2	2	1	1	2	2	1	0	2	0	2	2	0	27
Kiuchi <i>et al.</i> , (2006) [35]	1	2	2	1	2	1	1	2	2	1	1	2	2	1	0	2	0	2	2	2	29
Renno <i>et al.</i> , (2007) [12]	1	2	1	1	2	1	1	2	2	1	1	2	2	1	0	2	0	2	2	2	28
Umemura <i>et al.</i> , (2008) [7]	1	2	2	1	2	1	1	2	2	1	1	2	2	1	1	2	0	1	2	1	28
Shiguemoto <i>et al.</i> , (2012) [13]	1	2	1	1	2	1	1	2	2	1	1	2	2	1	2	2	0	1	2	0	27
Jung <i>et al.</i> , (2014) [34]	1	2	2	1	0	1	1	2	2	1	1	1	2	1	0	2	0	1	2	0	23
Stringhetta-Garcia <i>et al.</i> , (2016) [8]	1	2	1	1	2	1	2	2	2	1	1	2	2	1	0	2	0	2	2	2	29
Mosti <i>et al.</i> , (2016)[32]	1	2	1	1	2	2	1	2	2	1	1	2	2	1	2	2	0	2	2	2	31
Souza <i>et al.</i> , (2017) [31]	1	2	2	1	2	1	1	1	2	1	1	2	2	1	0	2	0	2	2	2	28
Okubo <i>et al.</i> , (2017) [33]	1	2	1	1	2	1	2	2	2	1	1	2	2	1	2	2	0	2	2	2	31
Gomes <i>et al.</i> , (2018) [15]	1	2	2	1	2	1	1	2	1	1	1	2	2	1	2	2	0	1	2	2	29
Stunes <i>et al.</i> , (2020) [9]	1	2	2	1	2	1	1	2	2	1	1	2	2	1	1	2	0	1	2	2	29
Hajisadeghi <i>et al.</i> , (2021) [18]	1	2	1	1	2	1	2	2	2	1	1	2	2	0	0	2	0	1	2	2	27
Tang <i>et al.</i> , (2022) [10]	1	2	1	1	2	1	1	2	2	1	1	2	2	1	2	2	0	2	2	2	30
Galdino-Alves <i>et al.</i> , (2022) [19]	1	2	2	1	2	2	2	2	2	2	1	2	2	1	1	2	0	1	2	2	32
Deriggi-Pisani <i>et al.</i> , (2023) [11]	1	2	2	1	2	1	1	2	2	1	1	2	2	0	2	2	0	1	2	2	29
Wang <i>et al.</i> , (2023) [14]	1	2	1	1	2	1	2	2	2	1	1	2	2	1	0	2	0	2	2	2	29
Category score (quality obtained)	18	36	28	18	34	20	23	35	34	19	17	35	36	16	17	36	0	28	36	27	513
Maximum score expected (quality expected)	12	24	24	12	24	24	24	24	24	24	12	24	24	12	24	24	24	24	24	24	432
Ratio quality score/ maximum score	2	2	1	2	1	1	1	1	1	1	1	1	2	1	1	2	0	1	2	1	1.18

A: title; B: abstract; C: introduction-background; D: introduction-objectives; E: methods-ethical statement; F: study design; G: experimental procedure; H: experimental animals; I: housing and husbandry; J: sample size; K: allocation; L: experimental outcomes; M: statistics; N: results baseline data; O: number analyzed; P: outcome, and estimation; Q: adverse events; R: discussion- interpretation/scientific implications; S: general applicability/relevance; T: funding; Total: represents total scoreained by each manuscript out of a maximum of 36 points

CAPÍTULO 3 – ARTIGO 2

Comparing Aerobic and Resistance Training in Estrogen Deficiency: Bone and Muscle Adaptations

Submitted to Climacteric Journal (on January 21st)

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Comparing Aerobic and Resistance Training in Estrogen Deficiency: Bone and Muscle Adaptations

Abstract: The reduction in bone mass and deterioration of bone microarchitecture are hallmark consequences of estrogen deficiency during menopause, increasing susceptibility to osteopenia and osteoporosis. Physical exercise is recognized as an effective non-pharmacological strategy; however, the comparative effects of different exercise modalities on bone remodeling and muscle–bone crosstalk remain unclear. This study investigated the effects of aerobic and resistance training on femoral bone metabolism and skeletal muscle adaptations in ovariectomized (OVX) mice. Sixty-four female C57BL/6J mice underwent ovariectomy or sham surgery and were allocated to sedentary, aerobic (treadmill running), or resistance (ladder climbing) training protocols for eight weeks. Bone histological and histochemical analyses, skeletal muscle morphology, and gene expression in bone and muscle tissues were evaluated. Both training modalities significantly increased osteoblast number and reduced osteoclast density in OVX animals compared with sedentary controls ($p < 0.05$), accompanied by a reduction in the RANKL/OPG ratio ($p < 0.001$). Resistance training induced more pronounced effects, including higher osteocyte density ($p < 0.01$), greater RUNX2 expression ($p < 0.001$), and stronger suppression of osteoclastogenesis. In parallel, resistance training significantly increased muscle fiber cross-sectional area ($p < 0.05$) and upregulated IGF-1 ($p < 0.01$) and FNDC5 expression ($p < 0.05$). These findings demonstrate that exercise mitigates estrogen deficiency–induced bone deterioration, with resistance training exerting superior effects on bone remodeling and musculoskeletal integration.

Keywords: estrogen deficiency; bone remodeling; resistance training; muscle–bone crosstalk

PLS

Loss of estrogen during menopause weakens bones and muscles, increasing the risk of osteoporosis and fractures. Physical exercise is widely recommended, but it is still unclear whether different types of exercise offer distinct benefits. In this study, we compared aerobic exercise (running) and resistance exercise (climbing with weights) in a mouse model of menopause. We found that both types of training helped protect bone health by increasing bone-forming cells and reducing bone-breaking cells. However, resistance training produced stronger benefits, leading to better preservation of bone structure, improved communication between muscle and bone, and greater muscle strength-related adaptations. These results suggest that while any regular exercise is beneficial during estrogen deficiency, resistance training may be especially effective for maintaining bone and muscle health with aging.

