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LILIAN BASEGGIO

EFEITOS DO EXERCÍCIO FÍSICO E ÁCIDO URSÓLICO EM RINS DE
RATOS SUBMETIDOS À MODELO DE INFLAMAÇÃO POR
LIPOPOLISSACARÍDEO

CHAPECÓ - SC

2025

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Dissertação apresentada ao Programa de Pós
Graduação em Ciências Biomédicas da
Universidade Federal da Fronteira Sul (UFFS),
como requisito para obtenção do título de Mestre
em Ciências Biomédicas.

Orientador: Prof. Dr.^a Andréia Machado Cardoso

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A todos, o meu muito obrigada.

Seja o que você quer ser,
porque você possui apenas uma vida
e nela só se tem uma chance de fazer aquilo que quer.
Tenha felicidade bastante para fazê-la doce.
Dificuldades para fazê-la forte.
Tristeza para fazê-la humana.
E esperança suficiente para fazê-la feliz.
As pessoas mais felizes não têm as melhores coisas.
Elas sabem fazer o melhor das oportunidades que aparecem em seus caminhos.
A felicidade aparece para aqueles que choram.
Para aqueles que se machucam.
Para aqueles que buscam e tentam sempre.
E para aqueles que reconhecem a importância das pessoas que passam por suas vidas.
(Clarice Lispector)

RESUMO

A inflamação crônica de baixo grau é um componente central da fisiopatologia da doença renal crônica (DRC), promovendo disfunção endotelial, fibrose e perda progressiva da função renal. Entre as vias moleculares envolvidas, a sinalização purinérgica emerge como um mediador central entre o estresse inflamatório e as respostas metabólicas renais. Nesta dissertação, o papel do exercício físico (EF) como modulador dessas respostas foi abordado sob duas perspectivas complementares. O primeiro estudo, uma revisão narrativa da literatura, reuniu evidências sobre os efeitos do EF no perfil inflamatório da DRC, destacando sua capacidade de modular citocinas inflamatórias e investigando a participação do sistema purinérgico nesse contexto. O segundo estudo, de caráter experimental, além de abordar o EF, investigou o ácido ursólico (AU) - triterpeno pentacíclico fitoquímico derivado de algumas plantas. Para isso utilizou modelo animal, com 65 ratos Wistar, machos, que foram submetidos a um protocolo de EF resistido e tratados com AU ou solução salina por via oral durante o período de seis semanas. Na última semana do protocolo, foi administrado diariamente lipopolissacarídeos (LPS) por via intraperitoneal para indução de resposta inflamatória. Os animais foram distribuídos em oito grupos: salina (SAL, $n = 9$), EF ($n = 8$), AU ($n = 8$), EF + AU ($n = 8$), LPS ($n = 8$), EF + LPS ($n = 8$), AU + LPS ($n = 8$), EF + AU + LPS ($n = 8$). Após, foram submetidos à eutanásia e procedida a coleta dos rins e do soro para análise do sistema purinérgico, níveis de irisina e parâmetros bioquímicos. Os resultados demonstraram que o exercício físico destacou-se como a intervenção mais eficaz em restaurar a homeostase purinérgica. A associação de EF e AU potencializou esses efeitos, reforçando o caráter sinérgico entre a ação metabólica do exercício e a bioatividade anti-inflamatória do triterpeno. Em conjunto, os dois estudos sustentam que o exercício físico exerce papel central na modulação da inflamação sistêmica e renal, atuando também sobre a sinalização purinérgica como via de interface entre metabolismo e resposta imune. A associação com compostos bioativos naturais, como o ácido ursólico, emerge como uma abordagem promissora para o desenvolvimento de estratégias não farmacológicas voltadas à prevenção e ao tratamento de lesões renais de origem inflamatória.

Palavras-chave: Ácido ursólico; exercício físico; injúria renal aguda.

ABSTRACT

Chronic low-grade inflammation is a central component of the pathophysiology of chronic kidney disease (CKD), promoting endothelial dysfunction, fibrosis, and the progressive loss of renal function. Among the molecular pathways involved, purinergic signaling emerges as a central mediator between inflammatory stress and renal metabolic responses. In this dissertation, the role of exercise training (ET) as a modulator of these responses was addressed from two complementary perspectives. The first study, a narrative literature review, gathered evidence on the effects of ET on the inflammatory profile of CKD, highlighting its ability to modulate inflammatory cytokines and investigating the involvement of the purinergic system in this context. The second, an experimental study, investigated both ET and ursolic acid (UA)—a phytochemical pentacyclic triterpene derived from various plants. For this purpose, an animal model was utilized, employing 65 male Wistar rats subjected to a resistance ET protocol and treated with UA or saline solution orally for six weeks. During the final week of the protocol, lipopolysaccharides (LPS) were administered daily via intraperitoneal injection to induce an inflammatory response. The animals were distributed into eight groups: saline (SAL, $n = 9$), ET ($n = 8$), UA ($n = 8$), ET + UA ($n = 8$), LPS ($n = 8$), ET + LPS ($n = 8$), UA + LPS ($n = 8$), and ET + UA + LPS ($n = 8$). Subsequently, the animals were euthanized, and kidneys and serum were collected for analysis of the purinergic system, irisin levels, and biochemical parameters. The results demonstrated that exercise training stood out as the most effective intervention in restoring purinergic homeostasis. The combination of ET and UA potentiated these effects, reinforcing the synergistic nature between the metabolic action of exercise and the anti-inflammatory bioactivity of the triterpene. Together, both studies sustain that exercise training plays a pivotal role in modulating systemic and renal inflammation, also acting on purinergic signaling as an interface pathway between metabolism and the immune response. The association with natural bioactive compounds, such as ursolic acid, emerges as a promising approach for developing non-pharmacological strategies aimed at the prevention and treatment of inflammatory-based renal injuries.

Keywords: Ursolic acid; physical exercise; acute kidney injury.

LISTA DE ABREVIATURAS E SIGLAS

ADO	Adesonina
ADP	Adenosina difosfato
AMP	Adenosina monofosfato
AMPK	Proteína quinase ativada por AMP
ATP	Adenosina trifosfato
AU	Ácido ursólico
cAMP	Monofosfato cíclico de adenosina
CD73	Ecto-5'-nucleotidase
DAMP	Padrão molecular associado ao dano
DRC	Doença Renal Crônica
EF	Exercício Físico
ENTPD1	Ectonucleosídeo trifosfato difosfohidrolase-1
ICAM-1	Moléculas de adesão intercelular
IKK	Via de sinalização I κ B kinase
IL-1	Interleucina 1
IL-10	Interleucina 10
IL-1 β	Interleucina 1 beta
IL-6	Interleucina 6
IRA	Injúria Renal Aguda
LPS	Lipopolissacarídeo
MAPK	Via de proteínas quinases ativadas por mitógenos
NF- κ B	Fator nuclear kappa B
TGF- β 1	Fator de Crescimento transformador beta 1
TLR	Receptor toll like
TLR-4	Receptor toll-like 4
TNF- α	Fator de Necrose Tumoral alfa
UCP2	Proteína desacopladora mitocondrial 2
UDP	Uridina difosfato
UTP	Uridina trifosfato
VCAM	Moléculas de adesão celular vascular

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1 INTRODUÇÃO

A lesão renal aguda é uma condição clínica com potencial devastador, caracterizada por declínio da função renal e dano às células tubulares renais. Um dos modelos de indução mais conhecidos para estudo é a injeção intraperitoneal de lipopolissacarídeos (LPS). Trata-se de um componente celular normal da parede celular das bactérias Gram-negativas, que leva à tempestade de citocinas inflamatórias, mudanças hemodinâmicas e vasculares que por fim, pode provocar disfunção renal (Chen *et al.*, 2018).

A sinalização purinérgica tem importante papel, tanto na função renal normal quanto nos mecanismos patológicos, promovendo desde a regulação do feedback tubuloglomerular, mediação do tônus vascular renal até mediação inflamatória, através de receptores específicos (Jost; Bizuti; Silva, 2021). No meio renal, o LPS promove a liberação de citocinas inflamatórias pela via de sinalização Toll like receptor 4 (TLR4), principalmente através do Fator de Necrose Tumoral alfa (TNF- α) e da Interleucina-1 β (IL-1 β), contribuindo para a ativação e dano de células epiteliais tubulares, consequentemente provocando hipoperfusão por dano à microcirculação renal (Chen *et al.*, 2018).

São encontrados receptores P1 e P2 ao longo de todos os segmentos do néfron e células renais, além de ectonucleotidases que mediam a ação dos sinalizadores. Em tecidos com aumento da fibrose, são encontradas maiores concentrações de adenosina trifosfato (ATP), adenosina difosfato (ADP), uridina trifosfato (UTP) e uridina difosfato (UDP). Outro fator que afeta a sinalização purinérgica no rim é a enzima ectonucleosídeo trifosfato difosfohidrolase-1 (ENTPD1 ou CD39), expressa pelos linfócitos T reguladores, e que atua no papel reparador em resposta a injúria renal crônica (Menzies *et al.*, 2017).

Considerando o potencial de dano que o prejuízo à função renal é capaz de promover, é importante considerar estratégias de prevenção que possam mediar a redução desses mecanismos de dano, como o exercício físico (EF) e o uso de ácido úrsólico (AU). Nesse sentido, cada vez mais se entende o papel do EF como terapêutico, para prevenção e tratamento de diversas doenças e condições, como na redução do risco cardiovascular, melhora do desempenho físico em tarefas diárias, redução da atrofia muscular, redução de caquexia e hipertensão bem como a promoção de um ambiente

anti-inflamatório, com a redução de IL 6 (interleucina 6) e IL 10 (interleucina 10) e a diminuição da ativação de linfócitos T e monócitos (Wilkinson; Shur; Smith, 2016).

Uma revisão sistemática de Asad e colaboradores (2020) demonstrou que o exercício, especialmente o resistido, possui um potente efeito anti-inflamatório, atuando principalmente pela redução da gordura visceral. Essa diminuição leva à menor secreção de adipocinas pró-inflamatórias, à liberação de IL-6, à redução na produção de citocinas inflamatórias secundárias e à diminuição da expressão de receptores toll-like em monócitos e macrófagos, além de reduzir os níveis de TNF- α . Paralelamente, a contração muscular promove a liberação de ATP, que induz um estado pró-inflamatório inicial, mas que é modulado pela ação de ectonucleotidases – enzimas que hidrolisam ATP em ADP e, finalmente, em adenosina monofosfato (AMP), esta última com propriedades anti-inflamatórias. Tais mecanismos podem estar relacionados ao controle de processos inflamatórios agudos que, quando recorrentes ou prolongados, contribuem para a progressão da Injúria Renal Aguda (IRA) para a Doença Renal Crônica (DRC).

Além disso, outra potencial intervenção com ação nefroprotetora seria o uso de substâncias com essa capacidade, como o AU. Suas inúmeras atividades biológicas têm sido estudadas devido aos potenciais benefícios à saúde, incluindo desordens metabólicas e obesidade, doenças cardiovasculares, câncer e doenças neurológicas.

A ação anti-inflamatória do AU está relacionada a supressão de NF- κ B, que é uma família de fatores de transcrição de regulam a expressão de genes envolvidos na gênese tumoral, adesão de moléculas, quimiocinas, citocinas pró inflamatórias, genes e ciclos celular (Nguyen *et al.*, 2021). Vários desses achados apontam o AU como potencial adjuvante terapêutico no tratamento de doenças renais (Ma *et al.*, 2014; Pai *et al.*, 2012; Tripathi; Alshahrani, 2021; Zhou *et al.*, 2010). Sobre a ação do AU sobre o sistema oxidativo ou purinérgico pouco é descrito na literatura.

Outro meio pelo qual a ação tanto do AU quanto do exercício físico podem estar relacionados à nefroproteção relaciona-se a liberação de irisina, uma miocina secretada pelo tecido muscular em resposta ao exercício físico (Formigari *et al.*, 2022), e que também tem sua circulação aumentada em resposta à suplementação com AU, conforme demonstrado por Bang e colaboradores (Bang *et al.*, 2014). A ação da irisina neutraliza os efeitos de reprogramação metabólica induzidas pelo Fator de Crescimento transformador beta 1 (TGF- β 1) nas células tubulares renais, suprimindo progressivamente o dano renal e fibrose, aumenta o consumo de oxigênio mitocondrial e maximiza a capacidade respiratória celular, bem como a respiração acoplada a ATP

em células tubulares (Peng *et al.*, 2017). Em outros estudos, também foi demonstrado o efeito nefroprotetor da irisina pela redução da morte celular tubular via p53 (Liu *et al.*, 2020) e aumento da proteína desacopladora mitocondrial 2 (UCP2) (Zhang *et al.*, 2020), bem como no estudo de Formigari e colaboradores (Formigari *et al.*, 2022) a proteção renal induzida pelo exercício físico na nefropatia diabética também relacionou-se ao eixo irisina/proteína quinase ativada por AMP (AMPK).

Apesar da relação com a produção energética celular e a nefroproteção demonstrada nesses estudos, pouco se sabe sobre o papel do sistema purinérgico na nefroproteção induzida pela atividade física e ácido ursólico, ou sobre sua associação. Assim, o presente estudo se justifica na importância premente de encontrar intervenções eficazes na prevenção de lesões renais. Pois, apesar dos avanços terapêuticos, há uma lacuna significativa no que diz respeito a intervenções que promovam prevenção à injúria renal.

A literatura recente tem explorado amplamente os benefícios do EF e AU, cujas propriedades anti inflamatórias e nefroprotetoras se mostram promissoras. O EF e o AU, através da liberação do hormônio irisina e da modulação inflamatória apresentam significativo potencial de redução de danos renais. No entanto, a combinação desses dois agentes e os possíveis efeitos sinérgicos não foram ainda relatados.

Deste modo, este estudo evidencia a necessidade de esclarecer os possíveis efeitos nefroprotetores da combinação entre EF e AU, utilizando um modelo animal para avaliar parâmetros bioquímicos, inflamatórios e purinérgicos. A pesquisa busca compreender como essas intervenções podem prevenir a progressão da lesão renal aguda para a doença renal crônica, elucidando os mecanismos de ação envolvidos. O objetivo final é fornecer uma base para uma nova estratégia terapêutica que possa melhorar os desfechos clínicos de pacientes com essa condição.

2 OBJETIVOS

Avaliar os efeitos nefroprotetores do exercício físico (EF) e do ácido ursólico (AU) – isolados e em associação – em um modelo animal de inflamação, e revisar o papel do EF na modulação inflamatória da Doença Renal Crônica (DRC).

2.1 OBJETIVOS ESPECÍFICOS

- Avaliar os efeitos de um protocolo de EF e AU em ratos submetidos a protocolo de inflamação renal sobre:
 - Expressão gênica de componentes da sinalização purinérgica em rins;
 - Níveis de citocinas pró e anti-inflamatórias;
 - Níveis de irisina;
 - Perfil de células sanguíneas expressas através de hemograma.
- Redigir um artigo de revisão sobre o papel do exercício físico no perfil inflamatório sobre a DRC.

3 REVISÃO DE LITERATURA

3.1 RIM E INFLAMAÇÃO

Os rins são órgãos vitais para a homeostase do corpo humano, têm formato oval e situam-se na parede posterior do abdome, no retroperitônio, uma de cada lado da coluna vertebral (Moore, 2019). São supridos pelas artérias renais, derivadas da aorta abdominal, e a drenagem venosa realizada pelas veias reais, que se unem a veia cava inferior. Já o plexo nervoso renal é suprido por fibras dos nervos esplâncnicos abdominopélvicos.

Muito além da excreção de metabólitos através da urina, os rins são órgãos reguladores, tendo papel fundamental na regulação da osmolalidade e do volume dos líquidos corporais, balanço hidroeletrólítico e balanço ácido-básico, bem como é um importante órgão endócrino, responsável pela produção e secreção de renina, calcitriol e eritropoetina (Koeppen; Stanton, 2025).

Diferentes tipos de doenças renais podem afetar o funcionamento desses órgãos, e apesar de sua grande reserva funcional, o acúmulo de danos estruturais e funcionais podem progredir para a perda total de funcionalidade dos rins. No caso específico da DRC, essas alterações são marcadamente irreversíveis e evoluem ao longo do tempo, sendo uma síndrome clínica que afeta milhões de pessoas em todo o mundo, com prevalência que chega a 10% de toda a população pesquisada em 11 países (Sundström *et al.*, 2022). Suas principais causas incluem diabetes, hipertensão, glomerulonefrite crônica, pielonefrite crônica, uso prolongado de anti-inflamatórios não esteroidais, condições autoimunes, doença renal policística, Doença de Alport, malformações congênitas e a persistência de injúrias renais agudas (Ammirati, 2020).

O modelo de indução inflamatória através da aplicação de LPS está bem estabelecido na literatura como um potente indutor de injúria em órgão alvo, que pode ser descrito como a infusão de componente da membrana celular de bactérias gram-negativas, que gera um processo inflamatório sistêmico análogo a fase inicial do quadro clínico de sepse, agregando anormalidade morfológicas, injúria vascular, resposta inflamatória e por fim, disfunção renal (Pinto *et al.*, 2012; Salama; Elgohary; Fahmy, 2023).

O LPS desencadeia uma tempestade de citocinas inflamatórias, inicialmente interage com o TLR-4 nas células tubulares epiteliais renais, e ativa as vias de

sinalização I κ B kinase (IKK)/fator nuclear kappa B (NF- κ B) e a via de proteínas quinases ativadas por mitógenos (MAPK), levando a uma regulação positiva de citocinas pró inflamatórias, como o TNF- α e a interleucina 1 (IL-1) e IL-6 (Lee *et al.*, 2024; Pinto *et al.*, 2012; Salama; Elgohary; Fahmy, 2023).

A lesão endotelial ocorrida em decorrência da inflamação induzida por LPS estimula a formação de moléculas de adesão intercelular 1 (ICAM-1), moléculas de adesão celular vascular (VCAM) e as selectinas P e E, que promovem interação entre leucócitos e endotélio, além de adesão plaquetária e obstrução mecânica da microvasculatura renal. Isso leva a um segundo mecanismo de lesão renal através de isquemia/reperfusão, pois a hipoperfusão gerada por este mecanismo leva a baixa disponibilidade de oxigênio, levando à lesão das células do epitélio tubular, apoptose e necrose tubular aguda (Pinto *et al.*, 2012).

Além disso, as lesões geradas nesse contexto promovem disfunção mitocondrial, com redução de síntese de novas moléculas de ATP, assim, a célula tenta compensar sua demanda energética através da exportação de ATP estocado na matriz mitocondrial para o citoplasma através da proteína carreadora de ATP/ADP. Processo que aumenta os níveis extracelulares de ATP, que liga-se aos receptores P2X que são responsáveis por mediar inflamação e dano tecidual (Mishra *et al.*, 2024).

Por fim, o processo de injúria aguda no tecido renal aumenta o risco de progressão para DRC, que ocorre em decorrência de alterações irreversíveis na função e estrutura renal, que podem ser desencadeadas pelo processo aqui descrito. (Ammirati, 2020; Lee *et al.*, 2024).

3.2 SISTEMA PURINÉRGICO NO RIM

O Sistema Purinérgico é um complexo de enzimas, nucleotídeos e receptores que modula a homeostase e a fisiopatologia do corpo humano e, conseqüentemente, dos rins (Cardoso; Manfredi; Maciel, 2021). Os principais mediadores incluem o ATP e seus derivados de hidrólise, como o ADP, AMP e Adenosina (ADO), atuando como mensageiros extracelulares essenciais (Burnstock, 2017). A ativação de seus receptores específicos (P1 e P2) no tecido renal regula criticamente as respostas inflamatórias, imunes e vasculares. Devido a essa ampla regulação de vias fundamentais, a desregulação purinérgica tem sido cada vez mais reconhecida como um elemento chave na patogênese e progressão da DRC (Di Virgilio; Vuerich, 2015; Vallon; Rieg, 2011).

As moléculas sinalizadoras compõem-se pelas purinas (ATP, ADP, AMP e ADO) e pelas pirimidinas (UDP e UTP), que se ligam principalmente aos receptores da família P2, com exceção da ADO que se liga aos receptores do tipo P1 (Cardoso; Manfredi; Maciel, 2021). As células renais liberam ATP e UTP no espaço extracelular, um fato que é regulado e facilitado por diversos sistemas de transporte que envolvem exocitose por vesículas e lisossomas, ou mediadas por canais via conexinas ou panexinas. Esses sinalizadores extracelulares costumam ter meia vida curta devido ao rápido catabolismo (Menzies *et al.*, 2017).

O catabolismo rápido dos nucleotídeos é promovido pelas ectonucleotidases, enzimas responsáveis pela hidrólise dos nucleotídeos em nucleosídeos. Estas estão englobadas em quatro famílias de genes: ectonucleotídeopirofosfato/fosfodiesterases (ENPPs), fosfatase alcalina, ectonucleosídeotrifosfato difosfohidrolases (ENTPDases) e 5'-nucleotidase (Cardoso; Manfredi; Maciel, 2021).

A vasculatura e microvasculatura renal expressam a ENTPD1, que hidrolisa o ATP em ADP e AMP, reduzindo rapidamente a sinalização purinérgica (Menzies *et al.*, 2017). Nos rins, essa enzima está expressa no endotélio vascular, nos ductos coletores do córtex e medula renal, e também no segmento ascendente da alça de Henle e do glomérulo (Kishore; Robson; Dwyer, 2018). Também há expressão renal da ecto-5'-nucleotidase (CD73), responsável pela conversão de AMP em ADO, sendo predominantemente encontrada nos glomérulos e nos fibroblastos peritubulares. Entretanto, seu papel é considerado menos importante do que o da ENTPD1 no contexto do funcionamento renal (Dwyer; Kishore; Robson, 2020).

A ADO atua nos receptores P1, que são ligados à proteína G e subdividem-se em A1, A2A, A2B e A3 (Cardoso; Manfredi; Maciel, 2021). De maneira geral, os receptores do tipo P1 têm ação opostora às respostas celulares geradas pela ação dos receptores P2, atuando como reguladores dos níveis de adenosina monofosfato cíclico (cAMP). A ativação dos receptores A1 e A3 leva à redução de cAMP intracelular, enquanto que os receptores A2A e A2B o aumentam. O receptor A2A, expresso em glomérulos, é reconhecido por seus efeitos anti-inflamatórios e nefroprotetores, atuando na inibição da síntese de citocinas e na promoção da vasodilatação. O receptor A2B, expresso em glomérulos, túbulos (especialmente em pacientes com DRC) e na vasculatura renal, é associado à proteção tecidual em condições de estresse. Enquanto o A3 é encontrado nas arteríolas aferentes e túbulos proximais, se relaciona à efeitos prejudiciais durante a IRA (Dwyer; Kishore; Robson, 2020; Wissmann *et al.*, 2025).

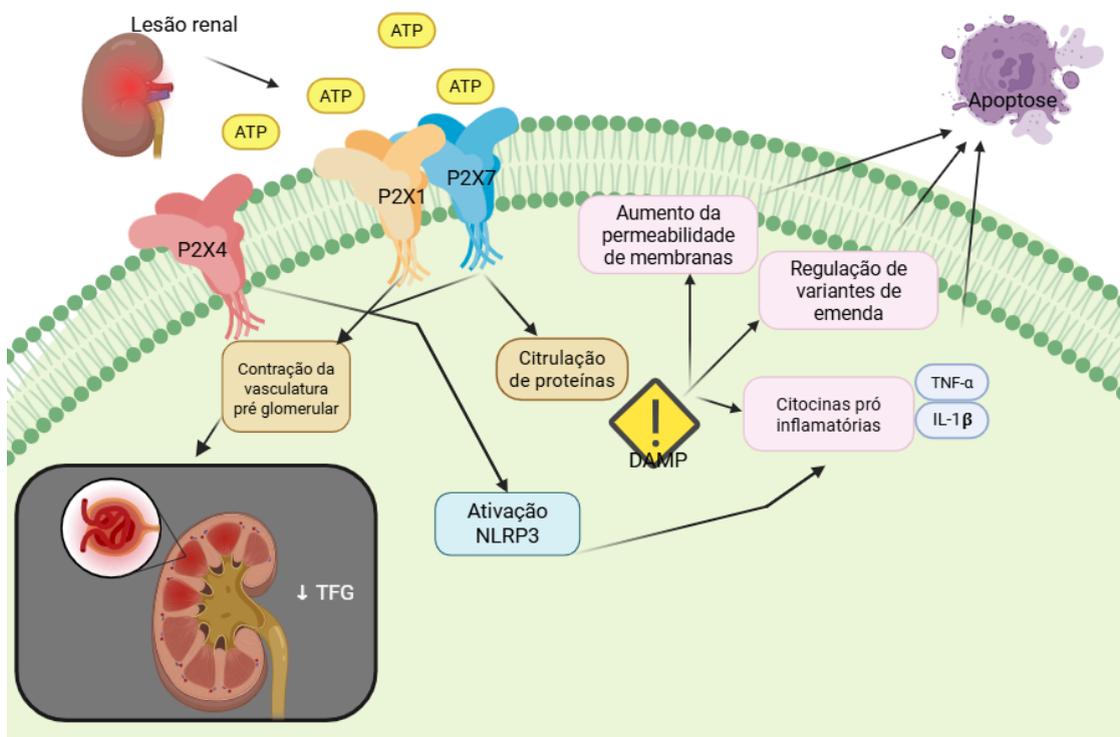
Em contraste com os receptores P1, os receptores P2 representam a principal via de ação para os nucleotídeos ATP e UTP no rim, sendo divididos em duas grandes famílias: P2Y, receptores metabotrópicos ligados à proteína G; e P2X, receptores ionotrópicos ligados a canais na membrana plasmática (Cardoso; Manfredi; Maciel, 2021; Kishore; Robson; Dwyer, 2018). Nos rins, os receptores purinérgicos do tipo P2 estão expressos em todos os segmentos do néfron, sistema de ductos coletores e células renais, e desempenham um papel crucial na regulação das funções renais e vasculares, participando na liberação e regulação de ATP e UTP extracelulares, influenciando processos como o transporte de íons e a resposta inflamatória (Menzies *et al.*, 2017).

O fluxo de sódio é fortemente suprimido pela ativação dos receptores P2R em vários segmentos no néfron, integrando-se a sistemas de adaptação hormonal mais lentos, como o sistema renina-angiotensina-aldosterona. No túbulo proximal, os nucleotídeos extracelulares inibem os transportadores de sódio, e a borda em escova expressa ectonucleotidases que terminam essa sinalização (Menzies *et al.*, 2017; Mishra *et al.*, 2024). No néfron distal, mudanças no fluxo ou na osmolaridade do fluido tubular promovem secreção de nucleotídeos, um processo que depende da ativação do canal transitório TRPV4 osmosensor. Essa ativação culmina na síntese endotelial de óxido nítrico e subsequente inibição do cotransportador NKCC2 apical e da atividade Na-K-ATPase (Menzies *et al.*, 2017).

A vasculatura renal responde a estímulos por meio de receptores P2X (P2X1, P2X4, P2X7), mecanismo que é exemplificado na figura 1. A exposição desses receptores ao ATP leva à contração da vasculatura pré-glomerular, diminuindo o fluxo sanguíneo renal e a taxa de filtração glomerular (TFG). Em um contexto patológico, os receptores P2X4 e P2X7 são cruciais, pois estão implicados em condições como hipertensão e glomerulonefrite (Mishra *et al.*, 2024). O P2X7 é um dos receptores mais estudados e sua superexpressão está associada a vasoconstrição potente das arteríolas pré-glomerulares, injúria glomerular, inflamação e apoptose celular (Menzies *et al.*, 2017). O P2X7 ativa vias que levam à citrulinização das proteínas, que sinalizam como Padrão Molecular Associado ao Dano (DAMP), resultando em reação inflamatória e indução de permeabilidade de membrana (Mishra *et al.*, 2024). Tipicamente, o ATP extracelular se liga ao P2X7 em macrófagos, resultando na ativação de inflamassomas e liberação de citocinas pró-inflamatórias (TNF- α , IL-18 e IL-1 β) (Menzies *et al.*, 2017; Mishra *et al.*, 2024). Além disso, o P2X4 atua na pro-inflamação e está envolvido na

ativação do inflamassoma NLRP3, mecanismos críticos na patogênese de lesões (Mishra et al., 2024).

Figura 1 - Mecanismos centrais da hiperativação purinérgica na fisiopatologia renal



Legenda: A figura ilustra as vias de sinalização pró-inflamatórias e deletérias disparadas pelo aumento do ATP extracelular no tecido renal, um sinal de lesão celular. O ATP ativa os receptores P2X4 e P2X7, desencadeando três vias patológicas interconectadas: 1. Resposta Vasoconstritora: A ativação de P2X1 e P2X7 na vasculatura pré-glomerular promove contração, resultando na diminuição do Fluxo Sanguíneo Renal e da TFG. 2. Dano Celular/Apoptose: O receptor P2X7 ativa vias que levam à citrulinação de proteínas e sinalizam como DAMP, culminando no aumento da permeabilidade de membranas e na apoptose celular. 3. Resposta Inflamatória: A ativação do P2X4 contribui para a ativação do inflamassoma NLRP3 e a subsequente liberação de citocinas pró-inflamatórias (demonstrados como exemplo o TNF- α , IL1 β), amplificando a inflamação e a injúria renal.

Fonte: elaborado pela autora (através do aplicativo Bio Render).

Estudos com modelos animais confirmam essa dualidade funcional. Ratos knockout para o receptor P2X7 evidenciaram um aumento nos macrófagos intersticiais, redução de miofibroblastos, diminuição da deposição de colágeno e da expressão de TFG- β no interstício renal, bem como redução da apoptose celular (Gonçalves et al., 2006). De forma análoga, em modelos de glomerulonefrite induzida por

nefrotoxicidade, o uso de antagonistas de P2X7 demonstrou redução significativa da infiltração macrofágica e da proteinúria (Jr., 2007).

Em relação aos receptores do tipo P2Y, há dominância da expressão do receptor P2Y1 nos podócitos e células endoteliais, cuja ativação promove vasodilatação dependente do endotélio (Vallon; Stockand; Rieg, 2012). Ratos knockout para P2Y1R parecem estar protegidos de injúria por nefrotoxicidade aguda, demonstrando preservação da função renal, diminuição da rarefação capilar e fibrose (Menzies *et al.*, 2017). O P2Y2 se associa à formação de cAMP relacionada à vasopressina e reabsorção de água. O aumento de *NaCl* ativa os receptores P2Y2 apicais, o que diminui a abertura de canais de sódio *ENaC*, facilitando a excreção de sódio (Vallon; Stockand; Rieg, 2012). Por fim, o P2Y12R, embora menos estudado no contexto renal, tem efeito sobre a inflamação, podendo estar relacionado à regulação da endocitose de células dendríticas. O ticagrelor, um inibidor potente do P2Y12R, já demonstrou efeitos benéficos relacionados à vasodilatação coronariana (Thomas; Storey, 2017), e em outro estudo, demonstrou efeito protetor renal em relação à injúria renal aguda, pela diminuição da atividade de plaquetas e atenuação da apoptose nas células renais (Li *et al.*, 2019).

Em resumo, o sistema purinérgico apresenta um paradoxo funcional no rim: subunidades como P2X4 e P2X7 promovem vasoconstrição e inflamação deletérias, enquanto P2Y1, P2Y12 e o receptor A2A atuam na manutenção da perfusão e na modulação anti-inflamatória. A modulação desse equilíbrio por intervenções de baixo custo, como o EF e o AU, é uma estratégia terapêutica promissora. No entanto, até o presente momento, não havia estudos que investigassem de forma integrada os efeitos combinados do EF e do AU na modulação específica da sinalização purinérgica renal em um modelo de inflamação de baixo grau. Desta forma, este trabalho se propôs a avaliar os possíveis efeitos nefroprotetores dessa associação, fornecendo evidências moleculares sobre o potencial sinérgico dessas intervenções no restabelecimento do equilíbrio purinérgico e no controle da inflamação renal.

3.3 EXERCÍCIO FÍSICO E DOENÇA RENAL

O EF é amplamente reconhecido por seu efeito anti-inflamatório e tem importante papel na redução de doenças cardiovasculares, como a hipertensão arterial sistêmica, ajuda no controle de peso e de dislipidemia, mantém a massa óssea, melhora

a absorção de glicose, oxigênio e nutrientes no músculo esquelético, melhora a capacidade respiratória e aumenta os níveis de ATP (Sabet; Soltani; Khaksari, 2022).

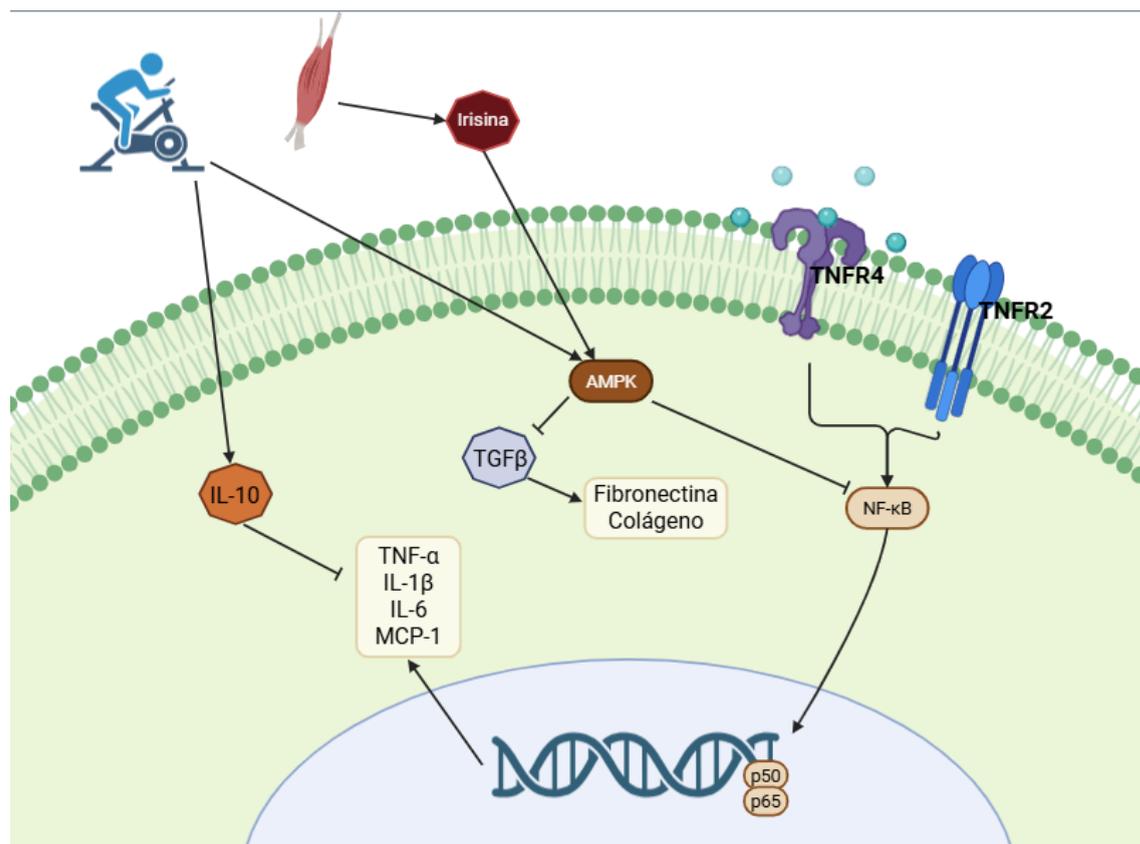
Os efeitos benéficos na DRC são bem estabelecidos na literatura, incluindo melhora da capacidade aeróbica, funcionamento muscular, função cardiovascular, capacidade de caminhar e qualidade de vida (Cheema *et al.*, 2014; Heiwe; Jacobson, 2014). No entanto, o potencial efeito sobre a lesão renal aguda apresenta menos resultados, embora promissores. O aumento transitório da IL-6 gerado pela contração muscular, leva à atenuação do aumento de TNF- α , IL1- β e outras citocinas pró inflamatórias após o estímulo, redução da expressão de TLR em monócitos e macrófagos (Asad; Burton; March, 2020; Miyagi *et al.*, 2014).

O exercício físico também estimula a produção de IL-10, cuja ação é anti inflamatória, e inibe a translocação do NF- κ B (p65 e p50) para o núcleo, o que leva à redução da expressão de citocinas pró inflamatórias (TNF- α , IL-6, IL-1 β , MCP-1), mecanismo que pode ser visualizado no figura 2. Melhora as concentrações de ureia e creatinina, além de haver evidências de redução na infiltração macrocítica, o que representa importante efeito anti fibrótico, uma vez que está envolvida na liberação de TGF-B, endotelina e angiotensina II, que são moléculas fibrogênicas (Francescato *et al.*, 2018).

A atividade física, quando realizada de maneira regular, estimula a produção de insulin-like growth factor I (IGF-1) e ativa diversas vias de sinalização, como a PI3K e Akt-mTOR. O que inibe o sinal de indução de atrofia e acaba levando a hipertrofia muscular, e também induz autofagia celular, que realiza importante papel na manutenção da homeostase do músculo esquelético, uma vez que é responsável pela “limpeza” de componentes intracelulares danificados e pela remoção de patógenos (Asad; Burton; March, 2020; Sabet; Soltani; Khaksari, 2022).

Além disso, a atividade gera consumo de ATP, o que aumenta a produção de AMP e leva a ativação da AMPK, que realiza a fosforilação da PGC1 α , que é um importante ativador da biogênese mitocondrial. O aumento do cálcio intracelular regula negativamente a expressão de genes também através da AMPK (Sabet; Soltani; Khaksari, 2022).

Figura 2 - Mecanismos relacionados à inflamação e fibrose



Legenda: A figura exemplifica como a atividade física estimula a produção de IL-10 que participa da regulação da expressão de fatores inflamatórios. Também mostra como a AMPK inibe as vias da NF-κB e o TGF-β, atuando na regulação da resposta inflamatória e na fibrose.

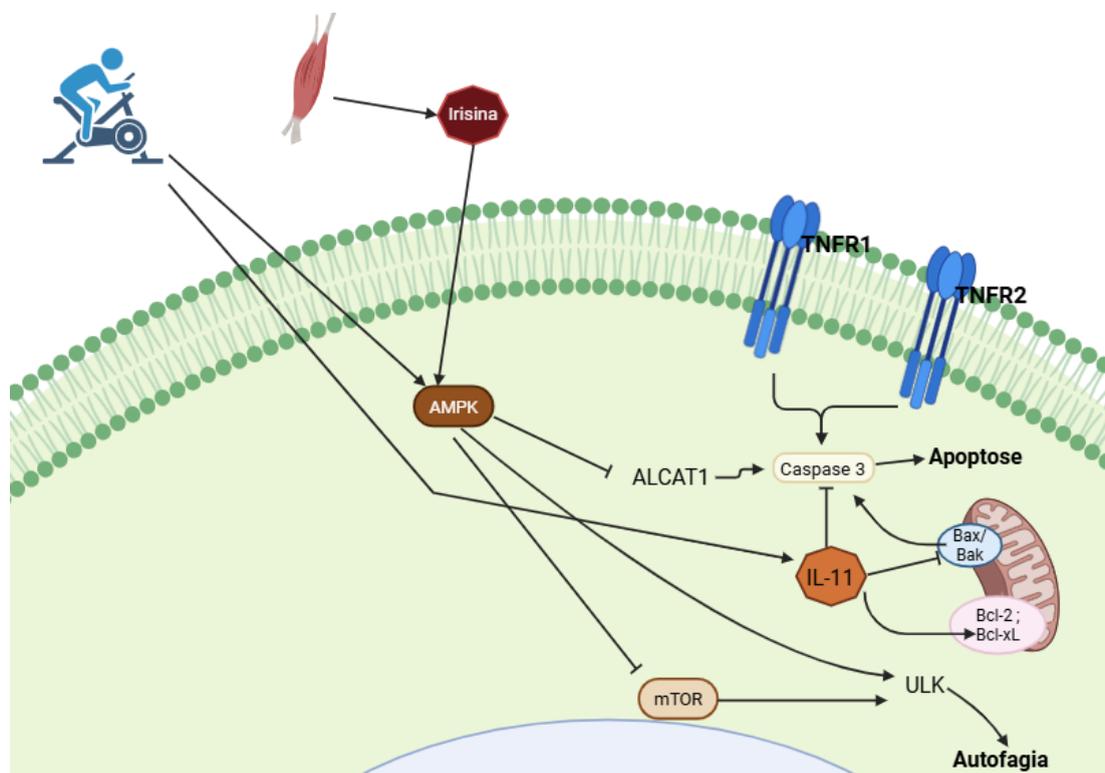
Fonte: elaborado pela autora (através do aplicativo Bio Render).

Também estimula a produção de irisina, que estimula a AMPK que, por sua vez, inibe o TGF-β e a ALCAT1, o primeiro responsável pelos efeitos fibróticos e o segundo relacionado à apoptose. Além disso, a ativação da AMPK pela ação da irisina também aumenta a expressão da unc-51 like kinase 1 (ULK1), levando ao início do processo de autofagia, e inibe o bloqueio ao ULK1 realizado pela via PI3K/Akt/mTOR. Outra citocina que é estimulada pela atividade física é a IL-11, que diminui a expressão de proteínas pró apoptóticas, como caspase-3, Bax e Bak, e aumenta expressão de proteínas anti apoptóticas, como Bcl2 e Bcl-xl (figura 3) (Malheiro *et al.*, 2024).

Considerando outros mecanismos, o exercício também proporciona alterações na hemodinâmica e excreção proteica renal. Sendo capaz de aumentar a fração de filtração, mesmo considerando a grande redução na TFG durante a injúria renal, o que leva a transferência de metabólitos e substâncias através da membrana glomerular. Também melhora o processamento tubular e taxa de excreção, aumenta a reabsorção de fluido

tubular, o que pode limitar o processo de desidratação. (Sabet; Soltani; Khaksari, 2022) Especificamente, o exercício físico resistido tem papel na regulação da síntese proteica, contribuindo para hipertrofia ao aumentar a área transversal das fibras musculares (Gould *et al.*, 2014).

Figura 3 - Mecanismos de apoptose e autofagia



Legenda: A figura exemplifica como a atividade física estimula a produção de IL-11, que inibe o processo de apoptose mediado por proteínas. Bem como o estímulo de irisina que ativa a AMPK e inibe a via mTOR, relacionados ao processo de autofagia.

Fonte: elaborado pela autora (através do aplicativo Bio Render).

Especificamente o exercício aeróbico parece não ser capaz de mitigar a resposta inflamatória provocada pelo LPS, pelo menos neste estudo realizado em 2020 em que o EF foi realizado pelo período de 4 semanas, onde foi encontrada piora da injúria renal induzida pelo TLR4 (Húngaro *et al.*, 2020).

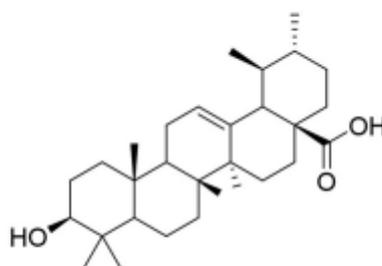
Assim, mesmo o exercício físico demonstrando potencial para ser uma intervenção de baixo custo com grande capacidade de modificar massa muscular, força e funcionamento físico. Ainda são necessários mais estudos para consolidar seus efeitos protetores sobre a recuperação após lesão renal aguda, determinar os mecanismos da ação nesse processo, e se, de fato, há possível redução no risco de progressão para DRC.

3.4 ÁCIDO URSÓLICO

Potenciais intervenções terapêuticas que surgem nos últimos anos incluem o uso de substâncias com papel nefroprotetor. O AU é uma substância que apresenta inúmeras atividades biológicas e benefícios potenciais, especialmente em distúrbios metabólicos, obesidade, doenças cardiovasculares, câncer e desordens neurológicas.

O AU é classificado como fitoquímico, ou seja, um metabólito secundário de plantas, assim chamado devido ao fato de ser encontrado em pouca quantidade e considerado um nutriente não essencial para os processos metabólicos em plantas. Os fitoquímicos englobam um grande número de componentes, estando o AU classificado no grupo dos triterpenóides (ácido carboxílico pentacíclico triterpenoide, cuja estrutura química pode ser observada na figura 4). É sintetizado a partir da via do ácido mevalônico, similar a hormônios esteróides em plantas e mamíferos. E é encontrado no revestimento ceroso de maçãs e outras frutas, alecrim e azeite (Nguyen *et al.*, 2021).

Figura 4 – Estrutura química do ácido ursólico



Ursolic acid

Fonte: adaptado de NGUYEN, et al (2021)

A ação anti-inflamatória do AU está relacionada a supressão de NF-κB, que é uma família de fatores de transcrição de regulam a expressão de genes envolvidos na

gênese tumoral, adesão de moléculas, quimiocinas, citocinas pró inflamatórias, genes e ciclos celular (Nguyen *et al.*, 2021). A ação do AU foi descrita como capaz de inibir a I κ B kinase e a fosforilação de p65 em várias linhagens celulares tumorogênicas, além de regular a lipoxigenase, MMP-9 e a iNOS. No entanto, também foi encontrado que o AU aumenta a liberação de IL-1 β em cultura de macrófagos peritoneais de camundongos, através do receptor CD36, que é responsável por mediar o processo de fagocitose e apoptose celular (Ikeda *et al.*, 2007).

Em estudos com animais, evidências sugerem diminuição de dor neuropática (Bhat *et al.*, 2016), efeito protetor contra doença renal induzida pelo diabetes (Zhou *et al.*, 2010), contra nefrotoxicidade induzida por cisplatina (Tripathi; Alshahrani, 2021) tetracloreto de carbono (Ma *et al.*, 2014) e gentamicina (Pai *et al.*, 2012). Mais recentemente, foi demonstrado efeitos na supressão de hiperglicemia, proteção da função renal, redução de ferroptose, e inativação da via JAK2/STAT3 em um modelo de nefropatia diabética (Zhou *et al.*, 2025).

Na população humana, no entanto, não há pesquisas avaliando especificamente a suplementação de AU e lesão renal. Em um estudo clínico duplo cego randomizado foi encontrado a remissão transitória de síndrome metabólica em 50% dos pacientes que receberam a intervenção (150 mg diários de AU), bem como redução de peso, IMC, circunferência abdominal, glicemia plasmática e aumento de sensibilidade insulínica, porém o estudo não encontrou mudanças na expressão de citocinas inflamatórias (Ramírez-Rodríguez *et al.*, 2017). Em outro ensaio clínico, a suplementação de AU ou placebo associado a treinamento resistido de alta intensidade em homens saudáveis, demonstrou aumento dos níveis plasmáticos de irisina, diminuição de proteína C reativa, IL-6 e TNF- α no grupo que recebeu suplementação (Asghari *et al.*, 2020).

Em suma, o AU se destaca como uma potencial intervenção terapêutica nefroprotetora, devido às suas diversas atividades biológicas e benefícios em distúrbios metabólicos, obesidade, doenças cardiovasculares, câncer e desordens neurológicas. O presente estudo visa aprofundar o entendimento sobre a capacidade do ácido ursólico de melhorar o perfil inflamatório na inflamação aguda induzida por LPS, consolidando seu potencial como uma intervenção terapêutica promissora para doenças renais e inflamatórias.

4 METODOLOGIA

4.1 ARTIGO DE REVISÃO

O primeiro resultado deste estudo foi delineado como uma revisão narrativa da literatura, com o objetivo de sintetizar as evidências atuais sobre o papel do exercício físico no perfil inflamatório da DRC. O levantamento bibliográfico foi realizado nas bases de dados eletrônicas PubMed, SciELO e Web of Science. A estratégia de busca utilizou a combinação de descritores relacionados à doença renal e mediadores inflamatórios, empregando os termos em inglês: 'renal injury' OR 'kidney' OR 'kidney disease' combinados pelo operador booleano AND com 'Interleukin' e suas variações específicas (ex: IL-6), bem como termos associados à sinalização purinérgica e exercício físico.

Para a seleção dos estudos, foram aplicados critérios de inclusão e exclusão visando identificar ensaios clínicos e modelos animais que abordassem protocolos de exercício (aeróbico, resistido ou combinado) e sua influência na modulação de citocinas pró e anti-inflamatórias. Inicialmente, as duplicatas foram removidas e os títulos e resumos foram analisados quanto à relevância temática. Os 33 artigos que atenderam integralmente aos critérios de elegibilidade foram lidos na íntegra e seus dados extraídos e sistematizados em tabelas comparativas. A análise dos resultados focou na interação entre o exercício físico e a modulação de marcadores inflamatórios correlacionando esses achados aos mecanismos da via de sinalização purinérgica.