Introduction

Estrogen deficiency resulting from menopause is one of the main determinants of accelerated bone loss and deterioration of bone microarchitecture, significantly increasing the risk of osteopenia, osteoporosis, and fragility fractures [1–3]. This process is characterized by an imbalance in bone remodeling, with predominance of osteoclast-mediated bone resorption and impairment of osteoblastic activity, ultimately leading to a progressive reduction in bone structural strength [4,5]. In experimental research, ovariectomy (OVX) consistently reproduces the hormonal and skeletal alterations observed in postmenopausal women and is therefore widely used to investigate pathophysiological mechanisms and intervention strategies targeting estrogen deficiency–induced bone loss [6,7].

Among non-pharmacological strategies, physical exercise has been widely recognized as an effective approach for preserving bone health throughout life [2,8–10]. The mechanical stimulus generated during exercise acts directly on bone tissue through mechanobiological mechanisms, whereby compressive, tensile, and shear forces are converted into biochemical signals by bone cells [11–13]. These signals activate intracellular pathways related to osteoblast proliferation and differentiation, extracellular matrix production, and reduction of osteoclast activity, involving calcium-dependent signaling, prostaglandin release, and regulation of pathways such as Wnt/ β -catenin [12,13]. Consequently, physical exercise contributes to the maintenance of bone mass and skeletal structural integrity. However, the osteogenic response to exercise is highly dependent on the characteristics of the mechanical stimulus, including load magnitude, frequency, and mode of application, which can differentially modulate cellular activity and signaling pathways involved in bone remodeling.

Resistance and aerobic training impose distinct mechanical stimuli on bone tissue, resulting in specific cellular and molecular responses [14]. Resistance training, characterized by higher loads and greater levels of muscle contraction, promotes increased bone deformation and osteocyte activation, favoring more pronounced osteogenic responses. This stimulus has been associated with activation of RUNX2 [14], an essential transcription factor for osteoblast differentiation, and increased expression of osteoprotegerin (OPG), contributing to the inhibition of osteoclastogenesis [12,13]. In contrast, aerobic training involves repetitive stimuli of lower contractile magnitude but may modulate bone metabolism through regulation of the immune system and reduction of reactive oxygen species (ROS), important

mediators of osteoclastogenesis [15]. Despite the beneficial effects of both modalities, the mechanisms by which different mechanical loading patterns regulate RUNX2, OPG, and bone remodeling, particularly under estrogen-deficient conditions, remain only partially understood.

Despite the growing recognition of muscle–bone interactions, studies integrating bone cellular responses, gene expression related to osteogenesis and osteoclastogenesis, and skeletal muscle morphological adaptations following different exercise training modalities in OVX models remain scarce. Therefore, experimental approaches combining histological, histochemical, molecular, and muscular analyses are essential for a more comprehensive understanding of the effects of physical exercise on bone metabolism under menopause-like conditions. Accordingly, the present study aimed to investigate the effects of resistance and aerobic training on femoral bone remodeling, gene expression of osteogenic and osteoclastogenic markers, osteocyte density, and skeletal muscle adaptations in ovariectomized and SHAM mice.

Methods

Animal models

This study included 64 female C57BL/6J mice (*Mus musculus*), 6.5 weeks old, initially weighing 20.9 ± 0.8 g, obtained from the Central Animal Facility of the Federal University of Minas Gerais (UFMG). The experimental protocol was approved by the Animal Ethics Committee of the Federal University of Lavras (UFLA) (under protocol no. 013/21). Throughout the study, the animals were housed in the animal facility of the Department of Nutrition at UFLA under controlled conditions of temperature (22 ± 2 °C), humidity (45 ± 15 %), and a 12 h light/12 h dark cycle. Standard commercial chow and water were provided ad libitum.

Ovariectomy procedures

After a one-week acclimation period, the animals were 7.5 weeks old, an age at which female mice typically become sexually mature. They were paired into two groups according to body weight.

Subsequently, the mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) intraperitoneally. Half of the animals underwent bilateral ovariectomy (OVX), while the other half were subjected to the same surgical procedure without ovary removal (SHAM group). After a two-week postoperative recovery, the animals in each group were assigned to acclimation for their respective physical training protocols (treadmill running or ladder climbing) and euthanized with isoflurane and subsequently decapitated at the end of the experiment. The timeline is illustrated in Figure 1.

Physical Training Protocol

Fatigue test and training protocols

Before the beginning of the training period, all animals underwent two fatigue tests to assess baseline physical capacity [16,17]. The maximum incremental load or speed test were performed to determine the highest load or running speed each mouse could achieve. For the ladder-climbing test, a fabric bag containing a standardized load was attached to the tail, starting at 5 g and increased by 1 g after each successful climb until concentric failure. For the treadmill test, mice began running at $6 \text{ m} \cdot \text{min}^{-1}$, with speed increments of $3 \text{ m} \cdot \text{min}^{-1}$ every 3 min, until they were unable to maintain the pace, remaining on the shock grid for 10 s at the end of the treadmill.

Forty-eight hours after the incremental test, the animals performed an endurance test at 80% of their maximum load or speed. Mice assigned to the resistance protocol climbed the ladder carrying 80% of their maximal load until failure, whereas mice assigned to the aerobic protocol ran on the treadmill at 80% of their maximal speed until exhaustion. Workload during ladder climbing was calculated based on body mass, carried load, ladder length, ladder slope, and number of climbs, while treadmill workload was calculated using body mass, running speed, treadmill incline, and total running time [16,17]. The results of these tests were used to characterize baseline physical capacity and to prescribe the subsequent training protocols.

The resistance and aerobic training protocols were then carried out over 8 weeks and were individualized according to the animals' performance in the fatigue tests. Training intensity was prescribed as a percentage of the maximum load (ladder climbing) or maximum speed (treadmill running). During weeks 1 and 5, training intensity was set at 60% of maximum load or speed, with a workload of 60%. In weeks 2 and 6, intensity was increased to 70%, with a workload of 70%. During weeks 3 and 7, intensity remained at 70%, while workload was increased to 80%. In weeks 4 and 8, training intensity was set at 80%, with a workload of 90%.