4.2 DELINEAMENTO EXPERIMENTAL

Este estudo é oriundo de um projeto guarda-chuva desenvolvido em parceria com a UFSM. Para a realização do experimento foram utilizados 65 ratos de 200 a 230g (6 a 7 semanas de idade), advindos do Biotério Central da UFSM. Os animais foram ambientados durante sete dias. A alimentação ofertada diariamente com ração comercial, água a vontade e ambiente com temperatura e umidade controlada (22°-24°; em torno de 50% - 60% UR).

Os animais foram distribuídos aleatoriamente em oito grupos experimentais, conforme descrito abaixo:

- Grupo I (Grupo SAL – n=9): Os animais não praticaram exercício físico, receberam NaCl 0,9% por gavagem, durante seis semanas, e NaCl 0,9% intraperitoneal, durante oito dias;

- Grupo II (Grupo EF – n=8): Os animais foram submetidos a prática do exercício físico, receberam NaCl 0,9% por gavagem, durante seis semanas, e NaCl 0,9% intraperitoneal, durante oito dias;

- Grupo III (Grupo AU – n=8): Os animais não foram submetidos a prática de exercício físico, receberam AU (5mg/kg) por gavagem, durante seis semanas, e NaCl 0,9% intraperitoneal, durante oito dias;

- Grupo IV (Grupo EF + AU – n=8): Os animais foram submetidos a prática de exercício físico, receberam AU (5 mg/kg) por gavagem, durante seis semanas, e NaCl 0,9% intraperitoneal, durante oito dias;

- Grupo V (Grupo LPS – n=8): Os animais não foram submetidos a prática do exercício físico, receberam NaCl 0,9% por gavagem, durante seis semanas, e LPS (250µg/kg) intraperitoneal, durante oito dias;

- Grupo VI (Grupo LPS + EF – n=8): Os animais foram submetidos a prática de exercício físico, receberão NaCl 0,9% por gavagem, durante seis semanas, e LPS (250µg/kg) intraperitoneal, durante oito dias;

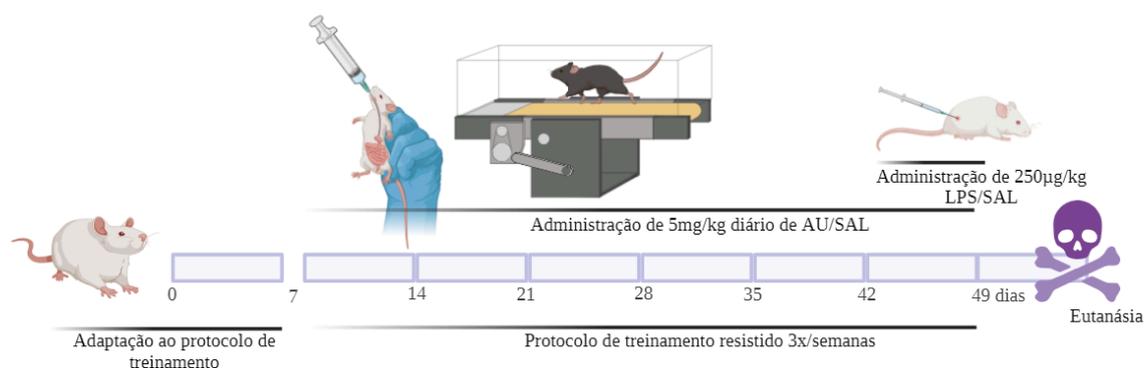
- Grupo VII (Grupo LPS + AU – n=8): Os animais não foram submetidos a prática do exercício físico, receberam AU (5 mg/kg) por gavagem, durante seis semanas, e receberam LPS (250 µg/kg) intraperitoneal, durante oito dias;

- Grupo VIII (Grupo LPS + EF +AU – n=8): Os animais foram submetidos a prática de exercício físico, receberam AU (5mg/kg) por gavagem, durante seis semanas, e receberam LPS (250 µg/kg) intraperitoneal, durante oito dias.

O protocolo de exercício resistido aconteceu durante o período de sete semanas, realizado três vezes por semana (segunda-feira, quarta-feira e sexta-feira) e está demonstrado na figura 5. Os animais foram pesados uma vez por semana durante todo o período do protocolo experimental. O momento da pesagem ocorreu antes do início da aplicação do protocolo de atividade física, utilizando uma balança digital SF-400 (Partyreal). Além disso, foi administrado o ácido ursólico (5mg/kg), antes do protocolo de exercício, diariamente, sete vezes por semana, durante seis semanas e o grupo controle recebeu NaCl 0,9% (SAL), por gavagem. Na última semana do protocolo de exercício físico, os animais receberam LPS (250 µg/kg) por via intraperitoneal ou então, NaCl 0,9% pela mesma via e foi aferido a temperatura e pesagem antes da aplicação do

LPS. A aplicação do LPS foi realizada pela manhã e após uma hora da aplicação do LPS, os animais foram submetidos à administração do AU. Este protocolo de tratamento baseia-se em um estudo já realizado e publicado por DORNELLES et al. (2020). Tendo em vista que o tempo de meia-vida do AU é de 4,5 horas (LIAO et al., 2005), o protocolo de treinamento físico foi realizado logo após a administração do ácido.

Figura 5: Desenho experimental



Fonte: (Elaborado pela autora através do aplicativo Bio Render).

Após a realização do delineamento experimental, os animais foram submetidos à eutanásia por aprofundamento anestésico inalatório com isofluorano. Eles foram acondicionados dentro de uma câmara de anestésico, na qual havia ambiente saturado de anestésico inalatório, e assim os animais não sentiam nenhum tipo de dor ou maus tratos, a morte foi confirmada através da ausência dos batimentos cardíacos e pela ausência dos movimentos respiratórios.

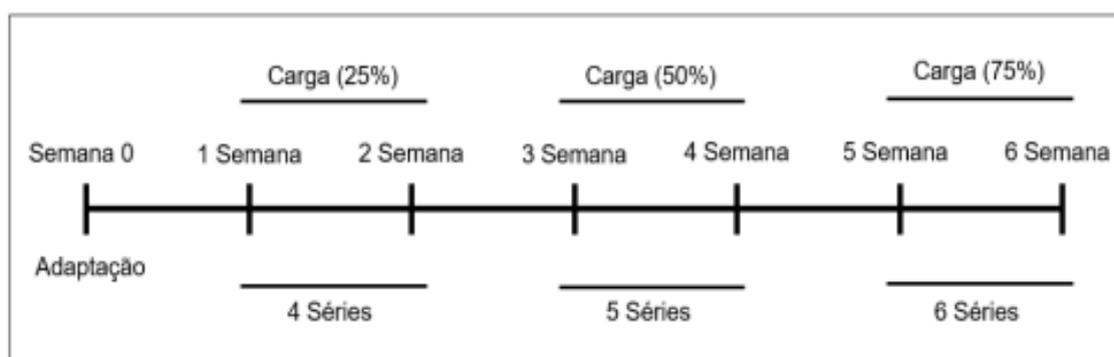
4.3 PROTOCOLO DE EXERCÍCIO FÍSICO

Os animais foram submetidos ao protocolo de exercício físico, de acordo com SCHEFFER et al. (2012) o qual foi adaptado, sendo considerado um protocolo de hipertrofia muscular. Para a realização do protocolo foi utilizada uma escada com 34 degraus com inclinação de 80°. Os ratos foram submetidos a um processo de adaptação com a duração de uma semana, sem acréscimo de peso. Foram colocados na parte

inferior da escada e incentivados a subir até o topo com um toque na cauda, seguido de período de repouso entre 30 e 60 segundos.

O treinamento foi realizado durante seis semanas mais uma semana inicial de adaptação, e a carga teve aumento progressivo que foi de 25% na primeira e segunda semana, 50% na terceira e quarta semana e 75% na quinta e sexta semana, a qual foi calculada de acordo com o peso do animal. Cada subida até o topo da escada foi contabilizada como uma repetição, no protocolo utilizado são 8 a 10 repetições, conforme as condições do animal, e um conjunto de séries que vai de 4 a 6 séries, com intervalo para descanso de dois minutos (Figura 6).

Figura 6 - Protocolo de exercício físico



Fonte: adaptado de Dornelles, 2020.

4.4 ÁCIDO URSÓLICO

Foi utilizado AU (Sigma-Aldrich) na dose de 5 mg/kg (BHAT et al., 2016; TRIPATHI; ALSHAHRANI, 2021), por via oral, uma vez ao dia, iniciado com o protocolo de exercício físico até o último dia. O tratamento teve duração total de quarenta e dois dias. O AU foi suspenso em solução salina e administrado via gavagem. A suspensão foi homogeneizada em vórtex antes da administração a cada animal para obtenção de uma solução homogênea. Esse protocolo de tratamento baseia-se em estudos anteriores com esse composto natural (BHAT et al., 2016; TRIPATHI; ALSHAHRANI, 2021).

4.5 LIPOPOLISSACARÍDEO

Para indução da resposta inflamatória sistêmica, na última semana do experimento foi utilizada injeção intraperitoneal diária de lipopolissacarídeos de *Escherichia coli* (Sigma-Aldrich, O55-B5) diluído em solução salina na dose de 250 µg/Kg, uma vez ao dia, durante oito dias. Esta dose foi selecionada de acordo com estudos prévios (Dornelles *et al.*, 2020; Salama; Elgohary; Fahmy, 2023).

4.6 COLETA DAS AMOSTRAS

Após o término do protocolo de treinamento, foi realizada a coleta do rim e do sangue por via intracardíaca. O rim foi armazenado imerso em RNAlater em microtubos e congelado a -80° para posterior análise de expressão gênica.

Foi coletado 7mL de sangue de cada animal. Para realização do hemograma, 1 mL de sangue foi acondicionado em tubo com EDTA, 2 mL de plasma com EDTA congelado para as posteriores dosagens de irisina, e o restante (4 mL) foi coletado em tubos sem anticoagulantes e centrifugado (3.200 r.p.m. por 10 minutos) para separação do soro para as análises bioquímicas (citocinas e creatinina).

4.7 PARÂMETROS ANALISADOS

4.7.1 Citocinas

Para a determinação dos níveis de citocinas foi utilizado o kit BD™ Cytometric Bead Array (CBA), específico para IL-2, IL-4, IL-6, IL-10, IL-17a, TNF- α e IFN- γ . Os parâmetros inflamatórios foram realizados em parceria com a Universidade Federal de Santa Maria (UFSM).

4.7.2 Análise dos níveis de Irisina

Para realizar a análise da dosagem de irisina, foi utilizado o kit de imunoenensaio enzimático (EK-067-29, Phoenix Pharmaceuticals, Burlin) a partir do plasma centrifugado. O kit é utilizado para detectar um conjunto específico de peptídeos, com base no princípio da competitividade do imunoenensaio enzimático. O kit já foi validado para verificar o nível de irisina (WEN et al., 2013; LEE et al., 2014) com uma gama de detecção de entre 0,1 e 1000 ng/ml. O plasma foi coletado em tubos EDTA sob gelo, centrifugado a 4°C, 3000 rpm por 10 minutos e posteriormente separado em 2 alíquotas (0,5 ml em cada) para ser armazenado a -20°C, até sua posterior análise.

4.7.3 Hemograma

A contagem de eritrócitos, leucócitos e a concentração de hemoglobina foram determinados utilizando contador automatizado (Mindray®). A determinação do hematócrito foi obtida em centrifuga de microhematócrito na rotação de 14.000 r.p.m por 15 minutos. O volume corpuscular médio (VCM) e a concentração de hemoglobina corpuscular média (CHCM) foram determinados por cálculos indiretos.

4.7.4 Extração de RNA das amostras biológicas

Foi utilizado o reagente Trizol para o isolamento de DNA, RNA e proteínas das amostras, conforme as especificações do fabricante. O RNA isolado foi tratado com a enzima DNase a fim de evitar a contaminação com DNA, e posteriormente estocado em freezer a -80 °C. As concentrações dos RNAs foram determinadas utilizando o

espectrofotômetro Nanodrop® (Thermo Scientific), e a partir delas foram preparadas soluções de trabalho. Aproximadamente 200 ng dos RNAs obtidos foram aplicados em gel de agarose 2,0% e corados com brometo de etídeo, para análise da integridade. A retrotranscrição de RNAm ou RNA total para a síntese de cDNA foi realizada com o kit "Platus Transcriber RNase H- cDNA First Strand" (Sinapse Inc).

4.7.5 Expressão gênica por RT-qPCR

As reações de RT-qPCR foram realizadas no equipamento QIAquant 96 5plex (Qiagen), com sistema de detecção por SYBR Green (Solis Biodyne).

As condições padrão de PCR foram as seguintes: 50 °C (2 min), 95 °C (10 min); 40 ciclos de 94 °C (30 s), 58 °C (30 s) e 72 °C (1 min); seguido por uma curva de desnaturação padrão. Os primers foram projetados usando o software Primer Express v2.0 (Applied Biosystems), com base nas sequências de nucleotídeos disponíveis no banco de dados GenBank. Platinum SYBR Green qPCR SuperMix UDG com reagente ROX (Invitrogen), 1 mg/ml de cada primer específico, e uma diluição de 1:20 de cDNA foram usados em cada reação. Os valores médios de Ct de medições duplicadas foram usados para calcular a expressão do gene alvo, com normalização para controles internos (GAPDH). Uma curva padrão de cada gene foi utilizada para calcular a quantificação absoluta. Os resultados são expressos em número de cópias do gene.

O principais genes que foram amplificados para verificação da sua expressão nas diferentes amostras biológicas são: receptor P2X7, receptor P2X6, receptor P2Y1 (P2Y1), receptor P2Y12 (P2Y12), receptor A1 (A1), receptor A2A (A2A), receptor A2B (A2B).

4.8 ANÁLISE ESTATÍSTICA

Os dados obtidos foram primeiramente testados quanto à normalidade por meio do teste de Shapiro-Wilk. Dados com distribuição normal foram expressos como média \pm desvio padrão e analisados por ANOVA de uma via com pós-teste de Tukey. Dados sem distribuição normal foram expressos como mediana e intervalo interquartil e analisados pelo teste de Kruskal-Wallis com pós-teste de Dunn. Foi considerado estatisticamente significativo dados com $p < 0.05$. Foi utilizado o software Graphpad Prism® 9.0 para análise dos dados.

4.9 ÉTICA EM PESQUISA

Este protocolo experimental tem aprovação da Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA-UFSM), protocolo nº 6805060821, de acordo com a Legislação em vigor e os princípios éticos publicados pela Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL) e pelo Colégio Brasileiro de Experimentação Animal (COBEA).

Os animais foram avaliados diariamente para garantir o bem-estar e a saúde deles, conforme a lei 1.095/2019. Também foram acondicionados em gaiolas com temperatura e umidade controladas, alimentação diária e água em livre acesso.

5 RESULTADOS

ARTIGO DE REVISÃO

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The role of physical exercise on inflammatory profile in chronic renal disease: An overview of the current literature

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ABSTRACT

Chronic kidney disease (CKD) is an irreversible and progressive clinical syndrome that affects more than 800 million people worldwide, and is caused by structural kidney damage. This condition is associated with increased systemic inflammation, which may be related to an imbalance of purinergic signaling. Physical exercise can modulate the purinergic system and inflammation, and has been used as a possible therapy to mitigate and prevent the progression of CKD; however, the specific effects that each physical activity protocol generates on the expression of pro- and anti-inflammatory cytokines are not known, nor is there clear evidence about the specific involvement of purinergic signaling components in mediating these inflammatory responses. By analyzing exercise protocols in patients with CKD and their impact on cytokine expression, this review discusses how different protocols affect inflammatory cytokine expression in CKD patients, with a particular focus on the mediating role of purinergic signaling.

1. Introduction

Chronic Kidney Disease (CKD) is a clinical syndrome that affects millions of people worldwide. A recent study found a pooled prevalence of 10 % in the population of 11 countries [1]. This disease arises due to lasting changes in the function and structure of the kidneys and is marked by irreversibility and gradual evolution over time. Its main causes include diabetes, hypertension, chronic glomerulonephritis, chronic pyelonephritis, prolonged use of anti-inflammatory drugs, autoimmune conditions, polycystic kidney disease, Alport's disease, congenital malformations and the persistence of acute kidney injuries [2].

Increased inflammatory activity is one of the main systemic consequences observed in CKD patients. Furthermore, it is believed that a sedentary lifestyle can contribute to CKD progression [3]. Therefore, physical exercise offers several benefits to patients affected by the disease, as it is recommended to incorporate regular physical activity from the early stages [4], as it modulates the inflammatory profile, leading to improvements in both physical condition and mental health, reduces the mortality rate and provides a higher quality of life for these individuals [5].

CKD patients manifest chronic systemic inflammation, commonly characterized by increased levels of pro-inflammatory cytokines [6]. They also present significant intramuscular inflammation, which is believed to play a significant role in the loss of skeletal muscle mass, known as sarcopenia, a common occurrence in this condition [7]. Therefore, the modulation of systemic and intramuscular inflammation has emerged as a fundamental strategy for improving outcomes in CKD. Physical exercise is the main therapeutic tool in this process.

Purinergic signaling is based on the action of extracellular nucleotides and nucleosides, such as adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), and adenosine (ADO), which act in modulating various physiological processes [8]. Under pathological conditions, such as the presence of inflammation, ATP release is triggered, particularly during cellular death [9]. Extracellular ADO, on the other hand, acts as a signal of tissue damage, inhibiting immune cells through a negative feedback loop to prevent further damage cascades [10]. When studying the effects of physical exercise, evidence suggests that exercise can alter nucleotide and nucleoside extracellular levels, modulating coagulation, inflammation, and vascular activity [11], which can positively influence CKD.

Various exercise approaches can play a crucial role in reducing the

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chronic inflammation associated with CKD. A recent review addressed the benefits of moderate physical activity in acute kidney disease [12]. However, this review addresses a gap in the literature regarding the effects of physical exercise on the inflammatory parameters of the disease, which demonstrates the action of the main cytokines present in CKD, and seeks to relate these findings to purinergic signaling. The most recent review that analyzed the subject covered only four inflammatory markers - Interleukin 10 (IL-10), Interleukin 6 (IL-6), Tumor Necrosis Factor alpha (TNF- α), and C-Reactive Protein (CRP) [13], making our work a pioneer in analyzing other inflammatory markers. Given this background, the present study aims to review the current literature on the role of physical exercise in modulating purinergic signaling and inflammatory profiles in CKD focusing on eight pro-inflammatory cytokines: IL-6, TNF- α , Interleukin-1 β (IL-1 β), Interleukin-17a (IL-17a), Interleukin 8 (IL-8), Interleukin-18 (IL-18), Interleukin-12 (IL-12) and CRP; and three anti-inflammatory cytokines: IL-10, Interleukin 4 (IL-4), Interleukin-15 (IL-15). We also discuss exercise protocol types and their therapeutic effects related to inflammation in this disease.

2. Inflammation and chronic kidney disease

CKD is a growing global health problem [14]. It is characterized by persistent abnormalities in kidney structure or function lasting more than three months, which compromise renal function and overall physiological balance [4].

The role of inflammation in CKD has been studied since the late 1990s, mainly due to the release of interleukin-1 (IL-1), a pro-inflammatory cytokine, which has been linked to an increased mortality rate in patients undergoing chronic dialysis [15]. This interest is justified by the kidneys' fundamental role in maintaining body homeostasis; their dysfunction and cellular damage can ultimately result in organ failure [16].

There is a specific cytokine pattern in patients with CKD, with an increase in pro-inflammatory markers IL-1, IL-6, and TNF- α , and a decrease in anti-inflammatory mediators such as IL-2, IL-4, IL-5, IL-12, complement activity (CH50), and T-cell counts. Patients with this profile have a lower survival rate than patients with normal cytokine levels [17].

Monocytes and endothelial cells are major producers of cytokines, contributing to a pro-inflammatory environment in CKD. In addition, cytokines promote matrix metabolism, proliferation of resident cells, and procoagulant activity of the kidney endothelium [18]. They also mediate some effects through acute-phase proteins, such as CRP, fibrinogen, and albumin [19].

Therefore, systemic inflammation in kidney disease represents a risk factor for mortality and increases the chances of patients developing other complications, such as sarcopenia, vascular calcification, and other vascular disorders, along with depression, osteoporosis, frailty [20], and an increased risk of cardiovascular diseases [21].

3. Physical exercise, inflammation, and chronic kidney disease

Physical exercise has been increasingly investigated as a non-pharmacological strategy to manage CKD-related complications. Regular physical exercise may counteract these effects by enhancing physical functioning, increasing muscle mass, and reducing systemic inflammation [22].

Impaired kidney function in CKD leads to the accumulation of nitrogenous substances in the bloodstream that should be excreted in the urine, resulting in a state of uremic inflammation [23]. This condition activates the innate immune system [24], contributing to systemic inflammation through the increased production of chemokines and pro-inflammatory cytokines [23]. This chronic inflammatory state is a hallmark of cellular senescence [25], which aggravates systemic inflammatory damage [23].

Physical exercise has been shown to modulate intramuscular

inflammation, which is accentuated in CKD and may play a central role in skeletal muscle atrophy [26]. Patients with CKD typically exhibit reduced physical function and exercise capacity [27], which are both associated with an increased risk of mortality and comorbidities [28]. It is currently understood that exercise acts as an anti-inflammatory by reducing adipokines through changes in IL-6 levels [29]. However, whether similar mechanisms operate in patients with CKD remains unclear [26].

Different exercise protocols, including aerobic, resistance and a combination of both, have been investigated as therapeutic strategies for CKD, with the aim of evaluating their effects across a range of intensities and physiological outcomes. Aerobic exercise is characterized by training that improves cardiorespiratory fitness by increasing the efficiency of aerobic energy production, through the mobilization of large muscle groups and a rhythmic sequence. Resistance training, in contrast, focuses on improving muscular strength and endurance, typically through the use of weights or resistance machines [30].

Although aerobic exercise provides benefits, it does not significantly induce muscle hypertrophy, a critical factor for preserving muscle mass in CKD patients. In contrast, resistance training enhances protein synthesis and increases the cross-sectional area of muscle fibers [22]. It is important to note that physical exercise is a cost-effective intervention capable of improving muscle mass, strength, and physical performance [28]. Nevertheless, further studies are needed to better characterize the specific benefits of each type of exercise and to identify the most effective protocols for CKD patients.

4. Physical exercise, purinergic signaling and CKD

The purinergic system involves signaling mediated by ATP, ADP, and ADO, which act through specific receptors (P1 and P2) expressed in various cells and tissues [8]. Activation of P2X and P2Y receptors - which respond to purines and pyrimidines - as well as P1 ADO receptors, play a critical role in regulating inflammatory, immune, and vascular processes [31]. Chronic inflammation and oxidative stress are key factors in the pathogenesis of CKD [32]. Evidence suggests that the purinergic system, particularly through ADO, contributes to the regulation of renal function and CKD progression [33].

ADO, a crucial component of the purinergic system, is associated with the modulation of renal blood flow and the inflammatory response in the kidneys. In experimental CKD models, ADO has demonstrated both protective and detrimental effects, depending on the physiological context [34]. For instance, activation of A1 receptors may lead to vasoconstriction and a reduction in glomerular filtration rate (GFR), while activation of A2A receptors has anti-inflammatory effects and may be beneficial under certain conditions [35].

Inflammation is one of the main pathological mechanisms in CKD, and the purinergic system plays a fundamental role in this process, as illustrated in Fig. 1. Nucleotides, such as ATP, are released in response to cellular damage and stress, acting as danger-associated molecular patterns (DAMPs). These molecules bind to purinergic receptors on immune cells and trigger inflammatory responses [35]. For example, activation of P2X7 receptor promotes the release of pro-inflammatory cytokines such as IL-1 β and TNF- α , exacerbating renal inflammation [36]. Conversely, ADO, by binding to A2A receptors, can inhibit the release of inflammatory cytokines and exert anti-inflammatory effects, demonstrating a dual role depending on the specific receptor involved and the cellular microenvironment [37]. Fig. 1 demonstrates this mechanism.

Additionally, purinergic signaling contributes to tissue remodeling and fibrosis. Elevated levels of extracellular ATP, ADP, uridine triphosphate (UTP), and uridine diphosphate (UDP), along with heightened activity of P2 receptors - particularly in fibroblasts - stimulate extracellular matrix production [38].

Physical exercise is widely recognized for its benefits for overall health and specifically on CKD [39], furthermore, it may influence purinergic signaling pathways. Studies indicate that exercise may

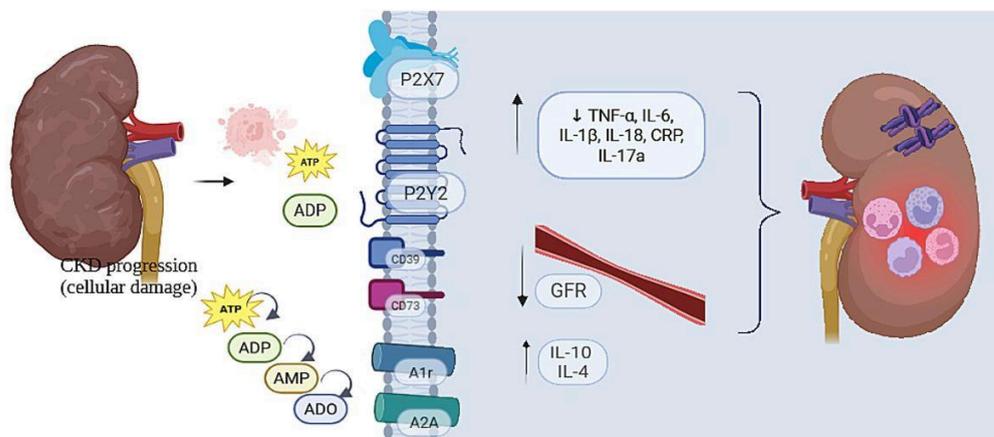


Fig. 1. - Schematic representation of how chronic kidney injury impacts purinergic signaling and contributes to inflammation. Cellular damage associated with CKD progression leads to the extracellular release of danger-associated molecular patterns (DAMPs), such as ATP and ADP. These nucleotides activate purinergic receptors (P2X7, P2Y2) on immune and renal cells, triggering pro-inflammatory signaling and the release of cytokines such as TNF- α , IL-6, IL-1 β , IL-18, CRP, and IL-17a. ATP and ADP are sequentially hydrolyzed by ectonucleotidases CD39 and CD73 into AMP and adenosine (ADO). ADO interacts with P1 receptors, such as A1 and A2A, mediating vasoregulatory and anti-inflammatory responses. While A1 receptor activation reduces glomerular filtration rate (GFR) through vasoconstriction, A2A activation promotes the release of anti-inflammatory cytokines like IL-10 and IL-4. The balance between ATP- and ADO-mediated signaling regulates renal inflammation and fibrosis, playing a critical role in CKD progression. This pathway underscores the critical role of purinergic signaling in orchestrating inflammatory responses that drive CKD progression. (Source: prepared by the authors.)

increase the release of ATP and ADO, thereby modulating inflammation and oxidative stress, both of which are critical components in CKD progression [40].

The interplay between the purinergic system and CKD is complex and multifaceted. The effects of adenosine and other nucleotides on renal function vary according to the receptors activated and the physiological context [35]. Notably, experimental models have shown that modulation of purinergic signaling, through P2X7 receptor antagonists or A2A receptor agonists, can attenuate inflammation and improve renal outcomes [41]. This suggests that targeting the purinergic system may be a promising therapeutic strategy to mitigate inflammation and slow CKD progression. Given the association between CKD, chronic inflammation, and altered purinergic signaling, the modulatory effects of exercise on these pathways support its potential as a complementary therapeutic strategy [41]. Regular physical activity may help manage CKD by reducing inflammation and enhancing purinergic signaling regulation [40].

Taken together, these findings highlight the potential of physical exercise as a modulator of purinergic signaling in CKD inflammatory regulation. While the underlying mechanisms remain to be fully elucidated, current evidence suggests that exercise may help mitigate inflammation and support renal health through these pathways. The following tables (Tables 1, 2 and 3) summarize the main findings from pre-clinical and clinical studies addressing this relationship.

5. The modulatory role of physical exercise on the pro-inflammatory profile and its relationship with purinergic signaling

5.1. IL-6

Interleukin 6 (IL-6) is a cytokine synthesized by almost all cell types, including monocytes and renal mesangial cells [46]. It plays an important role in metabolism and the regulation of inflammatory responses [73]. The inflammation cascade begins with the binding of circulating IL-6 to its soluble receptor (sIL-6R), which then interacts with gp130, a transmembrane glycoprotein expressed in all kidney cells. This IL-6

trans-signaling pathway is a key mechanism in triggering inflammation [74].

CKD is characterized by persistent systemic inflammation, leading to elevated levels of inflammatory cytokines, including IL-6. This increase is associated with impaired kidney function, dialysis treatment, or both. Since most dialysis patients have difficulty performing physical activities, evidence has shown both that aerobic and resistance exercise can reduce low-grade inflammation in this population [68].

Among the 33 articles selected for review, 27 analyzed IL-6: 19 studies used human subjects [21,26,44–48,54,56,61–64,66,68,70–73], and 8 used animal models [49,51,57,58,60,65,67,75]. Of these, 13 applied aerobic exercise protocols (Table 1) [21,45–49,51,54,56,58,60,72,75], 10 used resistance training (Table 2) [61–69,73] and 4 combined both modalities (Table 3) [26,70–72].

Eleven studies observed reduction in IL-6 following regular exercise interventions [46,51,54,56,60,61,64,66,68,70,75]. In contrast, two studies that assessed IL-6 after a single acute session of exercise found transient increases [45,47]. For example, in pre-dialysis CKD patients, a single 30-min session of aerobic exercise led to an immediate elevation in IL-6 levels [46]. A similar response was observed in children with CKD, where IL-6 increased after a 20-min aerobic session [47].

This initial rise in IL-6 is attributed to the activation of the “classic” IL-6 signaling pathway, in which IL-6 binds to its membrane-bound receptor (IL-6R), found in specific cell types such as renal cells [74]. This response appears to be linked to post-exercise metabolic adjustments, particularly those triggered by muscle glycogen depletion [29]. The classic pathway helps restore homeostasis by regulating glucose metabolism, and IL-6 levels typically return to baseline within an hour after exercise.

Simultaneously, acute exercise induces the release of anti-inflammatory cytokines such as interleukin 10 and IL-1 receptor antagonist (IL-1RA), along with cortisol from the adrenal glands [29]. During muscle contraction, IL-6 is produced through a TNF- α -independent pathway, acting as a myokine involved in metabolic regulation induced by exercise.

The transient rise in IL-6 is followed by an increase in anti-inflammatory cytokines, which in turn inhibit the production of TNF-

Table 1
Effects of resistance training on inflammatory profile in CKD.

Study	Design	Exercise Protocol	Pro-inflammatory Markers	Anti-inflammatory Markers
Asghar et al. 2007 [42]	Animal study: Male Fischer rats, aerobic vs. sedentary	Aerobic training (60 min/session, 5×/week, 12 weeks)	–	↑ IL-10
Ghosh et al. 2009 [43]	Animal study: 20 diabetic db/db and WT mice, exercised vs. sedentary	Aerobic training (60 min/day, 5×/week, 7 weeks)	↓ TNF-α	–
Wilund et al. 2010 [44]	Human clinical trial: 17 patients on hemodialysis (HD), aerobic vs. control	Aerobic cycling (progressive up to 45 min/session, 3×/week, 16 weeks)	= IL-6, = CRP	–
Van Craenenbroeck et al. 2014 [45]	Human acute study: 55 individuals (CKD, CHF, healthy), pre-post single session	Acute aerobic (8–10 min session)	↑ IL-6	–
Viana et al. 2014 [46]	Human clinical trial: 40 CKD patients, home-based walking vs. control	Aerobic training (30 min/session, 5×/week, 24 weeks)	↓ IL-6	↑ IL-10
Lau et al. 2015 [47]	Human study: 9 children/adolescents with CKD, pre-post single session	Acute aerobic (20 min at 50 % VO ₂ peak)	↑ IL-6, ↓ TNF-α	–
Peres et al. 2015 [48]	Human clinical trial: 9 CKD patients on hemodialysis, pre-post acute intervention	Acute aerobic (20 min during hemodialysis)	= IL-6, = TNF-α, ↑ IL-17	↑ IL-10
Shing et al. 2015 [49]	Animal study: 40 ApoE knockout mice, 4 groups (control, nephrectomy, with/without exercise)	Voluntary wheel running (12 weeks)	↑ IL-6, = TNF-α	= IL-10
Passos et al. 2016 [50]	Animal study: 20 hypertensive rats, 4 groups (sedentary/exercised ± extract)	Aerobic training (60 min/session, 5×/week, 8 weeks)	= IL-1β	↑ IL-10
Aqel et al. 2017 [51]	Animal study: Mice with lupus nephritis mutation and controls, 2 groups (aerobic vs. sedentary)	Aerobic training (45 min/session, 7×/week until terminal stage)	↓ IL-6, ↓ TNF-α, ↑ IL-1β	↓ IL-10
Dungey et al. 2017 [52]	Human clinical trial: 38 HD patients, exercise vs. usual care, with 16 healthy controls	Aerobic training (30 min/session, 3×/week, 24 weeks)	= IL-6, = TNF-α, = CRP	–
Huang et al. 2018 [53]	Animal study: 24 hypertensive rats, exercise vs. sedentary	Aerobic training (60 min/session, 5×/week, 12 weeks)	↓ IL-6	–
Ikizler et al. 2018 [54]	Human clinical trial: 111 CKD patients, 4 intervention groups	Aerobic training (30–45 min/session, 3×/week, 16 weeks)	↓ IL-6	–
Neves et al. 2021 [55]	Animal study: 40 Zucker rats, 4 groups (obese/lean ± NOS block ± aerobic)	Aerobic training (10 min/session, 5×/week, 2 weeks)	↑ IL-18, ↓ TNF-α, ↑ IL-17, ↓ CRP	↑ IL-10, ↑ IL-4
Aldahr et al. 2022 [56]	Human clinical trial: 80 obese T2D patients, aerobic vs. control	Aerobic training (40 min/session, 12 weeks)	↓ IL-6, ↓ TNF-α	↑ IL-10
Brito et al. 2022 [57]	Human clinical trial: 18 CKD patients, aerobic vs. control	Intradialytic aerobic (45 min/session, 3×/week, 12 weeks)	↓ TNF-α	–
da Silva et al. 2022 [58]	Animal study: 40 male Zucker rats, 5 groups (control, Metabolic Syndrome (MetS), MetS + Okra, MetS + Exercise Training, MetS + Exercise Training + Okra)	Aerobic training (60 min/session, 5×/week, 6 weeks)	↑ IL-6*, ↓ TNF-α	↑ IL-10
Highton et al. 2022 [59]	Human clinical trial: 40 HD patients, aerobic vs. control	Intradialytic aerobic (30 min/session, 3×/week, 24 weeks)	= IL-2, = IL-17	= IL-10
Zhou et al. 2022 [60]	Animal study: 30 diabetic and non diabetic mice, 3 groups (control, diabetic model, aerobic)	Aerobic training (60 min/session, 5×/week, 8 weeks)	↓ IL-6, ↓ TNF-α, ↓ IL-18, ↓ IL-1β	–

Effects of aerobic exercise protocols on pro-inflammatory and anti-inflammatory markers.

HD: hemodialysis; MetS: metabolic syndrome; IL-6: interleukin 6; TNF-α: tumor necrosis factor alpha; IL-10: interleukin 10; IL-18: interleukin 18; IL-1b: interleukin 1 beta; IL-17: interleukin 17; IL-15: interleukin 15; IL-4: interleukin 4; PCR: C-reactive protein; IL-12: interleukin 12; IL-2: interleukin 2. =: no alteration found in the study. †: elevation of the marker. ‡: decrease of the marker. *only in the MetS + ET + O group.

Table 2
– Effects of aerobic training on inflammatory profile in CKD.

Study	Design	Exercise Protocol	Pro-inflammatory Markers	Anti-inflammatory Markers
Castañeda et al. 2004 [61]	Human clinical trial: 26 CKD patients, resistance + low-protein diet vs. control	Resistance training (35 min/session, 3×/week, 12 weeks)	↓ IL-6, ↓ CRP	–
Cheema et al. 2011 [62]	Human clinical trial: 49 HD patients, resistance vs. control	Intradialytic resistance (2 sets of 10 exercises, 3×/week, 12 weeks)	= TNF-α, = IL-1β, = IL-6, = IL-8, = IL-12	= IL-10
Moraes et al. 2014 [63]	Human study: 37 HD patients, no control group	Resistance training (120 min/session, 3×/week, 24 weeks)	= IL-6, = TNF-α, ↓ CRP	–
Watson et al. 2017 [64]	Human clinical trial: 38 CKD patients, resistance vs. control	Resistance training (3 sets of 10–12 reps, 3×/week, 8 weeks)	↓ IL-6, ↓ TNF-α	↑ IL-15
Souza et al. 2018 [65]	Animal study: 15 male Wistar rats, CKD vs. control ± training	Resistance training (12 min/session, 3×/week, 8 weeks)	↑ IL-6	↑ IL-10, ↑ IL-4
Corrêa et al. 2021 [66]	Human clinical trial: 105 patients, 3 groups (control, resistance, resistance + blood flow restriction)	Resistance training (alternating upper/lower limbs, 3×/week, 24 weeks)	↓ IL-6, ↓ IL-18, ↓ TNF-α, ↓ IL-17	↑ IL-10, ↑ IL-15
de Alcantara Santos et al. 2021 [67]	Animal study: 32 male Wistar rats, diabetic and non-diabetic ± training	Resistance training (8 ladder climbs/day, 5×/week, 8 weeks)	= IL-6, = TNF-α, ↓ IL-1β	↓ IL-10
Gadelha et al. 2021 [68]	Human clinical trial: 107 elderly HD patients, resistance vs. sedentary	Resistance training (40 min/session, 3×/week, 24 weeks)	↓ IL-6, ↓ TNF-α	↑ IL-10
Saud et al. 2021 [69]	Animal study: 40 male Wistar rats, 6 groups (sham, nephrectomized ± resistance pre/post)	Resistance training (8–12 reps/day, 5×/week, 8 weeks)	= IL-6, ↓ TNF-α	= IL-10

Effects of resistance training protocols on pro-inflammatory and anti-inflammatory markers. HD: hemodialysis; IL-6: interleukin 6; TNF-α: tumor necrosis factor alpha; IL-10: interleukin 10; IL-18: interleukin 18; IL-1b: interleukin 1 beta; IL-17: interleukin 17; IL-15: interleukin 15; IL-4: interleukin 4; PCR: C-reactive protein; IL-12: interleukin 12; IL-2: interleukin 2. =: no alteration found in the study. †: elevation of the marker. ‡: decrease of the marker.

Table 3
– Effects of combined training on inflammatory profile in CKD.

Study	Design	Exercise Protocol	Pro-inflammatory Markers	Anti-inflammatory Markers
Meléndez-Oliva et al. 2022 [70]	Human clinical trial: 71 HD patients, intradialytic vs. home-based combined training	Combined aerobic + resistance (30 min each, 3×/week, 16 weeks)	↓ CRP, ↓ IL-6, = TNF-α	–
Oliveros et al. 2011 [71]	Human clinical trial: 15 HD patients, combined training vs. control	Combined aerobic + resistance (30 min each, 3×/week, 16 weeks)	= TNF-α, = IL-6, = CRP	–
Figueiredo et al. 2018 [72]	Human clinical trial: 37 CKD patients, 3 groups (inspiratory, aerobic, combined)	Combined training (35 min/session, 3×/week, 8 weeks)	= IL-6	–
Watson et al. 2022 [26]	Human study: 84 CKD and non-CKD patients, aerobic and combined training	Aerobic + combined (30 min/session, 3×/week, 12 weeks)	= IL-6, ↑ TNF-α	–

Effects of combined exercise protocols on pro-inflammatory and anti-inflammatory markers. HD: hemodialysis; IL-6: interleukin 6; TNF-α: tumor necrosis factor alpha; IL-10: interleukin 10; IL-18: interleukin 18; IL-1β: interleukin 1 beta; IL-17: interleukin 17; IL-15: interleukin 15; IL-4: interleukin 4; PCR: C-reactive protein; IL-12: interleukin 12; IL-2: interleukin 2. =: no alteration find in the study. †: elevation of the marker. ‡: decrease of the marker.

α. This supports the hypothesis that IL-6 may play a compensatory, rather than purely pro-inflammatory, role in exercise-induced immune modulation [76].

On the other hand, some studies on chronic exercise reported no change in IL-6. For instance, resistance training in rats for 8 weeks using vertical ladder climbing showed no significant alteration in IL-6 levels [67]. Likewise, an 8-week combined exercise protocol in rats (sair climbing with and without weight) found no difference between experimental and control groups [69].

However, most studies employing chronic exercise protocols lasting more than 8 weeks observed a reduction in IL-6. In nephrectomized rats, 12 weeks of aerobic exercise led to reduced IL-6 levels [49], while 10 weeks of resistance training had a similar effect [75]. An 8-week aerobic treadmill program in CKD mice with a more intensive intervention, 5 sessions per week lasting 60 min each, also reduced IL-6 [60].

In human studies, 12 weeks of combined aerobic and resistance training performed 3 times per week did not significantly affect in IL-6 levels [26]. On the other hand, a 16-week protocol combining both modalities, with 60-min sessions three times per week, showed a significant reduction in IL-6 [70].

In this context, recent studies suggested that purinergic signaling may be one of the molecular mechanisms underlying the modulation of IL-6 by physical exercise. The ATP released during muscle contraction activates P2X and P2Y receptors, promoting IL-6 secretion by immune cells such as macrophages, thereby amplifying inflammation. Conversely, adenosine, a product of ATP hydrolysis, binds to A2A and A2B receptors and suppresses IL-6 production, exerting an anti-inflammatory effect [77–79]. Studies have shown that physical exercise reduces the expression of the P2X7 receptor and increases

extracellular adenosine levels, both of which may contribute to the observed decreases in IL-6 levels following chronic exercise in CKD models [80]. These findings support the hypothesis that the beneficial effects of exercise on IL-6 may, at least in part, be mediated by purinergic signaling mechanisms.

Taken together, the evidence suggested that to effectively reduce IL-6 in CKD patients, exercise must be maintained for at least 16 weeks at a sufficient frequency. However, further research is needed to determine the optimal duration and intensity of physical exercise for therapeutic use in CKD. It is also important to mention the dual role of IL-6, which may function both as a pro-inflammatory cytokine and a myokine with anti-inflammatory effects depending on the context.

5.2. TNF-α

Tumor Necrosis Factor alpha (TNF-α) is a pro-inflammatory cytokine belonging to the TNF superfamily, primarily secreted by macrophages and monocytes [81]. It is known for its inflammatory activity, and derives its name from its ability to induce rapid regression in some types of tumors. Beyond its cytotoxic effects, TNF-α activates several intracellular signaling cascades, including those regulating the transcription of other cytokines such as IL-1, IL-2, IL-6, and TNF-α itself [82].

Elevated levels of TNF-α are associated with the aggravation of chronic systemic inflammation, a characteristic of CKD patients [64]. The anti-inflammatory potential of physical exercise has been studied as a strategy to mitigate this process. Among the 33 articles selected for this review, 21 assessed TNF-α: 8 in animal models [43,49,51,55,58,60,67,69], and 14 in human trials [26,47,48,52,56,57,62–64,66,68,70,71]. Of these, 11 protocols applied aerobic exercise protocols (Table 1) [43,47–49,51,52,55–58,60], 7 investigated resistance training (Table 2) [62–64,66–69], and 2 employed combined aerobic and resistance exercises in hemodialysis patients (Table 3) [70,71]. A single study evaluated aerobic exercise separately and its combination with resistance exercise [26]. Notably, only 2 studies addressed acute exercise's effect [47,48], and the majority focused on chronic interventions.

When analyzing the studies that applied aerobic exercise protocols in animal models, the results were heterogeneous. In a study investigating diabetic nephropathy, the leading cause of CKD, diabetic mice performing motorized wheel activity for one hour, five days a week over eight weeks. Compared to sedentary controls, the exercised group showed a reduction in TNF-α levels [60]. Similar findings were observed in a trial comparing involving obese Zucker rats, where aerobic exercise reduced TNF-α concentrations relative to sedentary obese counterparts [55].

A separate study using a treadmill protocol (45 min per day for eight weeks) in a murine model of CKD induced by social stress reported that moderate aerobic exercise decreases TNF-α levels, providing protective effects in experimental lupus nephritis [51]. Additionally, aerobic protocols applied to diabetic rats for eight weeks [60] and type-2 diabetic nephropathy patients for three months [56] consistently decrease TNF-α concentrations.

Conversely, a six-week aerobic protocol produced a reduction in TNF-α only in the metabolic syndrome group, with no changes observed in the other exercise groups [58]. Furthermore, a voluntary exercise protocol in nephrectomized mice, designed to simulate CKD, failed to induce a reduction in TNF-α levels, potentially due to the insufficient training volume achieved by the animals [49].

The acute effects of aerobic exercise on TNF-α levels in individuals with CKD have been investigated through intradialytic sessions performed on a cycle ergometer. Some studies reported a reduction in TNF-α concentrations following a single exercise session in children, adolescents, and adults with CKD [47,48]. However, the acute inflammatory response to exercise remains controversial, with variations likely related to differences in exercise load, intensity, and patient fitness status [83].

Regarding chronic aerobic interventions, one study using a bicycle ergometer protocol in CKD patients (45-min sessions, three times per week for three months), observed inverse correlation between improved and reduced plasma TNF- α levels in the exercised group [57]. In contrast, two other trials employing six months of intradialytic cycling (30 min per session) reported no significant changes in cytokine levels [52,59]. These findings may reflect the inability of many hemodialysis patients to achieve the exercise intensity necessary to elicit systemic anti-inflammatory adaptations at rest [59].

The effects of resistance training on TNF- α levels in CKD are controversial. A study involving 12 weeks of intradialytic progressive resistance training found no significant changes in cytokine concentrations compared to the control group [84]. Similarly, a six-month intervention with 71 resistance sessions reported no alterations in inflammatory markers [63], corroborating the hypothesis that hemodialysis patients often do not reach the intensity threshold necessary to modulate systemic inflammation through exercise alone.

In contrast, a study involving elderly hemodialysis patients who performed progressive resistance training three times per week for 24 weeks observed a significant reduction in TNF- α levels [68]. Another trial in non-dialyzed CKD patients applied an eight-week resistance training program and noted an initial increase in TNF- α after the first session, followed by a reduction after the complete training period [64]. Similar results were obtained in a protocol involving combined upper and lower limb resistance exercises, performed three times per week over 24 weeks, which also demonstrated a decrease in TNF- α [66].

Conversely, in an animal model study, diabetic and non-diabetic rats subjected to resistance training (ladder climbing with progressive loads) for eight weeks showed no significant differences in TNF- α levels between groups [67], contradicting the previously mentioned human trials.

Finally, only a few studies have evaluated the effects of combined aerobic and resistance exercise on TNF- α levels in CKD patients. One study conducted with hemodialysis patients randomized participants into two groups: one performing intradialytic combined training and the other following a home-based exercise program, both for four months. Each session lasted 60 min, comprising 30 min of resistance and 30 min of aerobic activity. No significant changes in TNF- α levels were observed in either group after the intervention [70]. A similar result was reported in another study involving a 16-week combined protocol of cycling and intradialytic resistance exercises, which also showed no alterations in TNF- α concentrations [71].

Additionally, a trial that assessed both aerobic exercise alone and combined aerobic and resistance training in non-dialyzed CKD patients over 12 weeks found no reduction in systemic inflammation in any of the intervention groups, a finding that may be attributed to insufficient exercise intensity [26].