Based on their initial performance in the fatigue tests, the animals were redistributed into three groups with similar physical capacities: Sedentary, Resistance Training (ladder climbing), and Aerobic Training (treadmill running). The sedentary group was subdivided, with seven animals performing fatigue tests on the ladder and seven performing fatigue tests on the treadmill, allowing comparisons of performance gains throughout the experimental period.

Histological and Histochemical Analyses

Femurs and extensor digitorum longus (EDL) muscles were carefully dissected and fixed in 10% buffered formalin (pH 7.4) for 48 h. Subsequently, bone specimens were decalcified in 14% EDTA (pH 7.4) for 21 days before routine histological processing and paraffin embedding. Paraffin blocks were sectioned in the sagittal plane, and 4 μm -thick sections were obtained.

Femoral sections were stained for tartrate-resistant acid phosphatase (TRAP) to identify and quantify osteoclasts [18], and with Masson's trichrome to identify and quantify osteoblasts [17], according to the manufacturer's instructions. Additional sections were stained with hematoxylin and eosin (HE) for general morphological evaluation and determination of osteocyte density [18]. Histomorphometric analyses were performed in the distal metaphysis region, located 1 mm from the epiphyseal growth plate, by analyzing at least two trabeculae per section in four sections per animal, as previously described [19].

Osteoclasts were identified as multinucleated TRAP-positive cells located on the bone surface, whereas osteoblasts were identified as round to cuboidal cells with basophilic cytoplasm aligned along the bone surface in Masson's trichrome-stained sections [20,21]. Osteocyte density was determined from H&E-stained sections by counting the number of cells within osteocyte lacunae inside a standardized, delimited area using ImageJ software (NIH, USA). Results were expressed as the number of osteocytes per mm² [22].

EDL muscles were embedded in paraffin after fixation and sectioned for H&E staining. Slide images were acquired using a light microscope coupled to a digital camera and computer. Between five and twenty muscle fibers were randomly selected per section for measurement of muscle fiber thickness using ImageJ software [23,24].

Gene expression analysis (qPCR)

Femoral bone and gastrocnemius muscle samples were collected at euthanasia and immediately stored at -80 °C until processing. Total RNA was extracted exclusively using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. RNA integrity and quality were assessed using the Qubit™ RNA IQ Assay Kit (Life Technologies, Eugene, OR, USA) [18].

Complementary DNA (cDNA) was synthesized from total RNA using the Platus Transcriber RNase H- cDNA First Strand Kit (Sinapse Biotechnology, Maranhao, SP, Brazil), following the manufacturer's protocol. This system employs an RNase H- reverse transcriptase to improve full-length cDNA synthesis and reduce RNA degradation.

Quantitative real-time PCR (RT-qPCR) was performed using the CFX Opus Real-Time PCR Systems (Bio Rad Systems, Berkley, CA, USA) with SYBR™ Green Universal Master Mix (Applied Biosystems, Foster City, CA, USA). The amplification protocol consisted of an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 2 min. A melting curve analysis was performed at the end of each run to confirm amplification specificity. Primer sequences are provided in Table 1.

The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as the sole reference gene for normalization. GAPDH was selected due to its low intra-group variability and consistent cycle threshold (Ct) values across experimental conditions. Mean Ct values for GAPDH were 25.81 ± 0.37 (Sedentary OVX), 26.33 ± 0.59 (Sedentary SHAM), 21.06 ± 0.32 (Ladder climbing OVX), 26.00 ± 0.35 (Ladder climbing SHAM), 26.39 ± 0.44 (Treadmill OVX), and 20.88 ± 0.30 (Treadmill SHAM), indicating adequate stability for normalization.

Mean Ct values obtained from duplicate reactions were used to calculate relative target gene expression, normalized to GAPDH, using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Data were tested for normality using the Shapiro–Wilk test. According to the experimental design, one- or two-way analysis of variance (ANOVA) was applied, followed by the Student–Newman–Keuls or Tukey post hoc test when appropriate. All analyses were performed using GraphPad Prism (version 5.01; GraphPad Software, San Diego, CA, USA), and statistical significance was set at $p < 0.05$.

Results

Animal characteristics and physical performance

Body weight increased progressively throughout the experimental period in the sedentary groups ($p < 0.05$) (Fig. 2a), whereas no significant post-intervention body weight gain was observed in the trained groups.

Resistance training induced a progressive increase in maximum load across the experimental period, with higher values observed at weeks 4 and 8 compared with baseline, while no changes were observed in sedentary animals ($p < 0.05$) (Fig. 2b). Similarly, aerobic training resulted in a progressive increase in maximum running speed at weeks 4 and 8, whereas

sedentary animals showed minimal changes over time ($p < 0,05$) (Fig. 2c). Workload increased over time in trained groups for both ladder climbing and treadmill running protocols, reflecting the progressive nature of the training prescription, while sedentary groups displayed no consistent changes throughout the experimental period (Fig. 2d).

Effects of training on femoral bone histochemistry

TRAP staining revealed significant differences in osteoclast number among experimental groups (Fig. 3a–e). Sedentary OVX mice exhibited a higher number of osteoclasts compared with OVX mice subjected to resistance and aerobic training ($p < 0.01$). In SHAM animals, osteoclast number was lower than in OVX animals, and both resistance and aerobic training further reduced osteoclast density compared with sedentary SHAM controls ($p < 0.0001$).

Analysis of Masson's trichrome-stained sections showed significant differences in osteoblast number between groups (Fig. 3f–j). Both resistance and aerobic training increased osteoblast density compared with sedentary animals in OVX mice ($p < 0.05$). In SHAM animals, trained groups also exhibited higher osteoblast numbers compared with sedentary controls ($p < 0.01$).