In addition to the direct effects of physical exercise on TNF- α concentrations, emerging evidence suggests that purinergic signaling plays a pivotal role in modulating this cytokine. During muscle contraction and cellular stress, extracellular ATP is released, activating P2X and P2Y receptors, which can amplify pro-inflammatory responses. However, following ATP hydrolysis by ectonucleotidases such as Nucleoside Triphosphate Diphosphohydrolase (NTPDase) and 5'-nucleotidase, adenosine is produced, exerting predominantly anti-inflammatory effects via the A2A, A2B, and A3 receptors. Specifically, the activation of these adenosine receptors on macrophages has been shown to suppress TNF- α production, contributing to the attenuation of systemic inflammation [85]. This purinergic mechanism appears to be responsive to physical exercise, as studies report increased enzymatic activity of 5'-nucleotidase and higher adenosine levels post-exercise, correlating inversely with TNF- α concentrations [77]. Therefore, the anti-inflammatory benefits of aerobic and resistance exercise observed in CKD patients and experimental models may be partly mediated by exercise-induced modulation of purinergic signaling pathways, particularly through the downregulation of ATP availability and the

upregulation of adenosine-mediated anti-inflammatory signaling.

Based on the available evidence, it appears that both aerobic and resistance training protocols tend to reduce TNF- α levels in CKD patients and animal models, with the exception of intradialytic training protocols, which frequently fail to induce significant changes. Conversely, combined aerobic and resistance exercise protocols consistently show no effect on TNF- α concentrations. These findings suggest that exercise modality and training context, particularly the exercise intensity and the hemodialysis condition, are key factors influencing the inflammatory response. Further studies are warranted to better elucidate the mechanisms underlying TNF- α modulation by different types of exercise and to determine optimal training parameters for this population.

5.3. IL-1 β

Interleukin-1 β (IL-1 β) is a pro-inflammatory and pro-atherogenic cytokine involved in the pathogenesis of chronic inflammation and organ dysfunction, including kidney disease. Its main sources of secretion are B lymphocytes, monocytes, macrophages, neutrophils, dendritic cells, natural killer cells, and keratinocytes [86]. In CKD, macrophages play a key role in producing IL-1 β and other cytokines that contribute to renal inflammation and promote extracellular matrix deposition, ultimately leading to renal fibrosis. This process can be mitigated by the modulatory effects of physical activity on the inflammatory system, as exercise reduces the expression of pro-inflammatory cytokines such as IL-1 β , in addition to promoting chronic reductions in adipose tissue, which itself acts as a source of inflammatory mediators [12].

Among the 33 articles included in this review, only five assessed the expression of this cytokine [50,51,60,67,84] (Table 1 and Table 2). Three of these studies reported a reduction in IL-1 β levels in response to physical activity [51,60,67], while two found no statistically significant changes [50,84]. Notably, three studies observed an increase in baseline IL-1 β concentrations, which were subsequently reduced after the exercise interventions. These investigations, conducted in animal models, examined diabetic nephropathy, hypertensive nephropathy, and lupus nephritis [51,60,67]. Conversely, one study evaluating hypertensive rats did not report significant changes in IL-1 β levels following aerobic exercise, despite using a protocol similar to those in studies that showed positive outcomes [86]. The only human study on this cytokine involved a 12-week program of intradialytic resistance training and reported elevated IL-1 β at baseline but no significant differences following the intervention [84].

From a mechanistic perspective, the purinergic signaling system may also play a crucial role in regulating IL-1 β expression, particularly through the activation of P2X7 receptors. During tissue damage and inflammatory processes, elevated extracellular ATP levels activate P2X7 receptors on immune cells, leading to the assembly of the inflammasome complex and the consequent release of IL-1 β [80]. Physical exercise appears to modulate this pathway by reducing P2X7 receptor expression and activity, thereby limiting the ATP-mediated stimulus for IL-1 β production. Experimental studies suggest that post-exercise reductions in extracellular ATP concentrations, alongside increased activity of ectonucleotidases such as NTPDases and 5'-nucleotidase, promote an anti-inflammatory environment that suppresses the P2X7/IL-1 β axis [77,80]. This modulation may partly explain the reduction in IL-1 β levels observed in animal models subjected to exercise interventions and highlights purinergic signaling as a potential mediator of exercise-induced anti-inflammatory effects in CKD.

It is hypothesized that methodological differences, including variations in exercise type, intensity, sample characteristics, and timing of blood collection and analysis, may account for the inconsistencies observed among studies.

5.4. IL-17a

Interleukin-17a (IL-17a) is a pleiotropic cytokine produced by a

variety of cell types, including CD4+ $\alpha\beta$ T cells, $\gamma\delta$ T cells, natural killer cells, neutrophils, macrophages, dendritic cells, lymphoid tissue-inducing cells, mast cells, and plasma cells. It plays a crucial role in the inflammatory process by inducing the expression of chemokines and pro-inflammatory cytokines [87]. In CKD, IL-17a contributes to tissue damage and is associated with elevated IL-6 levels, thereby triggering a cascade of adverse inflammatory effects [66]. Notably, IL-17a levels are elevated in end-stage CKD due to the expansion of effector memory T cells capable of producing this cytokine [87].

Among the 33 articles included in this review, only four evaluated IL-17a levels [48,55,59,66], one in animal model [55] and three in human studies [48,59,66]. Three protocols assessed aerobic exercise interventions [48,55,59]: including two intradialytic protocols [48,59] and one resistance training protocol [66]. Of the intradialytic aerobic protocols, one examined chronic exercise effects [59] and one focused on acute responses [48]. Chronic intradialytic cycling performed over 24 weeks failed to produce significant anti-inflammatory effects, likely due to the inability of hemodialysis patients to achieve the necessary exercise intensity to modify systemic inflammation [59]. In contrast, acute intradialytic cycling increased IL-17a levels, potentially reflecting an exercise-induced inflammatory response exacerbated by the dialysis procedure itself [48].

In an animal model study, aerobic exercise performed for 60 min per day over eight weeks resulted in higher IL-17a levels in obese mice compared to lean controls, while obese mice subjected to aerobic training exhibited lower IL-17a levels relative to sedentary obese mice [55]. Regarding resistance training, a study involving CKD patients demonstrated a reduction in IL-17a concentrations in both the standard resistance exercise group and the group that combined resistance training with blood flow restriction, after 24 weeks of intervention [66]. Despite these findings, additional studies are required to confirm the potential of physical exercise to modulate IL-17a levels in CKD, as available evidence remains limited.

Interestingly, recent evidence suggests that the purinergic signaling system may contribute to the regulation of IL-17a production through adenosine-mediated anti-inflammatory mechanisms. Activation of the A2A adenosine receptor has been shown to suppress IL-17a synthesis by effector T cells in various inflammatory conditions [88]. Physical exercise promotes increased enzymatic activity of 5'-nucleotidase and elevated adenosine levels in the extracellular environment [77], which may contribute to the downregulation of IL-17a. This anti-inflammatory mechanism, driven by exercise-induced modulation of purinergic signaling, represents a potential pathway for controlling IL-17a-associated inflammation in CKD. However, direct investigations into the interaction between physical exercise, purinergic signaling, and IL-17a regulation in this specific population remain limited and warrant further study.

5.5. IL-8

Interleukin 8 (IL-8) is a cytokine primarily produced by monocytes and macrophages [89], and it is well known for its role in neutrophil activations and recruitment [90]. Patients with end-stage CKD undergoing hemodialysis typically present with elevated plasma IL-8 levels [57].

Among the 33 articles included in this review, only two assessed IL-8 levels in human studies: one involving a 12-week intradialytic resistance training protocol [57] and the other employing intradialytic cycling over 24 weeks [59]. In the trial evaluating resistance training, IL-8 concentrations were elevated at baseline relative to normative data but remained unchanged following the exercise intervention [57]. Similarly, the intradialytic cycling protocol did not significantly affect IL-8 levels [59]. Given the limited number of studies addressing this cytokine, further research is necessary to clarify the role of IL-8 in CKD and its potential modulation through physical exercise.

Although few studies have explored the relationship between

exercise and IL-8 levels in CKD, purinergic signaling may represent a relevant regulatory pathway. Evidence indicates that IL-8 secretion in monocytes is modulated by the activation of P2Y2 and P2Y6 receptors, which respond to extracellular nucleotides such as ATP and UDP released during cellular stress and inflammation [91]. Physical exercise has been shown to modulate extracellular ATP availability and promote the activity of ectonucleotidases, potentially reducing P2Y-mediated IL-8 release. Although this mechanism has not yet been directly investigated in CKD patients, it represents a plausible anti-inflammatory pathway through which exercise could attenuate IL-8-driven inflammatory responses, particularly in hemodialysis settings where basal IL-8 levels are elevated. Future studies should examine whether purinergic modulation contributes to exercise-induced inflammatory control in this context.

5.6. IL-18

Interleukin-18 (IL-18) is a pro-inflammatory cytokine primarily produced by activated macrophages [92] which regulates both innate and adaptive immune responses. It is expressed in various tissues and cell types, with increased levels typically observed in endogenous inflammatory processes such as sepsis. Additionally, IL-18 is considered a mediator and biomarker of acute kidney injury and contributes to the progression of chronic kidney disease [92].

Among the 33 articles selected for this review, only two evaluated IL-18 expression, and both reported a reduction in IL-18 levels in response to aerobic physical exercise protocols [55,60]. One study examined the effects of aerobic training in obese mice over two weeks and observed increased IL-18 levels in sedentary obese controls, with a subsequent reduction in trained obese animals compared to their sedentary counterparts, though not compared to lean controls [55]. Another study conducted in diabetic rats similarly found elevated IL-18 levels at baseline and a significant reduction following an aerobic training program [60].

Both studies suggest that aerobic exercise may effectively reduce systemic inflammation mediated by IL-18 in the context of metabolic and renal dysfunction. A proposed mechanism involves the suppression of the NF- κ B signaling pathway, which leads to the downregulation of NLRP3 inflammasome activation. Upon activation, this inflammasome complex assembles with ASC, promoting the conversion of procaspase-1 to its active form and driving the maturation and secretion of pro-inflammatory cytokines, including IL-18. Thus, aerobic training appears to attenuate NF- κ B activation and, consequently, the downstream pro-inflammatory signaling cascade [60].

Despite the limited evidence directly addressing the interaction between exercise and IL-18 in CKD, purinergic signaling may play a complementary role in modulating this cytokine. Experimental studies have demonstrated that activation of the A2A adenosine receptor inhibits IL-18 production induced by lipopolysaccharides in human monocytes [93]. Physical exercise enhances the activity of ectonucleotidases such as NTPDases and 5'-nucleotidase, thereby increasing extracellular adenosine concentrations [77]. This exercise-induced rise in adenosine could potentially downregulate IL-18 secretion via A2A receptor activation, contributing to the anti-inflammatory effects observed in animal models subjected to aerobic exercise. However, no direct evidence currently exists linking exercise-induced purinergic modulation to IL-18 regulation in CKD, warranting further mechanistic investigations.

5.7. IL-12

Interleukin-12 (IL-12) is a pro-inflammatory cytokine predominantly produced by dendritic cells and macrophages. It functions as a key mediator between the innate and adaptive immune systems by promoting the differentiation of CD4+ T cells and regulating the activity of various effector cells [94]. In patients with CKD, elevated IL-12 levels

have been reported, contributing to the maintenance of a pro-inflammatory environment [95].

Among the 33 articles included in this review, only one assessed IL-12 levels. This study investigated a 12-week intradialytic resistance exercise protocol and found no significant changes in IL-12 concentrations following the intervention [84]. The absence of changes may be attributed to the limited exercise intensity that hemodialysis patients are typically able to achieve, which might be insufficient to modulate systemic inflammation. Consequently, new protocols with varied exercise modalities, intensities, and durations are needed to better understand the effects of physical activity on IL-12 levels in this population.

While studies directly linking exercise to IL-12 modulation in CKD is scarce, purinergic signaling has been implicated in the regulation of this cytokine. Studies have shown that activation of adenosine receptors—specifically A2A, A2B, and A3 receptors on macrophages—leads to a reduction in IL-12 production, contributing to the suppression of pro-inflammatory immune responses [93]. Since physical exercise increases extracellular adenosine availability through enhanced ectonucleotidase activity [77], it is plausible that exercise-induced modulation of purinergic signaling may indirectly downregulate IL-12 levels. However, no studies to date have directly investigated this mechanism in CKD patients, highlighting an important gap to be addressed in future research.

5.8. CRP

C-reactive protein (CRP) is an acute-phase molecule produced in response to systemic inflammation [96]. Its plasma concentration increases significantly in inflammatory conditions, making it a widely used biomarker in clinical practice [97]. Patients with CKD commonly present with elevated serum CRP levels, and it is well established that physical exercise can reduce inflammatory markers, including CRP, in various populations [98].

Among the 33 studies included in this review, seven assessed the effects of physical exercise on CRP levels in CKD [44,52,55,61,63,70,71], with one study conducted in an animal model [55]. Four of them showed a reduction in CRP after subjecting CKD patients to physical activity [55,61,63,70], and three of them showed no significant changes in CRP levels [44,52,71].

The study that investigated an animal model of CKD [55] compared the effect of physical exercise on sedentary lean and obese rats compared to exercised obese rats, which underwent aerobic activity five times a day for two weeks, reducing CRP levels. Among the human trials, three studies demonstrated reductions in CRP levels. In one, CKD patients undergoing hemodialysis were divided into two groups: an intradialytic exercise group and a home-based exercise group. Both performed combined aerobic and resistance training three times per week for four months, resulting in decreased CRP concentrations [70]. Another study reported reduced CRP levels following a six-month intradialytic resistance training program in hemodialysis patients [63]. Additionally, a study in non-dialyzed CKD patients compared resistance exercise combined with a low-protein diet to a control group over 12 weeks, observing significant reductions in CRP in the intervention group [44].

Conversely, three studies found no significant changes in CRP levels. These involved intradialytic exercise protocols combining aerobic and resistance training for 16 weeks [71] as well as aerobic-only protocols lasting 16 weeks [44] or 24 weeks [52]. Given the relatively small number of studies available and the heterogeneity of findings, further research is necessary to confirm the effects of physical exercise on CRP levels in patients with CKD.

Although the relationship between exercise, purinergic signaling, and CRP regulation in CKD has not been extensively studied, emerging evidence suggests that purinergic pathways may influence systemic inflammatory markers, including CRP. A study investigating the effects of resistance training in hypertensive women demonstrated reductions in CRP levels associated with decreased ATP concentrations and adenosine

deaminase activity, suggesting an anti-inflammatory effect mediated through purinergic signaling [99]. Physical exercise enhances the activity of ectonucleotidases, leading to increased adenosine availability [77], which could potentially contribute to lowering CRP levels by modulating pro-inflammatory signaling pathways. While this mechanism has yet to be directly explored in CKD populations, it represents a promising area for future research aimed at elucidating how purinergic modulation through exercise might influence CRP and other inflammatory biomarkers.

5.9. Purinergic mechanisms in exercise-induced pro-inflammatory modulation in CKD

Recent literature has increasingly explored purinergic signaling as a mechanism underlying the effects of physical exercise on pro-inflammatory profiles. For instance, a 2024 study investigating the effects of physical activity protocols on sedentary, overweight, and physically active subjects reported a significant increase in the activity of NTPDases, Nucleotide Pyrophosphatase/Phosphodiesterases (NPP), and 5'-nucleotidase immediately after exercise, suggesting a potential link between purinergic signaling and immune modulation following physical activity [77].

Additionally, the study found decreased plasma levels of ATP and ADP after acute moderate-intensity exercise, which remained suppressed for at least one hour post-exercise, while ADO, inosine (INO), and uric acid (UA) levels increased—with only ADO remaining elevated after one hour. Regarding inflammatory activity, the authors identified a significant negative correlation between 5'-nucleotidase activity and TNF- α levels before, immediately after, and one hour following exercise, along with correlations between NPP activity and TNF- α , as well as between ADP, ADO, and IL-8 [77]. These findings highlight the dynamic role of purinergic signaling in regulating inflammatory responses during physical exercise, particularly through modulation of extracellular ATP, ADO, and their respective receptors.

During muscle contraction, ATP is released into the extracellular environment, where it can act as a co-transmitter with norepinephrine at sympathetic nerve terminals, subsequently activating P2 receptors [80]. This release promotes T cell activation, the secretion of cytokines and chemokines, reactive oxygen species production, and, paradoxically, reductions in chronic inflammation, particularly through enhanced ectonucleotidase activity and ATP hydrolysis [80]. Moderate-intensity aerobic exercise promotes ATP release from erythrocytes, lymphocytes, muscle cells, and endothelial cells, with purinergic enzymes rapidly metabolizing the increased nucleotide concentrations. It is believed that a purinergic response to acute physical exercise occurs similarly in individuals with diverse metabolic profiles, leading to transient modulation of NTPDase, 5'-nucleotidase, and NPP activities, which reduces extracellular ATP availability and downstream pro-inflammatory signaling [77].

Following physical activity, a reduction in P2X7 receptor expression has been observed in various cell types, decreasing ATP-mediated apoptosis and limiting the release of pro-inflammatory mediators such as IL-1 β and IL-6 [80]. The purinergic system—via ATP-activated P2X and P2Y receptors and adenosine receptors (A1, A2A, A2B, and A3)—plays a key role in modulating cytokines including IL-6 and TNF- α , with additional evidence implicating its involvement in the regulation of IL-1 β , IL-18, IL-17a, IL-8, IL-12, and CRP [79].

Specifically, P2X7 receptor activation by ATP enhances IL-6 production and secretion by immune cells such as macrophages, amplifying inflammatory responses [79]. Conversely, adenosine, acting through A2A and A2B receptors, typically suppresses IL-6 production, exerting anti-inflammatory effects [100]. In inflammatory conditions, excessive ATP release intensifies purinergic signaling, increasing IL-6 production and contributing to chronic inflammatory diseases [78]. As IL-6 plays a pivotal role in renal inflammation and fibrosis, its elevated levels are associated with worsening kidney function and increased mortality in

CKD patients [24]. Regarding TNF- α , studies have demonstrated that adenosine binding to A2A, A2B, and A3 receptors on macrophages inhibits this cytokine's production, indicating that adenosine generated through exercise-induced purinergic activity may be crucial in mitigating inflammation [85].

In the case of IL-1 β , its production is closely associated with P2X7 receptor activity, which is downregulated following exercise, limiting pro-inflammatory signaling in skeletal muscle fibers and leukocytes and reducing the release of IL-1 β and IL-6, alongside apoptosis processes [80]. Although less extensively investigated, the purinergic system has also been implicated in modulating other cytokines. For instance, stimulation of A2A adenosine receptors has been shown to suppress pro-inflammatory mediators such as IL-17 in inflammatory diseases like multiple sclerosis [88]. IL-8 secretion appears to be regulated by P2Y2 and P2Y6 receptors in monocytes, activated by extracellular UDP or lipopolysaccharides [91]. Similarly, IL-18 production is inhibited by A2A receptor activation in human monocytes exposed to LPS, while IL-12 is modulated by A2A, A2B, and A3 receptors on macrophages, leading to its suppression [93].

Lastly, in relation to CRP, a study on hypertensive women undergoing six months of resistance training found reductions in both CRP and ATP levels, alongside decreased adenosine deaminase activity, suggesting that regular exercise exerts anti-inflammatory effects partially mediated by purinergic signaling [99].

Collectively, these findings reinforce the role of the purinergic system in modulating the inflammatory response triggered by cellular injury and stress. By controlling extracellular nucleotide levels and promoting adenosine-mediated signaling, regular physical exercise may regulate immune function in CKD, contributing to reductions in pro-inflammatory cytokines and enhancing anti-inflammatory responses essential for disease management.

6. The modulatory role of physical exercise on the anti-inflammatory profile and its relationship with purinergic signaling

6.1. IL-10

Interleukin-10 (IL-10) is a cytokine primarily produced by monocytes and lymphocytes, known for its potent anti-inflammatory effects through the inhibition of macrophage activation and T cell proliferation [51]. In patients with CKD, IL-10 plasma levels tend to increase as a counter-regulatory response to persistent systemic inflammation, and lower IL-10 levels in hemodialysis patients have been associated with a greater risk of atherosclerosis and cardiovascular events. Evidence suggests that physical exercise exerts acute and chronic anti-inflammatory effects in CKD by modulating IL-10 and suppressing pro-inflammatory cytokines, notably through the inhibition of M1 monocyte/macrophage and Th1 lymphocyte phenotypes and the downregulation of co-stimulatory molecules such as MHC, thereby reducing the risk of cardiovascular and metabolic complications in this population [61].

Among the 33 articles included in this review, 16 evaluated IL-10 expression: seven in human studies [46,48,56,59,66,68,84] and nine in animal models [42,49–51,55,58,65,67,69], all involving aerobic and/or resistance exercise protocols. In human studies, four adopted aerobic exercise interventions [46,48,56,59] predominantly walking-based protocols, while three employed resistance training [66,68,84], with one study examining acute effects [48]. Among animal studies, three utilized resistance training [65,67,69], and six adopted aerobic protocols [42,49–51,55,58], all assessing chronic training effects. Most of these studies ($n = 11$) reported an increase in IL-10 following exercise, independent of exercise type.

Most studies conducted in human populations have reported an increase in IL-10 levels in response to physical exercise. However, one study found no significant difference in IL-10 concentrations before and

after a 12-week resistance training program performed three times per week in dialysis patients ($n = 49$) [84]. Similarly, another trial involving 40 hemodialysis patients undergoing a 12-week intradialytic resistance exercise program also reported no significant changes in IL-10 levels [59]. In contrast, a study using a similar protocol but with a larger sample size ($n = 107$) over 24 weeks observed a significant increase in IL-10 concentrations [68]. Additionally, a study investigating the acute effects of two 20-min sessions of aerobic exercise during hemodialysis found a post-exercise increase in IL-10, consistent with findings in healthy individuals following moderate-to-intense physical activity [48]. Three additional studies also reported increased IL-10 levels: two following aerobic exercise protocols lasting 12 [56] and 24 weeks [46], and one after a 24-week resistance training program [66].

In animal models, six studies reported elevated IL-10 after exercise [42,50,55,58,65,69], two showed no significant changes [49,51], and one reported a decrease [67]. Aerobic training protocols yielded more consistent results, with four of six studies showing increased IL-10 levels [42,50,55,58]. Among the two exceptions, one evaluated lupus nephritis combined with chronic social stress, which may have masked exercise-induced IL-10 elevations due to disease-specific baseline increases [51], while the other involved voluntary exercise, in which control and nephrectomized rats covered similar distances [49].

Conversely, resistance exercise protocols in animal models produced more conflicting results, with two of three studies reporting IL-10 reductions [67,69]. In one investigation involving diabetic and non-diabetic rats undergoing resistance training for eight weeks, renal inflammation decreased while IL-10, previously elevated in diabetic rats, declined after training [67]. Another study observed reduced IL-10 in sedentary control groups, which was reversed by exercise, indicating an improved inflammatory balance marked by elevated IL-10 and reduced TNF- α and TGF- β , potentially mitigating renal interstitial fibrosis via TNF- α modulation [65].

Importantly, purinergic signaling appears to be directly involved in the regulation of IL-10 in response to physical exercise. Adenosine, generated from ATP breakdown via ectonucleotidases such as NTPDases and 5'-nucleotidase, binds to A2A and A2B receptors, promoting IL-10 production in monocytes and macrophages and contributing to the suppression of pro-inflammatory cytokines [53,85]. Additionally, P2X7 receptor activation has been implicated in IL-10 modulation in inflammatory conditions [78]. A study involving six months of resistance training in hypertensive women demonstrated concurrent increases in IL-10 levels, reductions in ATP and adenosine deaminase activity, and lowered blood pressure, reinforcing the connection between exercise-induced purinergic modulation and anti-inflammatory cytokine regulation [99]. This suggests that regular physical exercise, by increasing ATP release and subsequently elevating extracellular adenosine levels, enhances IL-10 secretion through purinergic signaling pathways, representing a key mechanism by which exercise attenuates inflammation in CKD.

In human studies, IL-10 responses were more uniformly positive, with physical exercise consistently enhancing IL-10 levels and mitigating pro-inflammatory responses. In contrast, conflicting outcomes in animal models, particularly with resistance exercise, suggest that additional research is needed to clarify exercise-induced IL-10 modulation in CKD. Collectively, these findings support the concept that exercise promotes anti-inflammatory effects in CKD, with IL-10 playing a central role in this response.

6.2. IL-4

Interleukin 4 (IL-4) is a glycoprotein primarily produced by CD4+ T lymphocytes, mast cells, and basophils. It functions as a potent anti-inflammatory cytokine by inhibiting the synthesis of pro-inflammatory mediators [101].

Among the 33 articles included in this review, only two assessed the effects of physical exercise on IL-4 in CKD, both conducted in animal

models [55,65]. One study employed a 10-week resistance training protocol in nephrectomized rats, consisting of stair-climbing exercises with progressively increased loads [65]. The other study analyzed IL-4 levels in obese and lean rats following a two-week aerobic treadmill running protocol [55].