HE staining demonstrated significant differences in osteocyte density among groups (Fig. 3k–o). Resistance and aerobic training resulted in higher osteocyte density compared with sedentary animals in OVX mice ($p < 0.01$). In SHAM mice, osteocyte density was also higher in trained groups compared with sedentary controls ($p < 0.05$), with differences between training modalities as indicated in the figure.

Effects of training on skeletal muscle morphology

Histological analysis of EDL muscle cross-sections revealed differences in muscle area among experimental groups (Fig. 4a–d). Resistance-trained animals were associated with increased muscle fiber cross-sectional area compared with sedentary animals in OVX and SHAM mice ($p < 0.05$).

Gene expression in femur and gastrocnemius muscle

Femur

In femoral bone, significant differences were observed in the expression of osteogenic and osteoclast-related genes among experimental groups (Fig. 5a–d). RUNX2 expression (Fig. 5a) was significantly increased in trained groups compared with sedentary animals in both OVX and SHAM conditions, with resistance training inducing the highest expression levels ($p < 0.001$ to $p < 0.0001$). OPG expression (Fig. 5b) was significantly higher in resistance and aerobic trained mice compared with sedentary controls in OVX animals ($p < 0.001$). In SHAM mice, trained groups also showed increased OPG expression relative to sedentary animals ($p < 0.01$). RANKL expression (Fig. 5c) was significantly elevated in sedentary OVX mice compared with SHAM controls ($p < 0.01$). Both resistance and aerobic training reduced RANKL expression in OVX animals compared with sedentary OVX mice ($p < 0.05$), while differences among SHAM groups were less pronounced. Consequently, the RANKL/OPG ratio (Fig. 5d) was significantly higher in sedentary OVX mice compared with SHAM animals ($p < 0.0001$). Both resistance and aerobic training markedly reduced the RANKL/OPG ratio in OVX mice compared with sedentary OVX controls ($p < 0.001$), indicating a shift toward a less osteoclastogenic profile. In SHAM animals, trained groups also exhibited lower RANKL/OPG ratios compared with sedentary controls ($p < 0.05$).

Gastrocnemius

IGF-1 expression (Fig. 5e) was significantly increased in trained groups compared with sedentary animals in OVX mice ($p < 0.01$), with resistance training showing the highest expression levels. In SHAM animals, both training modalities also induced higher IGF-1 expression compared with sedentary controls ($p < 0.05$). Similarly, FNDC5 expression (Fig. 5f) was significantly elevated in resistance-trained OVX mice compared with sedentary OVX animals ($p < 0.05$). However, aerobic training showed a non-significant pattern. In SHAM animals, no significant differences were observed among groups.

Discussion

The present study demonstrated that physical training, regardless of modality, was able to modulate bone metabolism. Overall, exercise stimulated femoral osteogenesis, as evidenced by increased osteoblastogenesis and reduced bone resorption, reflected by the decreased number of osteoclasts. These effects are particularly relevant for the management of conditions associated with bone loss, such as osteopenia and osteoporosis, especially under hormonal deprivation scenarios, such as ovarian senescence induced by OVX [17]. Previous studies have shown that physical exercise improves bone quality and preserves bone microarchitecture, including experimental models of estrogen deficiency [16,17]. Additionally, evidence indicates that different exercise training protocols can reverse or attenuate structural and metabolic alterations associated with OVX-induced osteoporosis [25]. However, studies directly comparing different exercise modalities within the same experimental model, particularly under conditions of ovarian senescence, remain scarce.

The mechanical stimulus generated by training acts directly on the musculoskeletal system, including bone tissue, triggering adaptive responses mediated by mechanobiological mechanisms such as bone piezoelectricity [12,13,26]. These stimuli promote bone remodeling processes that not only preserve or reconstruct bone architecture but also meet the metabolic and physiological demands of the organism [12]. In this context, exercise-induced mechanical loading can be converted into biological signals capable of directly regulating osteoblastogenesis and osteoclastogenesis, which is consistent with the cellular changes observed in the present study [12,13,26].

Both training modalities promoted beneficial effects on bone metabolism, as significant increase in osteoblast number was observed compared with sedentary groups in both OVX and SHAM animals, indicating enhanced osteogenic activity associated with exercise. From a mechanobiological perspective, the mechanical stimulus induced by exercise is converted into biological signals that directly regulate bone cell activity, as osteoblasts respond to exercise-induced mechanical stretching with increased cellular proliferation and enhanced production of bone matrix, particularly type I collagen, while shear stress acts on mesenchymal stem cells to promote their differentiation into osteoblasts through actin cytoskeleton remodeling, highlighting the adaptive role of these cells in bone formation [12].

In contrast, TRAP analysis revealed that sedentary OVX animals exhibited a higher number of osteoclasts compared with sedentary SHAM animals, confirming the effect of estrogen deficiency on increased bone resorption. Notably, both resistance and aerobic training significantly reduced osteoclast number compared with their respective sedentary controls in OVX animals, and a similar reduction was observed in trained SHAM animals when compared with sedentary SHAM controls. Reduced estrogen levels favor a pro-resorptive environment characterized by increased production of pro-inflammatory cytokines, such as IL-6 and TNF- α , elevated generation of reactive oxygen species (ROS), and increased expression of sclerostin,

factors that intensify osteoclastogenesis and inhibit bone formation through suppression of the Wnt/ β -catenin pathway [27,28]. In addition, estrogens exert direct anabolic effects on bone via activation of the estrogen receptor ER α , leading to increased β -catenin levels, inhibition of osteoblast and osteocyte apoptosis, and induction of osteoclast apoptosis through FasL gene expression [29,30].

In contrast to what was observed for osteoclasts and osteoblasts, osteocyte density was significantly higher in the resistance training group, suggesting a more pronounced effect of this modality on the maturation and maintenance of the osteocytic network. This finding may be explained by the mechanosensory role of osteocytes, which account for approximately 95% of bone cells and are highly responsive to shear stress generated by interstitial fluid flow during mechanical loading [31]. Osteocytes detect these mechanical stimuli and convert them into biochemical signals through calcium-dependent pathways, leading to the rapid mobilization of intracellular Ca²⁺ and activation of downstream signaling cascades, including the release of ATP, nitric oxide, and prostaglandin E₂ (PGE₂). PGE₂ acts as a key anabolic mediator of bone formation and facilitates intercellular communication through gap junctions, amplifying the osteogenic response to mechanical loading, particularly under higher-magnitude stimuli such as resistance training [12,32].

Despite the clear cellular effects observed, bone remodeling is indirectly regulated through the OPG/RANK/RANKL axis, in which reduced RANKL expression and a concomitant increase in OPG limit osteoclastogenesis and contribute to the maintenance of bone integrity [33]. Sedentary ovariectomized animals exhibited a significant increase in the RANKL/OPG ratio, reflecting a microenvironment favorable to bone resorption. In contrast, both training protocols were able to reduce this ratio, with a more pronounced effect in the resistance training group. Notably, resistance training induced the highest RUNX2 expression, suggesting that higher-magnitude mechanical stimuli may preferentially activate osteoblast

differentiation pathways. These findings are consistent with previous evidence demonstrating that mechanical stimuli can modulate osteoclastogenic signaling independently of estrogen presence, thereby contributing to the preservation of bone mass and bone tissue microarchitecture [17].

In addition to the direct effects of mechanical loading on bone tissue, gene expression analyses in skeletal muscle indicate that physical exercise may modulate bone metabolism through endocrine and paracrine pathways, reinforcing the concept of skeletal muscle as a secretory organ [34,35]. Both training protocols increased IGF-1 expression in the gastrocnemius muscle, suggesting greater activation of anabolic pathways with well-established osteotropic potential [36]. Similarly, FNDC5 expression was significantly upregulated in the resistance training group, indicating a possible contribution of myokines and myohormones, such as irisin, to muscle–bone communication and the regulation of bone remodeling [37]. In this context, muscle contraction induced by resistance exercise has been associated with increased FNDC5/irisin signaling, which regulates osteocyte metabolism via α V-class receptors [38], and resistance training has been shown to increase FNDC5 gene expression [17], with higher irisin levels being associated with improvements in bone mineral density in murine models [39]. Additionally, mechanical loading stimulates the secretion of osteomuscular factors such as TGF- β and prostaglandin E₂ (PGE₂), further reinforcing the role of muscle-derived signals in bone remodeling [40]. Consistently, the improvement in muscle morphology observed in trained groups followed the same pattern, with increased muscle fiber cross-sectional area in both protocols, again with greater magnitude in resistance training, which may have enhanced mechanical load transmission to the femur and potentiated the osteogenic effects of exercise, particularly under conditions of estrogen deficiency [4,5].

The present study has limitations inherent to the use of an animal model, which may restrict the direct translation of the findings to humans and micro-computed tomography

(micro-CT) was not performed for densitometric and microstructural assessment. Notably, however, a previous study from our group evaluated femoral bone tissue by micro-CT under both aerobic and resistance training protocols, and those results corroborate the patterns observed in the present work. Accordingly, the integrated histological, histochemical, and molecular analyses provided complementary and internally consistent evidence, supporting our conclusions regarding the effects of aerobic and resistance training on bone metabolism under menopause-like conditions.

In conclusion, the present study demonstrated that both aerobic and resistance training favorably modulate bone metabolism under estrogen-deficient conditions, increasing osteogenic activity and reducing bone resorption in the femur. Notably, resistance training induced more pronounced effects on bone remodeling, reflected by greater osteoblast activity, reduced osteoclastogenesis, increased osteocyte density, and favorable modulation of the RANKL/OPG axis. These effects may be partially mediated by mechanical loading–induced cellular responses and by muscle–bone crosstalk, including increased expression of IGF-1 and FNDC5 in skeletal muscle. Together, these findings reinforce the relevance of exercise training as a non-pharmacological strategy for preserving musculoskeletal health during female aging.

Conflict of Interest

The authors report no conflicts of interest.

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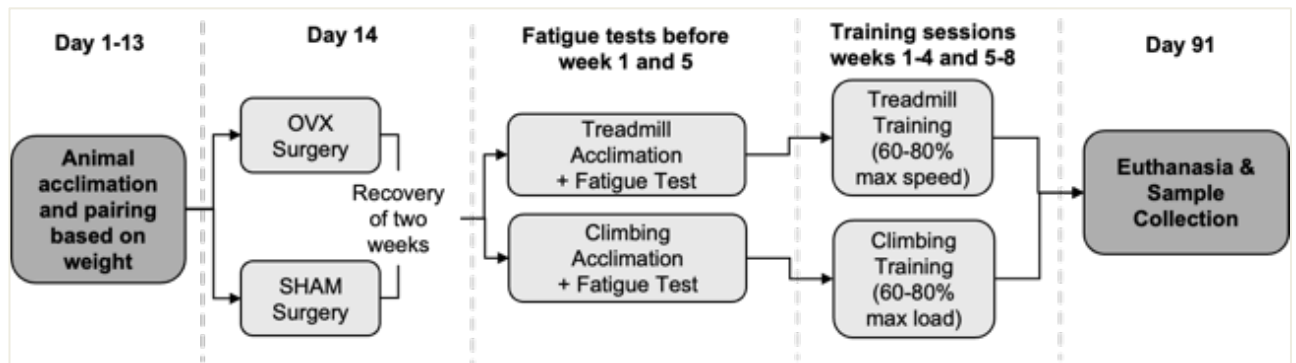


Figure 1. Experimental timeline.