Both investigations demonstrated increased IL-4 levels in the exercised groups compared to sedentary controls, supporting the hypothesis that physical exercise may exert anti-inflammatory effects in CKD through the upregulation of IL-4. From this perspective, recent research has shown that resistance training is associated with higher circulating T-cell counts at rest, potentially as an adaptive response to transient post-exercise pro-inflammatory states. This mechanism has been proposed to contribute to increased IL-4 and other anti-inflammatory cytokines following exercise [102].

In parallel, purinergic signaling has been identified as a relevant mechanism influencing IL-4 expression in inflammatory conditions. Experimental evidence suggests that activation of P2X and P2Y receptors by extracellular ATP can modulate IL-4 production in immune cells, including T lymphocytes and mast cells, with context-dependent effects according to the inflammatory microenvironment [78]. More consistently, adenosine — generated through the hydrolysis of ATP by ectonucleotidases — exerts anti-inflammatory effects via A2A and A2B receptors, promoting an environment favorable to IL-4 upregulation [100]. In renal tissue, IL-4 is proposed to enhance the activity of purinergic receptors linked to anti-inflammatory cytokine production while concurrently suppressing pro-inflammatory cytokines, potentially offering therapeutic benefits in inflammatory renal diseases [103].

Given the limited number of studies available and the promising mechanistic evidence, further research is necessary to confirm the effects of different exercise modalities on IL-4 levels in CKD, particularly in human populations, and to elucidate the role of purinergic signaling underlying this mechanism. Clarifying IL-4's anti-inflammatory function in this context may provide valuable insights for developing targeted, exercise-based strategies to improve inflammatory profiles in CKD patients.

6.3. IL-15

Interleukin-15 (IL-15) is a cytokine produced by bone marrow stromal cells and activated monocytes [104]. It is also highly expressed in skeletal muscle and, according to the literature, can be modulated by strength training, exerting anabolic effects and contributing to the reduction of adipose tissue [76]. Additionally, IL-15 levels may transiently increase following aerobic exercise [105].

In certain contexts, such as during infection or tissue injury, IL-15 promotes immune activation by stimulating natural killer (NK) cells and cytotoxic T cells. However, growing evidence suggests that IL-15 also exerts anti-inflammatory effects by modulating the activity of regulatory immune cells, including regulatory T lymphocytes [106].

Concerning CKD, IL-15 has been shown to prevent renal fibrosis by inhibiting collagen synthesis in myofibroblasts and reducing macrophage infiltration, particularly in experimental models such as unilateral ureteral obstruction. As a result, IL-15 has been proposed as a potential therapeutic agent for CKD [107].

Among the 33 articles selected for this review, only two evaluated the relationship between physical exercise, CKD, and IL-15 in human studies [64,66]. In one study, 24 weeks of resistance training with progressive intensity adjustments every eight weeks resulted in increased IL-15 levels in stage-2 CKD patients compared to a control group, suggesting a possible anti-inflammatory effect mediated by this cytokine [66]. Similarly, an eight-week resistance training protocol involving three sets of 10 to 12 repetitions per session also demonstrated elevated IL-15 concentrations relative to controls [64].

Although these findings are promising, it is important to note that further research is necessary to confirm the effects of physical exercise on IL-15 levels in CKD. While preliminary evidence highlights the

potential of IL-15 in mediating exercise-induced anti-inflammatory effects, its precise therapeutic role in CKD remains to be established.

In terms of biological mechanisms, purinergic signaling may also influence IL-15 regulation in response to physical exercise. Activation of P2X and P2Y receptors by extracellular ATP promotes the proliferation and activation of immune cells such as NK cells and T cells — key targets of IL-15 [78,79]. Moreover, adenosine, produced via the enzymatic degradation of ATP during exercise, exerts anti-inflammatory effects through A2A receptor activation, which may indirectly suppress excessive IL-15 expression while enhancing its immunoregulatory properties [100]. Although no studies have yet directly addressed this mechanism in CKD, these findings suggest that exercise-induced modulation of purinergic signaling could contribute to regulating IL-15-mediated responses, favoring an anti-inflammatory renal environment and potentially improving patient outcomes.

Given the limited number of studies available and the promising mechanistic evidence, further research is necessary to confirm the effects of different exercise modalities on IL-15 levels in CKD, particularly in human populations. Additionally, clarifying the role of purinergic signaling in this regulatory process may provide valuable insights for developing targeted, exercise-based strategies to improve inflammatory control and renal health in CKD patients.

6.4. Purinergic mechanisms in exercise-induced Anti-inflammatory modulation in CKD

The purinergic system also plays a significant role in modulating anti-inflammatory cytokines, primarily through the actions of adenosine, which is responsible for restraining excessive inflammatory responses [53]. Notably, IL-10 and other anti-inflammatory mediators are influenced by adenosine, which stimulates their release via binding to A2A and A2B receptors [99] as well as through the activation of P2X7 receptors [78]. Supporting this, a study involving six months of resistance training in hypertensive women demonstrated increased IL-10 levels, along with reductions in blood pressure, plasma ATP concentrations, and adenosine deaminase activity, suggesting that regular exercise can simultaneously modulate interleukin production and purinergic signaling activity [99].

Purinergic pathways are also involved in the regulation of interleukin-4 (IL-4), a cytokine with potent anti-inflammatory properties essential for T helper 2 (Th2) cell differentiation and the regulation of allergic responses. Activation of P2X and P2Y receptors by extracellular ATP can influence IL-4 production in immune cells such as T lymphocytes and mast cells, with effects dependent on the cell type and the local inflammatory microenvironment [78]. Furthermore, adenosine acting through A2A and A2B receptors fosters an anti-inflammatory environment by modulating IL-4 production [100]. In renal tissue, IL-4 may promote an anti-inflammatory response by enhancing purinergic receptor activity associated with increased anti-inflammatory cytokine production and the suppression of pro-inflammatory mediators [103].

Similarly, activation of P2X and P2Y receptors by extracellular ATP can affect IL-15 expression, promoting the proliferation and activation of T cells and NK cells, which are essential for immune defense [78,79]. Additionally, adenosine, through A2A receptor activation, exerts anti-inflammatory effects that can suppress excessive IL-15 production, thereby modulating the intensity and balance of immune responses [100]. This positions purinergic signaling as a critical intersection point in the regulation of IL-15 and the orchestration of inflammatory and immune processes.

As physical exercise is known to increase the production of anti-inflammatory interleukins — particularly in the context of regular aerobic training, which promotes positive immunological adaptations [108] — the interaction between exercise-induced ATP release, purinergic receptor activation, and subsequent modulation of cytokine production emerges as a promising mechanism for inflammation control. In this

regard, purinergic signaling may play a fundamental role in mediating the anti-inflammatory benefits of exercise in CKD, although further mechanistic studies are warranted to fully elucidate this relationship.

7. Other cytokines

In addition to the cytokines previously discussed, IL-33, IL-7, and IL-11 were also investigated. However, no studies evaluated the expression of IL-33 or IL-7 in response to physical exercise in CKD. IL-11, on the other hand, has been primarily associated with acute kidney injury (AKI), which lies outside the scope of this review.

IL-33 is predominantly found in epithelial barrier tissues and endothelial cells, especially within tubulointerstitial cells of the kidney. It mediates inflammatory and repair processes by binding to its receptor, ST2, which activates intracellular signaling pathways such as NF- κ B, JNK, p38, and ERK, ultimately stimulating neutrophils, eosinophils, Th2 cells, and innate lymphoid cells. Exogenous factors that induce renal injury, cell damage, or mechanical stress can upregulate IL-33, promoting both inflammation and tissue repair. Although IL-33 has been explored as a biomarker for kidney damage, recent evidence suggests that expression of its receptor ST2 may be more relevant in identifying CKD progression [109].

Interleukin-11 (IL-11) is a pleiotropic cytokine produced by various stromal cells, including fibroblasts, epithelial cells, and osteoblasts. It is upregulated by mechanical stress and plays roles in hematopoiesis, hepatocyte stimulation, and the regulation of acute-phase protein production [110]. IL-11 has been widely studied for its involvement in renal fibrosis and its potential therapeutic applications [111]. The study examining cisplatin-induced AKI in rats used an eight-week high-intensity interval training protocol and reported a significant increase in IL-11 levels in exercised groups. The authors concluded that IL-11 exerted nephroprotective, anti-apoptotic, and anti-inflammatory effects in this AKI model [112]. Given these findings, further research is necessary to determine whether IL-11 plays a comparable role in CKD and whether it can be modulated by physical exercise.

Interleukin-7 (IL-7) has been implicated in muscle hypertrophy and kidney function regulation. Expressed by skeletal muscle cells, IL-7 levels increase in response to chronic physical activity, with changes observed after two weeks of training [113]. Cellular studies demonstrated that IL-7 suppresses TGF- β receptor expression in tubular epithelial cells, thereby attenuating renal fibrosis [114].

Considering these characteristics and the limited available data, further research is warranted to better elucidate the roles of IL-33, IL-7, and IL-11 in CKD, particularly regarding their modulation by physical exercise and their potential relevance within exercise-based therapeutic approaches.

As these findings highlight important gaps in the current literature, future studies investigating the purinergic signaling pathways associated with these cytokines could provide valuable mechanistic insights into how exercise influences the inflammatory and regenerative processes in CKD.

8. Conclusion

The purinergic system plays a pivotal role in regulating inflammation, and its dysfunction has been closely associated with CKD. In this condition, purinergic signaling is often dysregulated, contributing to the chronic inflammatory state characteristic of CKD. Nucleotides and nucleosides such as ATP and ADO, key mediators of the purinergic system, modulate the release of both pro-inflammatory and anti-inflammatory cytokines. Physical exercise influences purinergic signaling by promoting the release of ADO, which attenuates inflammation through the upregulation of anti-inflammatory cytokines and suppression of pro-inflammatory mediators.

The evidence reviewed demonstrates that exercise protocols, particularly aerobic and resistance training sustained over eight weeks

or more, consistently increase anti-inflammatory IL-10, IL-4, and IL-15 while reducing proinflammatory TNF- α , IL-1 β , and CRP. TNF- α exhibited the most consistent reduction across different exercise types, whereas IL-6 and IL-17a showed response patterns dependent on protocol duration and intensity. Aerobic exercise protocols were most frequently studied and showed a stronger association with favorable anti-inflammatory profiles, although this likely reflects publication bias toward aerobic modalities. A visual summary of the mechanisms discussed throughout this review is illustrated in Fig. 2.

Despite these findings, substantial gaps remain in the literature, particularly regarding the effects of physical exercise on lesser-studied cytokines such as IL-8, IL-18, IL-12, IL-4 and IL-15, as well as on IL-11, IL-7, and IL-33, where no data in CKD populations were identified. Additionally, studies investigating the mechanistic involvement of purinergic signaling in these exercise-induced changes remain scarce.

Also, most of the studies included in this review do not classify participants according to KDIGO (Kidney Disease: Improving Global Outcomes) guidelines. Among those that do report KDIGO classification or estimated glomerular filtration rate (eGFR), none explore whether inflammatory responses to exercise vary according to disease stage. We suggest that physical exercise may yield greater benefits for patients in the early stages of CKD, as these individuals are more likely to tolerate and adhere to exercise protocols. This, in turn, could lead to more effective modulation of purinergic signaling and the associated pro-inflammatory profile, potentially slowing disease progression. However, this remains speculative, as most of the available studies focus on patients undergoing hemodialysis (stages IV and V). Only six studies included non-dialysis CKD patients [26,45,47,54,61,66], and none of them performed stratified analyses by CKD stage. Our review highlights a significant gap in the literature, emphasizing the need for future research investigating the effects of exercise on inflammatory markers and purinergic signaling across different stages of CKD as defined by KDIGO.

In this context, the interaction between physical exercise and the purinergic system emerges as a promising therapeutic avenue for improving inflammatory profiles and delaying CKD progression. This review reinforces the need for further research to refine and personalize exercise protocols for CKD patients, expand the investigation of less-studied cytokines, and clarify the molecular mechanisms — particularly the role of purinergic signaling — that underlie the anti-inflammatory benefits of physical activity in this population.

Glossary

Chronic kidney disease (CKD): progressive loss of renal function over time, characterized by structural damage and impaired filtration (e.g., reduced GFR or albuminuria), often leading to systemic complications.

Purinergic system: cell signaling network mediated by nucleotides (ATP/ADP) and nucleosides (adenosine), which regulates processes such as inflammation, immunity, and vascular function through specific receptors (P1 and P2).

Aerobic exercise: characterized by training that improves cardiorespiratory fitness by increasing the efficiency of aerobic energy production, through the mobilization of large muscle groups and a rhythmic sequence.

Resistance training: training that focuses on improving muscular strength and endurance, typically through the use of weights or resistance machines.

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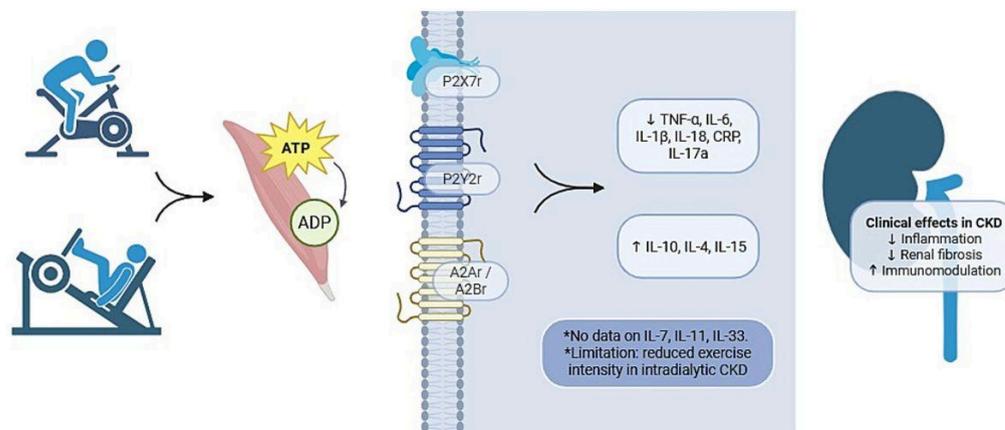


Fig. 2. Effects of physical exercise on inflammatory markers and the purinergic system. Schematic representation summarizing the main effects of physical exercise on inflammatory and anti-inflammatory cytokines in patients with chronic kidney disease (CKD), highlighting the modulatory role of purinergic signaling. Physical exercise (aerobic and resistance training) promotes extracellular ATP release by muscle, immune, and endothelial cells, followed by increased adenosine (ADO) generation via ectonucleotidases (NTPDase, 5'-NT). ATP activates P2X and P2Y receptors, stimulating immune responses, while ADO exerts anti-inflammatory effects via A2A and A2B receptors. These mechanisms contribute to the reduction of pro-inflammatory mediators (TNF- α , IL-6, IL-1 β , IL-8, IL-18, CRP, IL-17a) and the increase of anti-inflammatory cytokines (IL-10, IL-4, IL-15). Aerobic protocols demonstrated more consistent results, although research on resistance and intradialytic exercise remains limited. No studies evaluating the effects of physical exercise on IL-7, IL-11, and IL-33 in CKD were identified. Source: prepared by the authors.

CRedit authorship contribution statement

Lilian Baseggio: Writing – original draft, Investigation, Data curation. **Isabela Berton Wissmann:** Writing – original draft, Visualization, Investigation, Data curation. **Renata Cristina Daniel Coelho:** Writing – original draft, Methodology, Investigation. **Andreia Machado Cardoso:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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MANUSCRITO

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Synergistic Nephroprotection by Exercise Training and Ursolic Acid: Purinergic Signaling Modulation in LPS-Induced Kidney Injury

ABSTRACT

Low-grade inflammation is a key driver of renal dysfunction and is associated with purinergic system hyperactivation. While exercise training (ET) and ursolic acid (UA) possess anti-inflammatory properties, their synergistic impact on renal purinergic signaling remains unexplored. Male Wistar rats were subjected to a 6-week resistance ET protocol (ladder climbing, 3 sessions/week) and/or daily UA administration (5 mg/kg, via gavage). During the final week, low-grade inflammation was induced with lipopolysaccharide (LPS; 250 µg/kg/day, IP). Following euthanasia, systemic markers (irisin, cytokines via CBA), renal function (serum creatinine), and renal mRNA expression of purinergic receptors and enzymes (RT-qPCR) were evaluated. LPS administration induced systemic inflammation, reduced irisin, and promoted acute renal dysfunction, characterized by a significant increase in serum creatinine. ET effectively attenuated purinergic hyperactivation and normalized ATP-dependent inflammatory markers. Notably, the ET+UA combination demonstrated the most robust nephroprotective effect, serving as the only intervention to fully restore creatinine levels to baseline. This synergy optimized the ATP/adenosine balance and significantly suppressed pro-inflammatory cytokines, specifically TNF-alpha and IL-17a. We conclude that ET is a potent modulator of renal purinergic signaling, and its association with UA amplifies functional and immunomodulatory protection, establishing a promising non-pharmacological approach for the prevention of inflammation-related renal injury.

Keywords: Ursolic Acid; Exercise Training; Acute Kidney Injury; Purinergic System; Inflammation

INTRODUCTION

The kidneys represent a complex set of regulatory and homeostatic systems in the human body, participating in hydroelectrolytic and acid-base balance, waste excretion, and hormone production. Acute Kidney Injury (AKI) is a highly prevalent clinical syndrome associated with elevated rates of in-hospital morbidity and mortality, affecting approximately 21% of hospitalized patients, with mortality also reported around 21% (1). Given this scenario, the study of potential interventions that can modulate and prevent renal damage is of fundamental scientific and clinical importance.

The inflammatory induction model using lipopolysaccharide (LPS) is widely employed for the study of renal injury (2). LPS induces the release of inflammatory cytokines by activating the TLR4 receptor, which triggers the I κ B kinase (IKK)/nuclear factor kappa B (NF- κ B) and the mitogen-activated protein kinases (MAPK) signaling pathways. This leads to increased production and release of pro-inflammatory cytokines, notably Tumor Necrosis Factor-alpha (TNF- α) and Interleucina-1 β (IL-1 β) (2). This process, in turn, enhances oxidative stress and promotes the activation of tubular epithelial cells, whose damage leads to hypoperfusion and microcirculatory injury in the kidney (3).

In the context of renal inflammation, the role of purinergic signaling is being explored. It is known that tubular damage induced by LPS leads to the release of extracellular ATP, the main agonist of P2 purinergic receptors, which are widely expressed in renal tissue (4). These receptors, divided into the P2X (ion channels) and P2Y (G protein-coupled) families, along with adenosine receptors, regulate renal function, vasculature, and immune activity (5,6). The modulation of these receptors highlights the purinergic system as a potential therapeutic target in renal diseases, given that its regulation may reduce inflammation, monocyte infiltration, and proteinuria (7).

Ursolic acid (UA) — a pentacyclic triterpenoid found in various plants, such as apple peel, rosemary, and olive oil (8) — demonstrates protective action on renal function, reduction of ferroptosis, and inactivation of signaling pathways like JAK2/STAT3 (9). Its mechanism of interest lies in its ability to inhibit inflammatory pathways such as NF- κ B/I κ B kinase, which can be activated by LPS (10).

In parallel, exercise training (ET) constitutes an important anti-inflammatory strategy, acting through pathways similar to UA, such as the modulation of NF- κ B and AMPK pathways (11,12). It is also known to promote an increase in renal filtration

fraction, improve tubular processing and excretion rate, and enhance tubular fluid reabsorption (13).

Irisin, a myokine secreted in response to exercise, has been proposed as a potential link between muscle activity and renal protection. Given that its circulating levels may also be influenced by ursolic acid, we investigated its behavior alongside renal purinergic signaling as a possible biomarker of the overall metabolic response. Secreted by muscle tissue in response to exercise (14), irisin's circulation is also increased in response to UA supplementation (15). Furthermore, it demonstrates a nephroprotective effect by suppressing tubular damage and fibrosis, acting via UCP2 and the Irisin/AMPK axis (14,16).

Although UA and ET have been studied separately, there are no reports investigating the interaction between the two interventions or their combined protective effects on the kidneys. Thus, the combination of UA and ET may reveal potential synergies between the two therapies and offer a new perspective for preventing and attenuating renal damage induced by inflammation.

In view of the above, this study assessed the synergistic nephroprotective effects of the combination of ET and UA, focusing on the modulation of purinergic signaling, in an animal model of LPS-induced renal injury. The elucidation of these combined mechanisms may provide new avenues for the development of preventive and therapeutic strategies against inflammation-associated renal injury.

MATERIALS AND METHODS

Animal Procedures

The experimental protocol followed the legislation in force and the ethical principles established by the Brazilian Society of Laboratory Animal Science (SBCAL) and the Brazilian College of Animal Experimentation (COBEA). It was approved by the Ethics Committee for the Use of Animals of the Federal University of Santa Maria (CEUA-UFSM), protocol number 6805060821.

This was an experimental study conducted on male Wistar rats (n=65) with a mean weight of 269.4 g \pm 28.8 g, obtained from the Central Vivarium of UFSM. Animals were provided commercial feed and water ad libitum, maintained at a constant temperature (22° to 24°), and subjected to a 12-hour light-dark cycle.

The animals underwent a 7-week period of resistance exercise training on a ladder (34 steps, 80° inclination). Initially, there was a one-week adaptation period without added load. The training protocol consisted of 3 sessions per week, with 4 to 6 sets of 8 to 10 repetitions, and a 2-minute interval between sets. The load was progressively increased by 25% every 2 weeks. The animals were weighed once per week throughout the entire experimental protocol period. Weighing occurred before the start of the physical activity protocol, using a digital scale (SF-400, Partyreal®). Ursolic acid was administered at a dose of 5mg/kg by gavage once daily, 7 times per week; or saline solution was administered via the same route. In the last week of the protocol, the animals received LPS at a dose of 250 µg/kg via intraperitoneal injection once daily for seven days, or 0.9% NaCl via the same route. The experimental design is demonstrated in Figure 1.

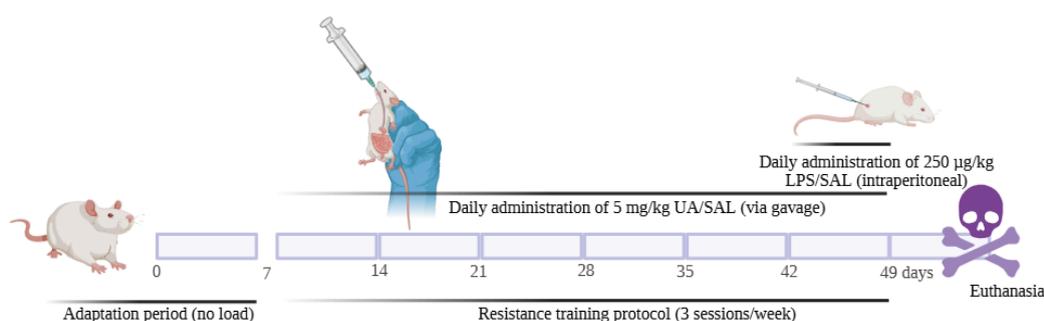


Figure 1. Experimental Design and Study Timeline. Schematic representation of the 7-week experimental protocol. Male Wistar rats were subjected to a 1-week adaptation period (no load) followed by 6 weeks of resistance exercise training (ET; 3 sessions/week) and daily administration of ursolic acid (UA; 5 mg/kg via gavage) or saline solution (SAL). During the final week (days 42–49), lipopolysaccharide (LPS; 250 µg/kg) or saline was administered daily via intraperitoneal injection to induce low-grade inflammation. Euthanasia was performed at the end of the 7th week for tissue and blood collection. Abbreviations: ET, exercise training; UA, ursolic acid; SAL, saline; LPS, lipopolysaccharide.

The animals were randomly allocated into eight groups, as described in Table 1.