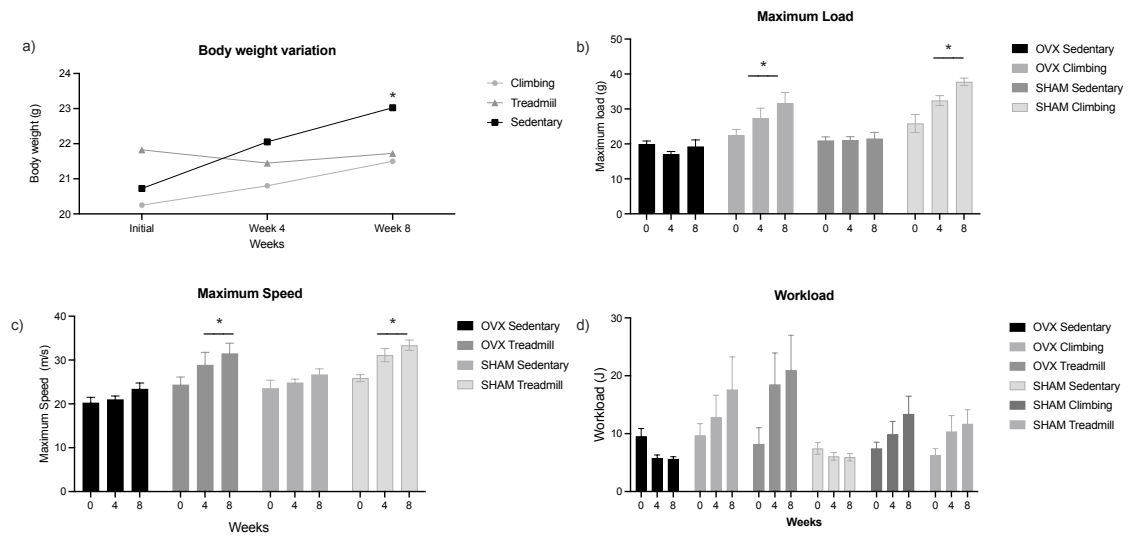


Figure 2. Animal characteristics. (a) Body weight variation throughout the experimental period (baseline, week 4, and week 8) in sedentary, resistance training (ladder climbing), and aerobic training (treadmill running) groups. (b) Maximum load achieved in the ladder-climbing incremental test at baseline, week 4, and week 8. (c) Maximum running speed achieved in the treadmill incremental test at baseline, week 4, and week 8. (d) Calculated workload for ladder-climbing and treadmill protocols at baseline, week 4, and week 8. Data are presented as mean $\pm$ SD. Statistical differences are indicated in the figure (* $p < 0.05$).

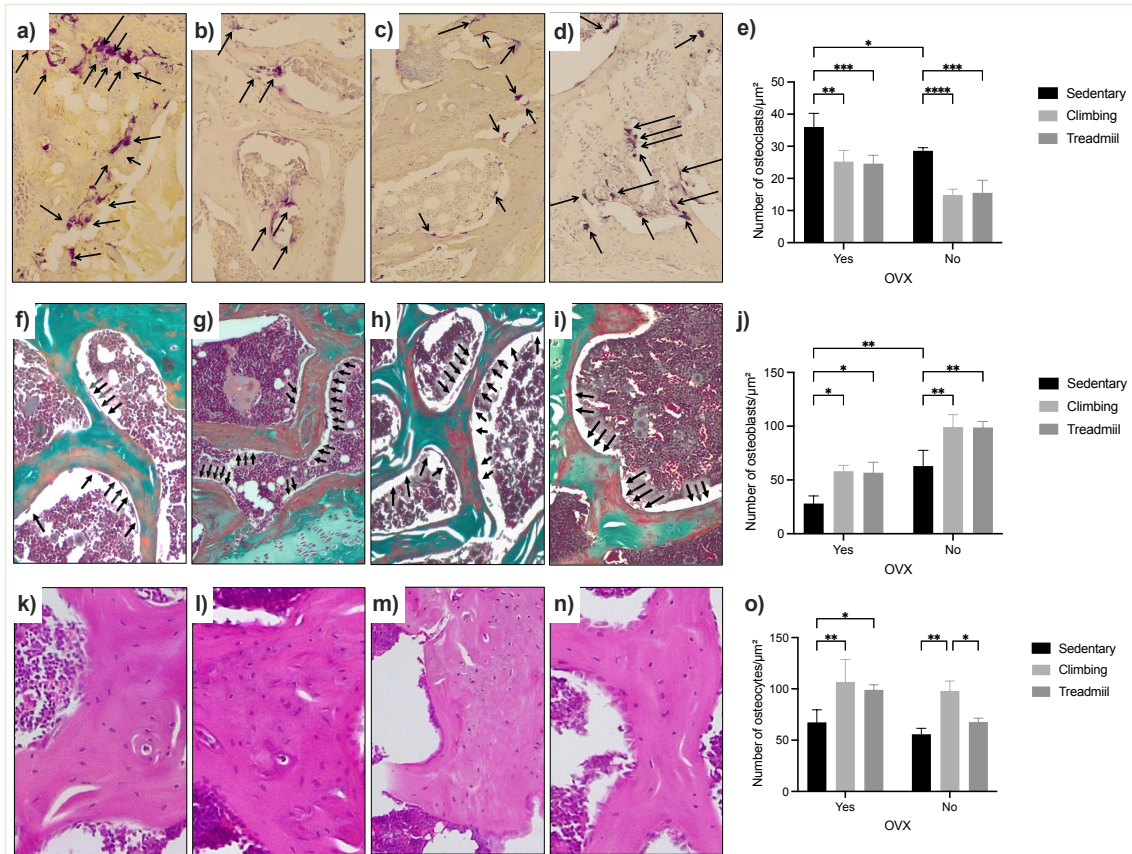


Figure 3. Femoral bone remodeling in OVX and SHAM mice. Representative histological images and quantitative analyses of femoral bone remodeling. TRAP-stained sections showing osteoclasts (arrows) in OVX sedentary, OVX resistance training (ladder climbing), OVX aerobic training (treadmill running) and SHAM sedentary groups, (a–d, respectively). (e) Osteoclast number (cells/mm²) in OVX and SHAM animals. Masson's trichrome–stained sections showing osteoblasts (arrows) in OVX sedentary, OVX resistance training (ladder climbing), OVX aerobic training (treadmill running) and SHAM sedentary groups, (f–i, respectively). (j) Osteoblast number (cells/mm²). H&E-stained sections used for osteocyte analysis in OVX sedentary, OVX resistance training (ladder climbing), OVX aerobic training (treadmill running) and SHAM sedentary groups (k–n, respectively). (o) Osteocyte density (cells/mm²). Data are presented as mean ± SD. Statistical differences are indicated in the figure (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ co).