Table 1. *Experimental Groups and Treatment Distribution*

	Exercise Training (Resistance)	Treatment (Gavage)	Injury (Intraperitoneal, IP)
SAL (n=9)	No	Saline Solution	Saline Solution
ET (n=8)	Yes	Saline Solution	Saline Solution
UA (n=8)	No	UA	Saline Solution
ET+UA (n=8)	Yes	UA	Saline Solution
LPS (n=8)	No	Saline Solution	LPS
LPS+ET (n=8)	Yes	Saline Solution	LPS
LPS+UA (n=8)	No	UA	LPS
LPS+ET+UA (n=8)	Yes	UA	LPS

Eight experimental groups of male Wistar rats (n = 65 total) were established to evaluate the synergistic effects of resistance exercise training (ET) and ursolic acid (UA) on an LPS-induced inflammatory model. Animals were followed over a 7-week protocol, including one week of adaptation and six weeks of intervention. UA, ursolic acid (5 mg/kg/day via gavage); ET, exercise training (3 sessions/week); IP, intraperitoneal injection; LPS, lipopolysaccharide (250 µg/kg/day during the final week); SAL, saline solution.

Euthanasia and Material Preparation

Upon completion of the protocol, animals were induced with general anesthesia 48 h after the final exercise session and euthanized by deepening the inhalation of isoflurane. Subsequently, blood samples were collected via the intracardiac route for biochemical analysis, and both kidneys were stored immersed in RNAlater in microtubes and frozen at -80°C for subsequent gene expression analysis.

Hematological Analysis

Erythrocyte and leukocyte counts, as well as hemoglobin concentration, were determined using an automated cell counter (Mindray®). Hematocrit was determined using a microhematocrit centrifuge at 14,000 r.p.m for 15 minutes. Mean Corpuscular

Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined by indirect calculation.

Biochemical Analysis

Serum cytokine levels were determined using the BD™ Cytometric Bead Array (CBA), specific for IL-2, IL-4, IL-6, IL-10, IL-17a, TNF- α , and IFN- γ . Serum creatinine levels were determined via an automated colorimetric assay using the Roche Cobas c 111 biochemical analyzer (Roche Diagnostics), according to the manufacturer's instructions. Results are expressed in milligrams per deciliter (mg/dL).

Irisin Analysis

Irisin levels were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (E-EL-R2625, Elabscience®) from centrifuged plasma. The kit utilizes the principle of competitive immunoassay to detect a specific set of peptides, with a detection range between 0.1 and 1000 ng/mL. The validity of the kit for irisin measurement has been previously demonstrated (17). Plasma was collected in EDTA tubes, kept on ice, centrifuged at 3.000 rpm for 10 minutes at 4°C, and subsequently separated into two aliquots (0.5 mL each) to be stored at -20°C until subsequent analysis.

Gene Expression Analysis

Initially, samples were treated with the Trizol reagent for DNA, RNA, and protein isolation, following the manufacturer's specifications. Isolated RNA was treated with DNase enzyme to prevent DNA contamination, and subsequently stored in a freezer at -80 °C. RNA concentrations were determined using a Nanodrop® spectrophotometer (Thermo Scientific), and working solutions were prepared from these concentrations. Approximately 200ng of the obtained RNAs were applied to a 2% agarose gel and stained with ethidium bromide for integrity analysis. Retrotranscription of mRNA or total RNA for cDNA synthesis was performed using the "Platus Transcriber RNase H- cDNA First Strand" kit (Sinapse Inc ®).

RT-qPCR reactions were carried out on a QIAquant 96 5plex instrument (Qiagen), using the Sybr Green detection system (Solis Biodyne ®). Target gene

expression was calculated based on mean Ct values from duplicate runs, normalized to the internal control *GAPDH*. Absolute quantification was performed using a standard curve analysis for each gene, with results reported as absolute gene copy numbers. The primary genes of the purinergic system were amplified to verify their expression in the different biological samples. After analyzing the gene dissociation temperatures, the expression of the target genes was compared among the groups of biological samples used.

The main genes amplified were: P2X1 receptor (*P2X1*), P2X2 receptor (*P2X2*), P2X3 receptor (*P2X3*), P2X4 receptor (*P2X4*), P2X5 receptor (*P2X5*), P2X6 receptor (*P2X6*), P2X7 receptor (*P2X7*), P2Y1 receptor (*P2Y1*), P2Y6 receptor (*P2Y6*), P2Y11 receptor (*P2Y11*), P2Y12 receptor (*P2Y12*), P2Y13 receptor (*P2Y13*), P2Y14 receptor (*P2Y14*), A1 receptor (*A1A*), A2A receptor (*A2A*), A2B receptor (*A2B*), ectonucleoside triphosphate diphosphohydrolase-1 enzyme (*NTPDase1*), 5'-nucleotidase enzyme (*CD73*), and adenosine deaminase (*ADA*).

Statistical Analysis

Data distribution was assessed using the Shapiro-Wilk test. For parametric data, results are expressed as mean \pm standard deviation (SD) and were analyzed using One-Way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. For non-parametric data, results are expressed as median and interquartile range (IQR) and were analyzed using the Kruskal-Wallis test followed by Dunn's post-hoc test. All statistical analyses were performed using GraphPad Prism software, version 8.0.2. A p-value < 0.05 was considered statistically significant.

RESULTS

Body Weight

All groups showed a statistically significant weight gain throughout the study, with the SAL group demonstrating the most expressive increase (mean gain of 154 g over the seven-week protocol).

When comparing body weight halfway through the study (prior to inflammatory induction) with the final weight (after LPS administration), the ET group showed a

significant increase. In contrast, the LPS+UA and LPS+ET+UA groups showed a significant decrease in body weight during the final phase of the study (indicated by the symbol & in Table 2). The remaining groups showed no statistically significant difference between the mid-point and the end of the protocol.

Table 2. *Evolution of body weight throughout the experimental protocol*

Groups	Initial Weight (g)	Mid-Study Weight (g)	Final Weight (g)
SAL (n=9)	275.1 ±18.66* ^{&}	434.8 ±28.46	429 ±28.71 [#]
ET (n=8)	258.6 ±32.97* ^{&}	404.1 ±37.35 ^{&}	410.8 ±38.68 [#]
UA (n=8)	279.1 ±12.3* ^{&}	431.3 ±32.95	416.1 ±40.95 [#]
ET+UA (n=8)	265.8 ±25.25* ^{&}	375 ±22.81	375.9 ±21.3 [#]
LPS (n=8)	272.4 ±27.4* ^{&}	420.4 ±66.99	407.3 ±57.84 [#]
LPS+ET (n=8)	275.1 ±17.14* ^{&}	378 ±25.57	375.9 ±20.9 [#]
LPS+UA (n=8)	288.8 ±21.54* ^{&}	410.9 ±40.6 ^{&}	397.3 ±42.08 [#]
LPS+ET+UA (n=8)	240 ±18.02* ^{&}	382.5 ±40.77 ^{&}	373.8 ±37.56 [#]

Data are presented as means ± SD. Sample size: n = 8–9 per group. Statistical significance was determined by One-Way ANOVA followed by Tukey's post-hoc test for intra-group comparisons between initial, mid-study (pre-LPS), and final time points. * p < 0.05 vs. Mid-study weight; [#] p < 0.05 vs. Initial weight; [&] p < 0.05 vs. Final weight. SAL, saline; ET, exercise training; UA, ursolic acid; LPS, lipopolysaccharide.

The greatest weight gain between the initial measurement and the mid-study point was observed in the SAL (160 g) and UA (152g) groups. LPS induction led to either weight loss or a reduction in expected weight gain in all groups that received the lipopolysaccharide. However, the LPS+ET group (exercise training alone) attenuated the LPS-induced weight loss, being the only group with inflammatory induction that did not show a significant difference between the mid-study and final time points.

The weight gain observed in each group during the protocol application is illustrated in Figure 2.

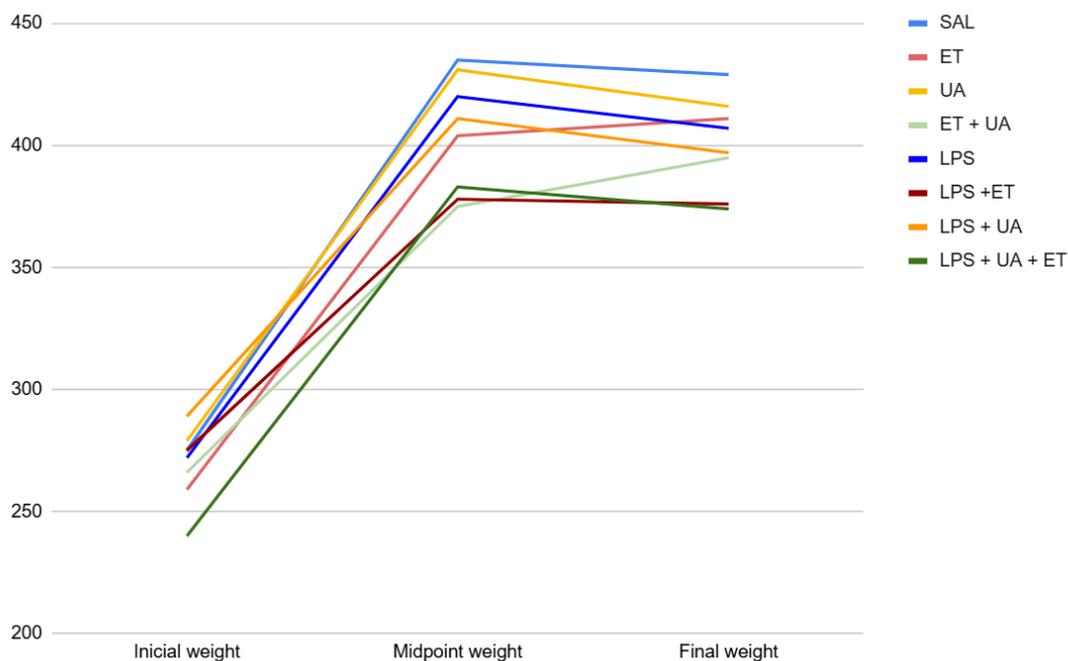


Figure 2. Evolution of Body Weight During the 7-Week Protocol. Line graph representing the mean body weight (g) of the eight experimental groups at the beginning (Initial), halfway (Midpoint, prior to LPS induction), and at the end of the study (Final). Data are expressed as mean \pm standard deviation (SD). Sample size: $n = 8-9$ per group. Abbreviations: SAL, saline; ET, exercise training; UA, ursolic acid; LPS, lipopolysaccharide.

Hematological Analysis

The LPS-induced injury model significantly affected the parameters of both the red and white blood cell series. Inflammatory induction caused a notable reduction in erythrocyte counts, hemoglobin, and hematocrit in all LPS-receiving groups, suggesting the development of inflammation-associated anemia, as observed in Figure 3.

Erythrocyte counts in the LPS group were significantly lower compared to the SAL ($p=0.0224$) and UA ($p=0.0036$) groups. The LPS+UA group was the most affected, showing diminished values compared to three control groups (SAL, ET, and UA; $p \leq 0.003$).

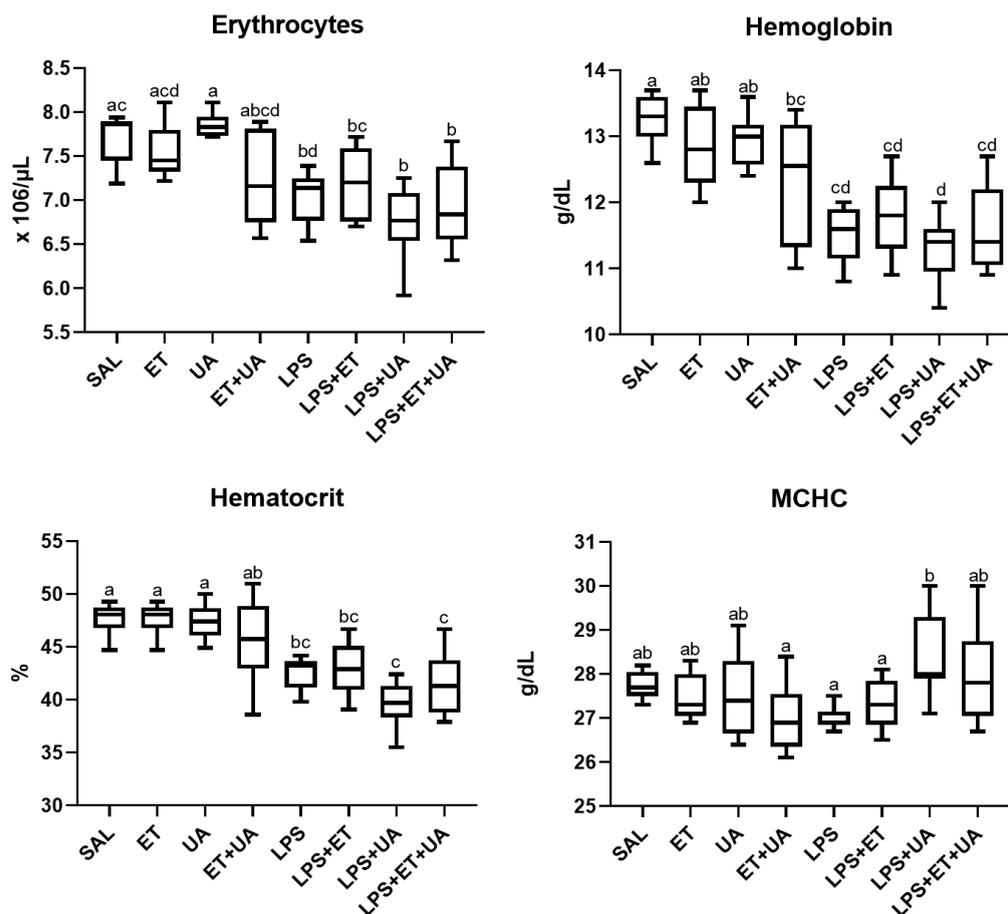


Figure 3. Impact of LPS-Induced Injury and Interventions on Red Blood Cell Series. Box-and-whisker plots showing (A) Erythrocyte count ($\times 10^6/\mu\text{L}$), (B) Hemoglobin concentration (g/dL), (C) Hematocrit percentage (%), and (D) Mean Corpuscular Hemoglobin Concentration (MCHC; g/dL). Boxes represent the median and interquartile range (25th to 75th percentiles); whiskers indicate the minimum and maximum values. Different lowercase letters indicate statistically significant differences between groups ($p < 0.05$) as determined by One-Way ANOVA followed by Tukey's post-hoc test. Sample size: $n = 8-9$ per group. Abbreviations: SAL, saline; ET, exercise training; UA, ursolic acid; LPS, lipopolysaccharide.

A similar pattern was observed for hemoglobin and hematocrit: the LPS, LPS+ET, LPS+UA, and LPS+ET+UA groups showed decreased values compared to the control groups (Supplementary Table). The LPS+UA group was statistically lower in hemoglobin relative to SAL, ET, and UA ($p \leq 0.003$).

Regarding the inflammatory response, the LPS group showed a significant increase in both the percentage and total count of monocytes compared to all non-LPS control groups (SAL, ET, UA, and ET+UA; all $p \leq 0.0206$) (Figure 4). This monocytosis, which is indicative of an inflammatory response, was maintained in the

treated groups: the LPS+UA and LPS+ET+UA groups presented significantly higher total monocyte counts than the UA group ($p \leq 0.0064$).

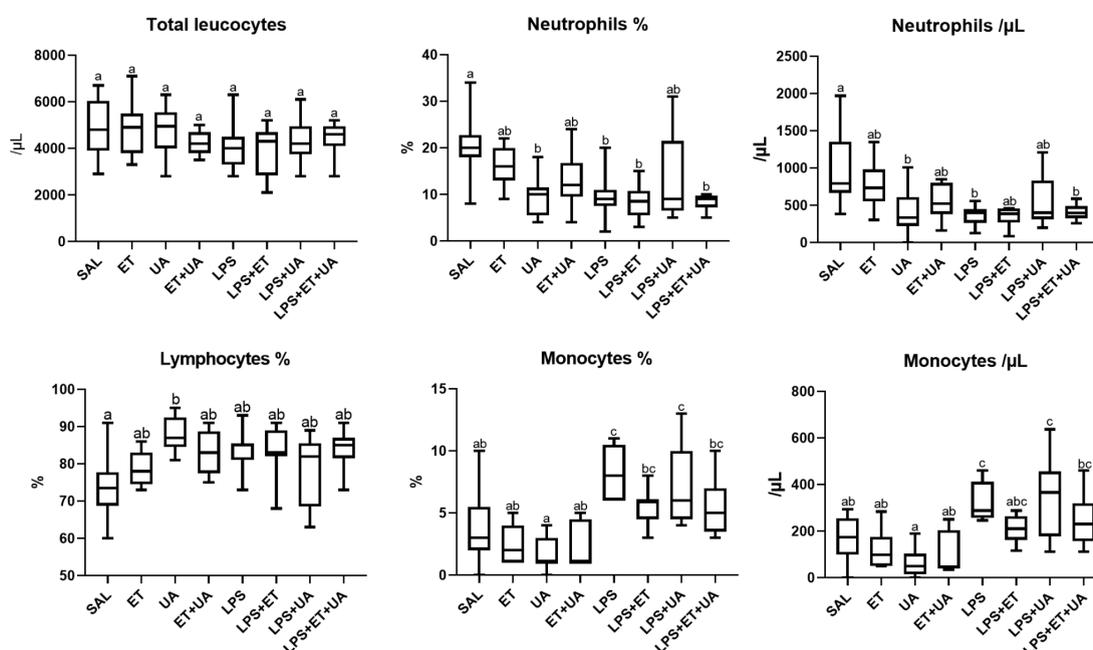


Figure 4. Impact of LPS-Induced Injury and Interventions on White Blood Cell Series. Box-and-whisker plots showing total and differential leukocyte counts. Boxes represent the median and interquartile range (IQR); whiskers show the minimum and maximum values. Different lowercase letters above the bars indicate statistical significance ($p < 0.05$). Data were analyzed using One-Way ANOVA (parametric) or Kruskal-Wallis followed by Dunn's test (non-parametric), depending on normality. Sample size: $n = 8-9$ per group. Abbreviations: SAL, saline; ET, exercise training; UA, ursolic acid; LPS, lipopolysaccharide.

Interestingly, the SAL group presented the highest neutrophil count, which was significantly higher than all LPS groups and the UA group (Supplementary Table). Total Plasma Protein values were reduced in the LPS, LPS+ET, LPS+UA, and LPS+ET+UA groups compared to the SAL group ($p \leq 0.0245$), suggesting a systemic effect of LPS on protein homeostasis (Figure 5). Other hematological parameters (MCV, platelets, total leukocytes, bands, total lymphocytes, eosinophils, and basophils) showed no statistically significant differences between the groups.

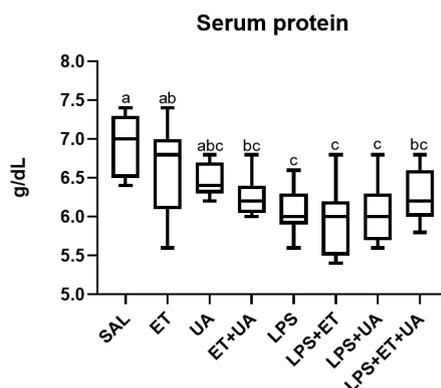


Figure 5. Total Serum Protein Levels. Box-and-whisker plots representing total protein concentration (g/dL) in serum. The median is indicated by the horizontal line within the box. Results were analyzed by One-Way ANOVA followed by Tukey's post-hoc test. Different letters indicate significant differences between groups ($p < 0.05$). Sample size: $n = 8-9$ per group. Abbreviations: SAL, saline; ET, exercise training; UA, ursolic acid; LPS, lipopolysaccharide.

Creatinine

Renal function results, as assessed by serum creatinine concentration, are presented in Figure 6. It was observed that the control (SAL), isolated exercise training (ET), isolated ursolic acid (UA), and the combined group without the inflammatory challenge (ET + UA) maintained baseline creatinine levels, with no statistical differences between them ($p > 0.05$).

LPS administration induced a sharp and significant increase in creatinine levels (1.374 ± 0.348 mg/dL) compared to the SAL group (0.488 ± 0.073 mg/dL), indicating the establishment of acute renal dysfunction in the experimental model. Isolated interventions with exercise (LPS + ET) and ursolic acid (LPS + UA) partially mitigated this increase (0.910 ± 0.143 mg/dL and 0.968 ± 0.099 mg/dL, respectively), showing significantly lower values than the LPS group, although levels remained higher than those of the SAL group. Notably, the association of exercise training with ursolic acid within the inflammatory context (LPS + ET + UA) demonstrated the most robust protective effect. This group exhibited creatinine levels (0.537 ± 0.068 mg/dL) statistically similar to the control group (SAL), suggesting that the synergy between exercise and the phytochemical was effective in preventing LPS-induced renal dysfunction.

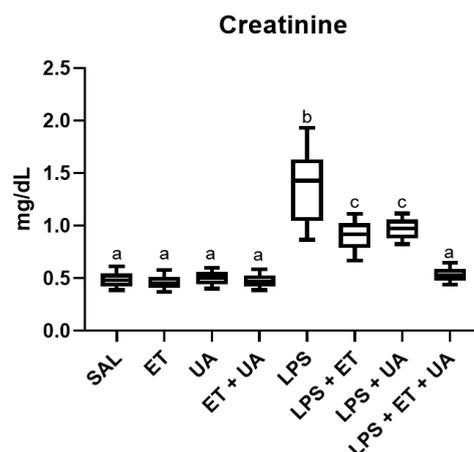


Figure 6. Evaluation of Renal Function via Serum Creatinine. Box-and-whisker plots representing creatinine levels (mg/dL) determined by an automated colorimetric assay (Roche Cobas c 111). Horizontal lines within the boxes represent the median. Different lowercase letters above the whiskers indicate statistically significant differences ($p < 0.05$) according to One-Way ANOVA followed by Tukey's post-hoc test. Sample size: $n = 8-9$ per group. Abbreviations: SAL, saline; ET, exercise training; UA, ursolic acid; LPS, lipopolysaccharide.

Irisin

LPS inflammatory induction led to a significant decrease in plasma irisin concentration (median of 2058 pg/mL (1163, 2144)) compared to all non-LPS control groups (SAL, ET, UA, and ET+UA; $p \leq 0.029$).

Intervention with exercise training (LPS+ET) alone prevented this reduction, maintaining irisin levels (3678 pg/mL (2825, 3749)) significantly greater than the LPS group ($p=0.040$) and not differing from the control groups.

In contrast, the LPS+UA group (2862 pg/mL (2806, 2893)) showed diminished levels compared to the exercise groups (ET and ET+UA; $p \leq 0.016$). The combination of the three interventions (LPS+ET+UA) did not show a statistically significant difference relative to any other group (3361 pg/mL (3305, 4342)), as visualized in Figure 7.

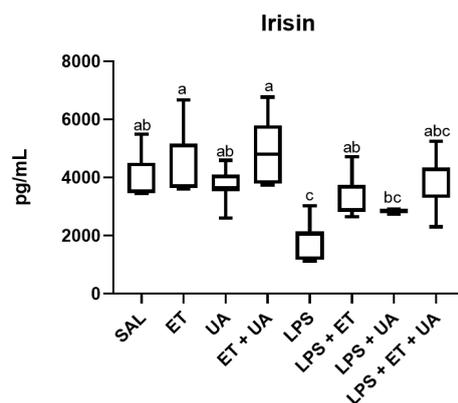


Figure 7. Plasma Irisin Concentrations. Box-and-whisker plots showing irisin levels (pg/mL) quantified by Enzyme-Linked Immunosorbent Assay (ELISA). The median, interquartile range, and min-max values are shown. Different letters indicate statistically significant differences ($p < 0.05$) using the Kruskal-Wallis test followed by Dunn's post-hoc analysis. Sample size: $n = 8-9$ per group. Abbreviations: SAL, saline; ET, exercise training; UA, ursolic acid; LPS, lipopolysaccharide.

Purinergic System

Out of the 21 components of the purinergic system analyzed, the expression of receptors P2X1, P2X2, P2X3, P2X5, P2Y4, P2Y6, A1, and A2B did not show a significant difference between the groups. The P2Y11 and P2Y14 receptors did not achieve amplification in the sample. The relevant findings are summarized in Figure 8.

The P2X4 receptor showed an expressive increase in the LPS group relative to all other groups in the study (48933 ± 2399 vs 8217 to 35973; $p < 0.0001$). The ET (LPS+ET) intervention attenuated this increase (19498 ± 2538 ; $p < 0.0001$ vs LPS), and the combined treatment (LPS+ET+UA) demonstrated the greatest attenuation (8217 ± 153 ; $p < 0.0001$ vs LPS).