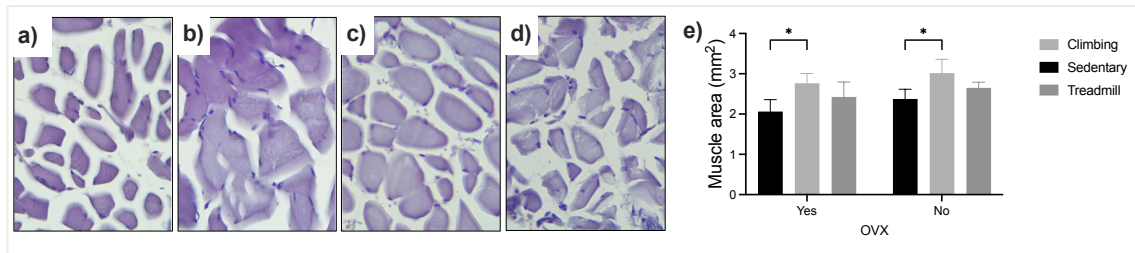


Figure 4. Representative hematoxylin and eosin–stained cross-sections of the extensor digitorum longus (EDL) muscle OVX sedentary, OVX resistance training (ladder climbing), OVX aerobic training (treadmill running) and SHAM sedentary groups, respectively (**a–d**, respectively). (**e**) Quantification of muscle fiber cross-sectional area (μm^2) in OVX and SHAM animals. Data are presented as mean $\pm$ SD. Statistical differences are indicated in the figure (* $p < 0.05$).

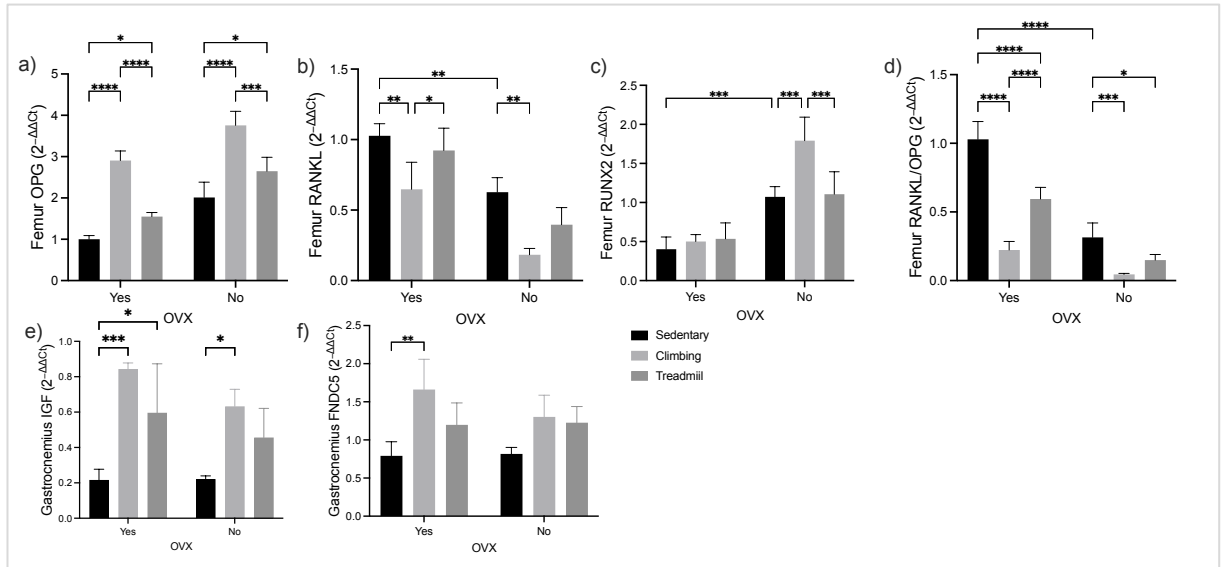


Figure 5. Effects of resistance and aerobic training on gene expression in femoral bone and gastrocnemius muscle of OVX and SHAM mice. Relative mRNA expression of bone-related genes in the femur (a–d) and muscle-related genes in the gastrocnemius muscle (e–f) from sedentary, resistance training (ladder climbing), and aerobic training (treadmill running) groups. Data are presented as mean $\pm$ SD. Statistical differences are indicated in the figure (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Table 1. Primer Sequence and Reaction Properties

Target Genes	<i>Sense</i> and <i>anti-sense</i> sequences
FNDC5	TGGGCAGGTGTTATAGCTCTC TATCTTGCTGCGGAGGAGAC
RANKL	CCTGAGGCCAGCCATT CTTGGCCCAGCCTCGAT
OPG	TACCTGGAGATCGAATTCTGCTT CCATCTGGACATTTTTTGCAA
RUNX2	AACCACAGAACCACAAGTGCG AAATGACTCGGTGGTCTCGG
GAPDH	ACGGCCGCATCTTCTTGCA CGCCCAAATCCGTTCACACCG

ANEXOS

ANEXO 1 – CERTIFICADO DE APROVAÇÃO DE PROJETO NO COMITÊ DE ÉTICA NO USO DE ANIMAIS.



PRÓ-REITORIA DE PESQUISA
COMISSÕES PERMANENTES
COMISSÃO DE ÉTICA NO USO DE ANIMAIS
Cx.P.3037 - Lavras – MG – 37200-000 – (35) 3829-5182 cba@ufla.br

CERTIFICADO

Certificamos que a proposta intitulada "Movimentação ortodôntica, menopausa e exercício físico – aspectos microtomográficos, imunohistoquímicos e moleculares em camundongos fêmea ovariectomizada " protocolo nº 013/21, sob a responsabilidade de Luciano José Pereira, Pedro Gustavo Machado, Luciana Botelho Ribeiro e Ivam Moreira de Oliveira Júnior, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto homem), para fins de ensino e/ou pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas edificadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), do Ministério da Ciência, Tecnologia e Inovação (MCTI), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da Pró-Reitoria de Pesquisa/UFLA, em reunião de 28/04/2022.

Vigência da autorização: de 01/08/2022 a 01/08/2024

Finalidade: () Ensino (x) Pesquisa Científica

Espécie/linhagem/raça: Camundongo

Número de animais aprovados: 112

Peso/Idade: 20g / 8 semanas

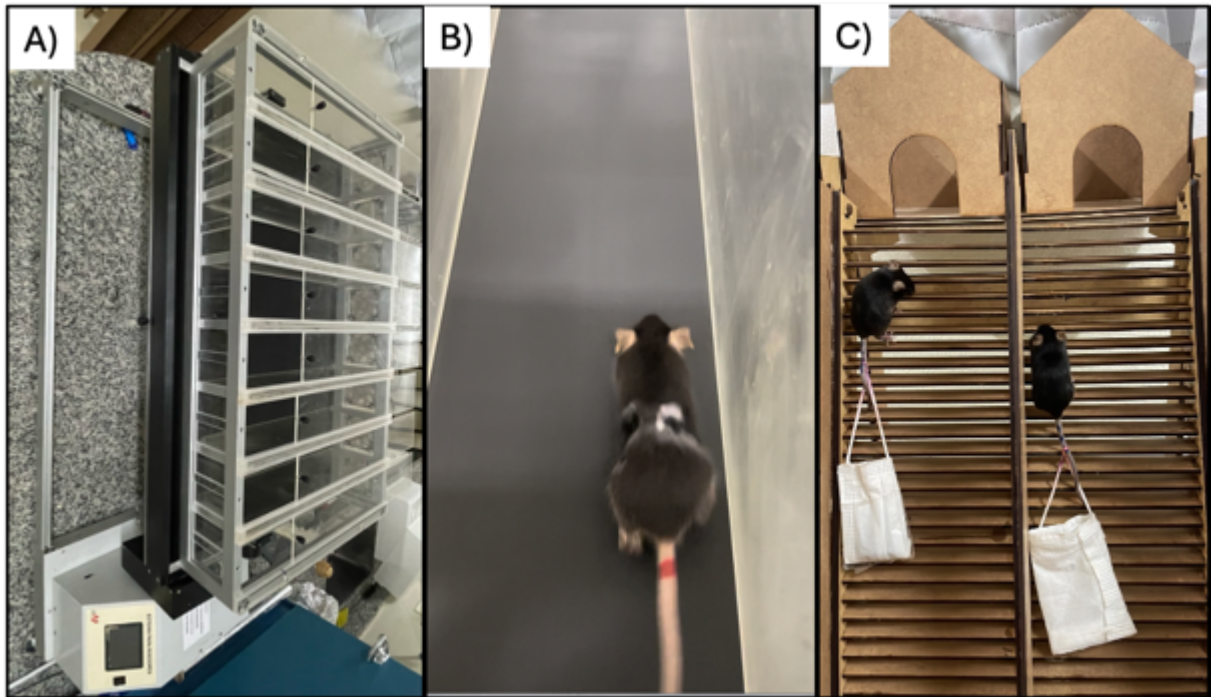
Sexo: fêmea

Origem dos animais (documento apresentado pelo pesquisador responsável e arquivado pela CEUA)

Rafael Neodini Remedio

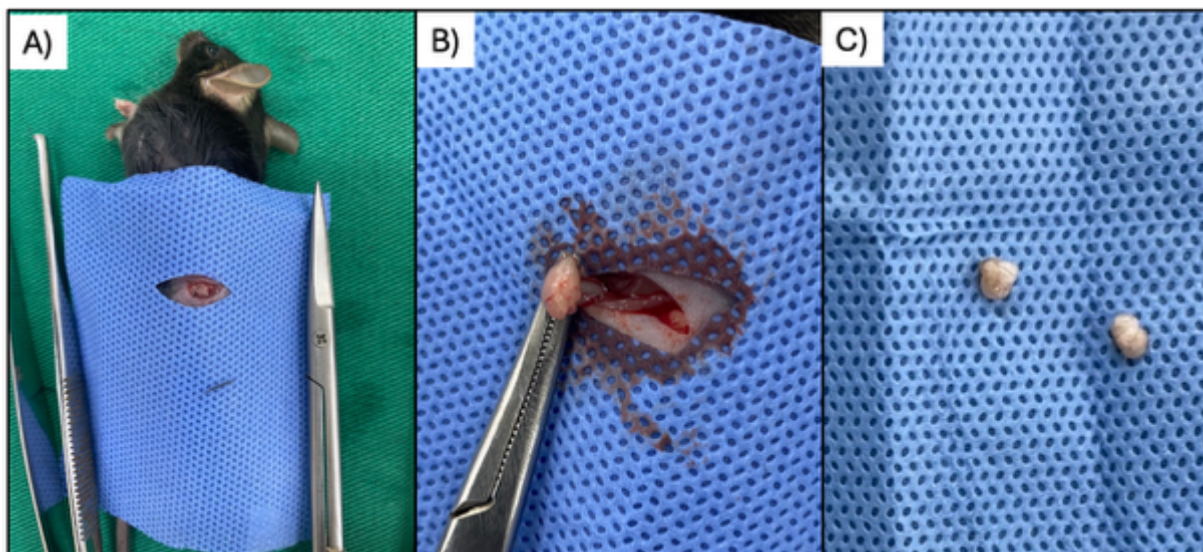
Prof. Rafael Neodini Remedio
Presidente da Comissão de Ética no Uso de Animais CEUA

ANEXO 2 – ESTEIRA (A-B) E ESCADAS (C) UTILIZADAS PARA OS TREINOS AERÓBICOS E DE RESISTÊNCIA, RESPECTIVAMENTE.



Fonte: Do autor (2026).

ANEXO 3 – ETAPAS DA CIRURGIA DE OVARIECTOMIA (OVX). A) INCISÃO BILATERAL, B) EXPOSIÇÃO DOS OVÁRIOS E C) OVÁRIOS REMOVIDOS.



Fonte: Do autor (2026).