The P2X7 receptor, another inflammatory marker, was also significantly elevated in the LPS group compared to the control groups (SAL, ET, and ET+UA; $p \leq 0.01$). UA treatment (LPS+UA) did not completely reverse this effect (elevated vs ET and ET+UA; $p < 0.0403$).

The expression of the P2Y1, P2Y2, P2Y12, and P2Y13 receptors was also significantly elevated in the LPS group compared to the control groups. The combined treatment (LPS+ET+UA) attenuated the increase of P2Y1 and P2Y13 compared to LPS alone ($p \leq 0.0411$).

Regarding the catabolism enzymes, the expressions of NTPDase1, CD73 and ADA were significantly increased in the LPS group compared to the majority of the control groups. The combined treatment (LPS+ET+UA) attenuated the increase of NTPDase1 compared to LPS alone ($p = 0.0124$).

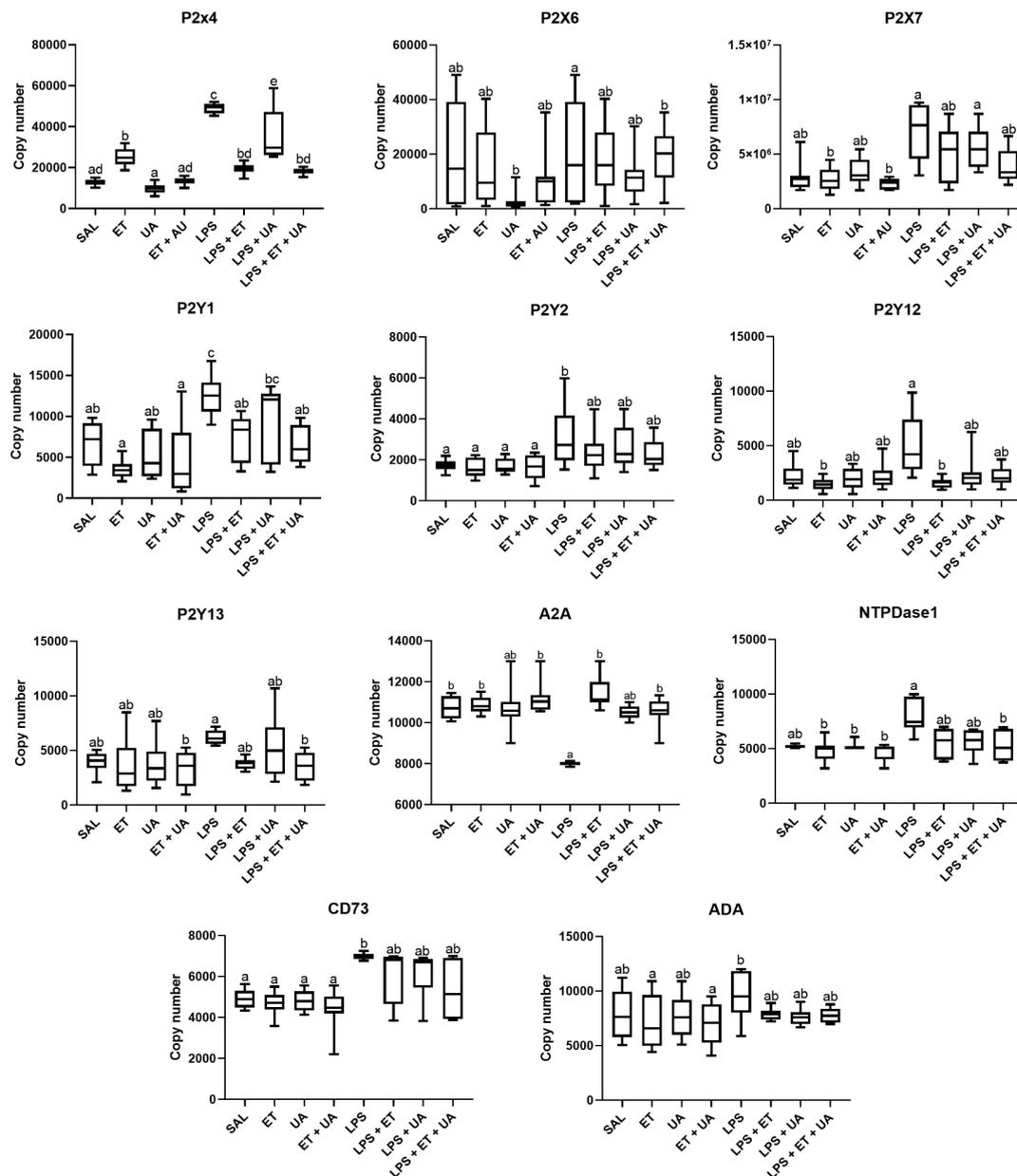


Figure 8. Relative mRNA Expression of Purinergic Receptors and Ectonucleotidases in Renal Tissue. Box-and-whisker plots illustrating the gene copy number of key purinergic signaling components (P2X4, P2X6, P2X7, P2Y1, P2Y2, P2Y12, P2Y13, A2A, NTPDase1, CD73, and ADA) determined by RT-qPCR and normalized to GAPDH. Different letters indicate statistically significant differences between groups ($p < 0.05$). Sample size: $n = 8-9$ per group. Abbreviations: SAL, saline; ET, exercise training; UA, ursolic acid; LPS, lipopolysaccharide; NTPDase1, ectonucleoside triphosphate diphosphohydrolase-1; CD73, 5'-nucleotidase; ADA, adenosine deaminase.

Cytokines

In the evaluation of systemic inflammatory mediators, only Tumor Necrosis Factor-alpha (TNF- α) and Interleukin-17a (IL-17a) showed significant results. Interleukins IL-2, IL-6, IL-4, IL-10, and IFN- γ showed no statistically significant differences between the groups.

The LPS group showed a significant increase in TNF- α (2956 pg/mL (2828, 3057)) compared to all non-LPS control groups ($p \leq 0.0014$). The exercise training (LPS+ET) intervention demonstrated an attenuation of this effect, being significantly lower than LPS alone ($p = 0.0068$). The LPS+UA group (2090 pg/mL (2002, 2157)) also showed lower values than LPS, but remained elevated relative to the controls (ET, UA, and ET+UA; $p \leq 0.0211$).

For IL-17a, the LPS group exhibited the highest increase (1713 pg/mL \pm 110.3) compared to all other groups ($p < 0.0001$). The interventions attenuated this increase, with the combined treatment (LPS+ET+UA) showing the lowest concentration among the LPS groups (1031 pg/mL \pm 52) and being significantly higher only relative to the LPS+ET (679.5 \pm 477; $p=0.0101$)

In summary, the interventions, especially exercise training and the combined therapy, attenuated the increase in LPS-induced inflammatory cytokines, as shown in Figure 9.

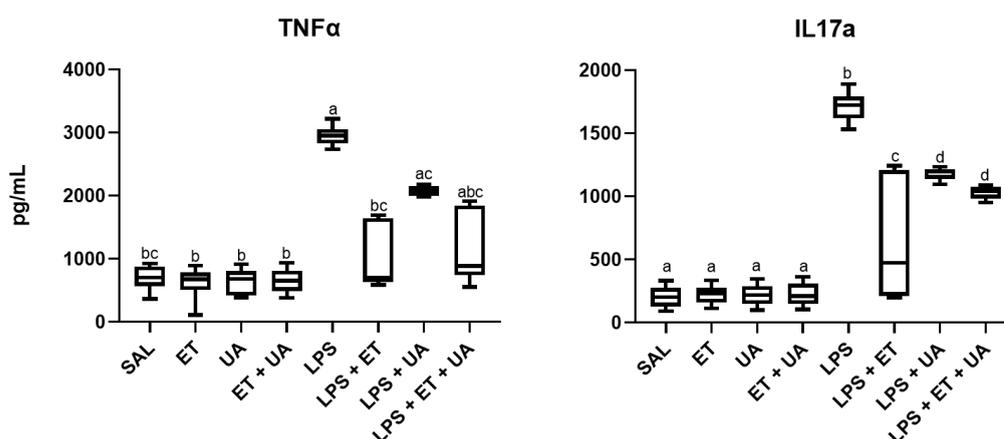


Figure 9. Serum Concentrations of Pro-inflammatory Cytokines. Box-and-whisker plots showing concentrations (pg/mL) of (A) Tumor Necrosis Factor-alpha (TNF- α) and (B) Interleukin-17a (IL-17a) determined by Cytometric Bead Array (CBA). Different lowercase letters represent significant differences between groups ($p < 0.05$). Sample size: $n = 8-9$ per group. Abbreviations: SAL, saline; ET, exercise training; UA, ursolic acid; LPS, lipopolysaccharide; TNF- α , tumor necrosis factor-alpha; IL-17a, interleukin-17a.

DISCUSSION

The present study is the first to evaluate in an integrated manner the effects of exercise training (ET) and ursolic acid (UA) on renal purinergic signaling in a low-grade inflammation model. This combination revealed synergistic interactions between the metabolic and purinergic pathways, offering new perspectives for non-pharmacological nephroprotective strategies.

The study findings demonstrate that the pro-inflammatory effect of LPS interferes with the weight gain process, reflecting the typical catabolic state of low-grade systemic inflammation, which can promote anorexia and protein degradation. Exercise training played an important role in mitigating these metabolic effects.

Regarding the hematological findings, significant alterations were observed in the red blood cell series, with a reduction in erythrocytes, hemoglobin, and hematocrit across all groups that received inflammatory induction. This suggests a negative impact of LPS on hematopoiesis and oxygen transport. The inflammatory dosage of LPS used in the study was not broad enough to cause total leukocytosis, which corroborates the study's objective of establishing a low-grade inflammation. However, despite no differences in total leukocyte counts, some subpopulations showed significant changes, such as a reduction in neutrophils and an increase in monocytes, thus confirming the inflammatory response.

Ursolic Acid Effects and Systemic Proteins

The groups that received UA administration showed ambiguous effects. In the literature, UA has been shown to stimulate the expression of the protein tyrosine phosphatase (SHP-1), which is abundantly expressed in hematopoietic cells and is involved in the negative regulation of the JAK/STAT signaling pathway (18), a process central to the immunological response provoked by infections (19). Exercise training also promoted a reduction in monocytes in the LPS groups and prevented excessive increases in leukocytes and monocytes.

The decrease in total serum proteins in the LPS, LPS+ET, and LPS+UA groups indicates compromised hepatic synthesis during the inflammatory response. There was a subtle reversal of this condition with the ET and UA combination, suggesting a mild synergistic effect in protein preservation.

The efficacy of the low-grade inflammation model and its direct impact on renal tissue were further corroborated by the assessment of renal function. The marked increase of approximately 181% in serum creatinine levels in the LPS group confirms the establishment of acute renal dysfunction. Notably, the LPS+ET+UA group was the only one to exhibit creatinine levels statistically similar to the control (SAL), demonstrating a synergistic functional protection. This finding suggests that the restoration of purinergic homeostasis and the reduction of pro-inflammatory cytokines (TNF- α and IL-17a) observed in this study are directly reflected in the preservation of the glomerular filtration rate and nephron integrity.

These systemic alterations confirm the efficacy of the low-grade inflammation model and establish the metabolic context upon which the main modifications in renal purinergic signaling were observed.

Systemic Metabolic Indicators: Irisin and Protein Homeostasis

Irisin stands out as a potential mediator of the observed effects, with exercise training (ET) being the main related factor. Irisin is encoded by the FNDC5 gene (20), and its action neutralizes the effects of metabolic reprogramming induced by TGF- β 1 in renal tubular cells (16). Furthermore, it promotes renal protection in diabetic nephropathy models via the irisin/AMPK axis (14).

The restoration of irisin levels by ET (LPS+ET group) parallels the improvements observed in the purinergic profile. However, our data suggest that while irisin may contribute to the metabolic environment, the synergy observed in the LPS+ET+UA group's renal function is likely driven by a broader modulation of ATP/adenosine balance and receptor expression (P2X4/P2X7 and A2A)

Thus, the restoration of irisin levels by exercise training may also have contributed to the purinergic modulation observed in low-grade inflammation, given that the irisin/AMPK axis is associated with the regulation of adenosine receptors and the attenuation of renal inflammatory responses.

In the evaluation of the purinergic system, LPS induction promoted the hyperactivation of several receptors and pathway enzymes, which were attenuated especially by the exercise training interventions.

Purinergic Receptors and Enzymes

The P2X4 and P2X7 receptors are crucial in inflammatory renal injury, as they are implicated in inflammasome activation and tubular cell death (4). Consistent with this, LPS induction markedly elevated the expression of both. However, exercise training (LPS+ET) and, notably, its association with ursolic acid (LPS+ET+UA), attenuated this hyperactivation, with P2X4 showing the greatest synergistic reversal. Specifically, the P2X7 receptor is known to be downregulated following physical activity, thereby decreasing ATP-mediated apoptosis and limiting the release of pro-inflammatory mediators such as TNF- α and IL-17a (21). The P2X4 receptor, in turn, participates in nitric oxide release and sodium reabsorption modulation in the distal nephron (4). In this study, the marked elevation of P2X4 in the LPS group was reversed by ET and the combination, suggesting that ET and UA inhibit pro-inflammatory ATP-dependent pathways and restore purinergic balance.

The increase in P2Y1 suggests endothelial dysfunction, which was reversed by the interventions—reinforcing the protective role of ET and UA on renal vascular integrity. P2Y1 activation contributes to glomerular injury, and knockout rats for this receptor are protected against acute nephrotoxic injury, showing preserved renal function and reduced fibrosis (22). P2Y2 receptors are known to participate in salt balance and blood pressure regulation (23). Although LPS increased its expression, no intervention significantly reversed this finding.

The P2Y12 receptor is involved in inflammation, and ET alone was shown to reverse the LPS-induced increase, aligning with the renal protective effect of P2Y12 inhibitors in acute kidney injury (22). The P2Y13 receptor, primarily activated by ADP, suggests an important role in the immune system due to its abundance in the spleen (25), which is corroborated by the increased expression observed with LPS. The capacity of ET to normalize P2Y12 and the combination with UA to suppress the increase in P2Y13 suggests a coordinated effect on endothelial and immune pathways, potentially preserving renal microcirculation.

The expression of the purinergic enzymes NTPDase1, CD73, and ADA was also evaluated. The NTPDase1/CD73 axis is responsible for increasing local adenosine concentration, which exerts anti-inflammatory and tissue-protective effects (6,26). The concomitant increase of NTPDase1, CD73, and ADA with LPS reflects the homeostatic attempt to control excess extracellular ATP. The tendency to normalization of

NTPDase1 by ET and UA - compared to ET, UA and ET+UA groups, suggests the reestablishment of the ATP/adenosine balance, which is essential for limiting the activation of pro-inflammatory receptors.

Specifically, the A2A receptor, found in the tubular epithelium and mesangial cells, is linked to immunosuppression and exerts a nephroprotective role through the promotion of vasodilation and improved renal perfusion (26,27). Thus, ET alone or in association with UA positively modulated A2A expression, preventing the decrease observed with LPS induction alone. Furthermore, the maintenance of A2A expression in the treated groups paralleled the reduction of IL-17a, pointing to an interaction between the adenosinergic system and Th17 lymphocyte regulation, thereby demonstrating the role of ET and the triterpenoid in maintaining protective adenosinergic signaling.

The P2X1, P2X2, P2X3, P2X5, P2Y4, P2Y6, A1, and A2B receptors did not present statistically significant differences, suggesting that these specific receptors may have a more limited role in the low-grade inflammatory process investigated in this study.

Integration of Purinergic Signaling and Inflammation

The effectiveness of purinergic modulation was systematically reflected in the reduction of inflammatory mediators. Increases in IL-17a and TNF- α were observed with inflammatory induction, with the LPS+ET group showing the greatest attenuation of TNF- α elevation, and the LPS+ET+UA group demonstrating the best suppression of IL-17a. These findings are consistent with the literature linking exercise training to the reduction of these cytokines in nephropathies (28,29). Although the benefit of isolated ursolic acid in reducing TNF- α was not demonstrated in this study, other works confirm its anti-inflammatory action in animal models (30). This decrease in TNF- α and IL-17a paralleled the reduction of P2X4/P2X7 and the restoration of A2A, indicating that the inflammatory control promoted by the interventions occurs, at least in part, via purinergic signaling.

Specifically, the A2A receptor—found in the tubular epithelium and mesangial cells, and linked to immunosuppression and vasodilation—was positively modulated by exercise training alone or in association with ursolic acid, preventing the decrease in its expression that occurred with isolated inflammatory induction. The activation of A2A receptors suppresses IL-17a synthesis (31), which corroborates the observed interaction

between the maintenance of A2A expression and the reduction of IL-17a in the treated groups.

Limitations

Among the study's limitations, we highlight the use of a low dose of ursolic acid, and the short intervention period. And although we included serum creatinine as a functional marker, future studies could explore more sensitive parameters of early renal injury. Nevertheless, the molecular analysis revealed robust alterations in purinergic signaling, paving the way for future studies that explore dose adjustments, exposure time, and models with more advanced renal damage.

Furthermore, while we observed a correlation between circulating irisin and the modulation of renal purinergic receptors, our study did not directly test the causal mechanisms. Additional experimental work is required to clarify the precise mechanistic link between exercise-induced irisin, the activation of the AMPK pathway in renal tissue, and the subsequent regulation of purinergic signaling. Future studies employing pharmacological inhibitors or knockdown models are necessary to confirm whether irisin acts as the primary driver of these renoprotective molecular changes.

Thus, exercise training—isolated or associated with ursolic acid—emerges as an effective modulator of renal purinergic signaling, reducing pro-inflammatory activation and preserving endothelial and adenosinergic mechanisms. Such evidence reinforces its translational potential as an adjuvant in therapies for inflammatory nephropathies.

CONCLUSION

In conclusion, the findings of this dissertation establish the renal purinergic system as a modifiable target for non-pharmacological interventions. It was demonstrated that LPS-induced inflammatory injury promoted systemic inflammation, anemia, a reduction in irisin, and pronounced acute renal dysfunction, as evidenced by elevated creatinine levels. While isolated exercise training was effective in mitigating these damages, the association with ursolic acid (LPS+ET+UA) potentiated nephroprotection, serving as the only intervention capable of fully restoring renal function to baseline levels. This synergy operates through a coordinated mechanism of

tubular and immunomodulatory protection, reducing the hyperactivation of P2X4 and P2X7 receptors and preserving the A2A adenosinergic pathway.

This study demonstrated that LPS-induced inflammatory injury promoted mild systemic inflammation, anemia, reduction in serum proteins and irisin, as well as marked renal purinergic hyperactivation. Exercise training was the most effective factor in reversing these effects, both in systemic and molecular parameters, by modulating purinergic receptors and preserving the balance between ATP and adenosine.

Among the receptors evaluated, P2X4 and P2X7 stood out, whose LPS-induced elevations were attenuated by exercise and ursolic acid, suggesting a reduction in ATP-dependent pro-inflammatory activation. This modulation of purinergic hyperactivation—marked by the overexpression of P2X4 and P2X7 and ATP/adenosine imbalance—was dramatically reversed by ET, with the ET+UA combination amplifying this effect at the molecular level. The synergy tends to normalize the expression of ectonucleotidases (NTPDase1), and restore the irisin/AMPK axis and the adenosinergic pathway through A2A, which paralleled the reduction of cytokines TNF- α and IL-17a. This action configures a coordinated mechanism of endothelial, anti-fibrotic, and immunomodulatory protection by these interventions in preventing the progression to fibrosis and renal dysfunction.

Crucially, these molecular improvements were reflected in the preservation of renal function; while LPS induction significantly elevated serum creatinine levels, the synergistic combination of exercise training and ursolic acid was the only intervention that fully restored this functional marker to baseline levels.

This study is pioneer in investigating the interaction between exercise training and ursolic acid on renal purinergic signaling, revealing a transformative potential for non-pharmacological nephroprotection. It opens the door for future research exploring different doses, exercise protocols, and inflammation models, in order to enhance the understanding and therapeutic potential of these interventions in the renal inflammatory context.

The data provide a solid preclinical basis for the development of hybrid protocols involving physical training and phytochemical supplementation as adjuvant strategies in the primary and secondary prevention of acute and chronic kidney injury, with potential for translational impact in future clinical trials.

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6 CONCLUSÃO

A presente dissertação evidencia o papel do exercício físico como modulador multifatorial da inflamação sistêmica e renal, integrando mecanismos metabólicos, imunes e purinérgicos. A revisão teórica confirmou que o exercício físico reduz citocinas pró-inflamatórias e melhora o perfil imune em pacientes com DRC, enquanto o estudo experimental demonstrou que tais efeitos se associam à modulação de receptores e enzimas da via purinérgica, com destaque para o restabelecimento do equilíbrio ATP/adenosina.

Fundamentalmente, os resultados demonstram que a disfunção renal provocada pelo LPS foi neutralizada pela intervenção combinada. Enquanto a indução inflamatória elevou drasticamente os níveis de creatinina sérica, a associação entre exercício físico e ácido ursólico foi a única estratégia capaz de restaurar plenamente a função renal aos níveis basais, confirmando o efeito nefroprotetor sinérgico desta abordagem.

A associação do exercício físico ao ácido ursólico mostrou efeitos complementares sobre a sinalização purinérgica, sugerindo potencial terapêutico sinérgico em modelos de inflamação de baixo grau. Esses achados fortalecem a compreensão do papel do sistema purinérgico como elo entre atividade física e proteção renal, além de apontarem caminhos para abordagens integradas de prevenção e manejo da DRC.

Assim, este conjunto de estudos contribui para o avanço do conhecimento sobre os mecanismos biológicos do exercício físico e destaca a importância de estratégias não farmacológicas no enfrentamento de doenças renais associadas à inflamação crônica.

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ANEXO A - Certificado Comissão de Ética no Uso de Animais



Comissão de Ética no Uso de Animais

da

Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "EFEITO DO EXERCÍCIO FÍSICO E DO ÁCIDO URSÓLICO NA LIBERAÇÃO DE IRISINA EM RATOS SUBMETIDOS A NEUROINFLAMAÇÃO POR LIPOPOLISSACARÍDEOS.", protocolada sob o CEUA nº 6805060821 (ID 003483), sob a responsabilidade de **Andréia Machado Cardoso e equipe; Cinthia Melazzo de Andrade; Erin John Rieger de Almeida; Guilherme Lopes Dornelles; Stéfani dos Santos Torres** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 17/08/2021.

We certify that the proposal "THE EFFECT OF PHYSICAL EXERCISE AND URSOLIC ACID ON THE RELEASE OF IRISIN IN RATS SUBMITTED TO NEUROINFLAMATION BY LIPOPOLYSACCHARIDE.", utilizing 104 Heterogenics rats (104 males), protocol number CEUA 6805060821 (ID 003483), under the responsibility of **Andréia Machado Cardoso and team; Cinthia Melazzo de Andrade; Erin John Rieger de Almeida; Guilherme Lopes Dornelles; Stéfani dos Santos Torres** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 08/17/2021.

Finalidade da Proposta: [Pesquisa](#)

Vigência da Proposta: de [09/2021](#) a [10/2022](#)

Área: [Departamento de Métodos Técnicas Desportivas](#)

Origem: [Biotério Central UFSM](#)

Espécie: [Ratos heterogênicos](#)

sexo: [Machos](#)

idade: [6 a 7 semanas](#)

N: [104](#)

Linhagem: [Wistar](#)

Peso: [200 a 230 g](#)

Local do experimento: experimento: Os animais serão mantidos no Biotério de experimentação de ratos ou camundongos, do Prédio 21. As análises laboratoriais serão desenvolvidas no Laboratório de Bioquímica e Estresse Oxidativo (Bio-Ox) e Laboratório de Análises Clínicas Veterinário (LACVet) da UFSM.

Santa Maria, 25 de novembro de 2021

Profa. Dra. Patrícia Severo do Nascimento
Presidente da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Presidente da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

APÊNDICE A

Tabela A1 - Material Suplementar do Manuscrito

(Continua)

Assessed variable	SAL (n=9)	ET (n=8)	UA (n=8)	ET +UA (n=8)	LPS (n=8)	LPS+ET (n=8)	LPS+UA (n=8)	LPS+Et+U A (n=8)
Erythrocytes x10 ⁹ /L	7,689 ±0,2780	7,554 ±0,2919	7,859 ±0,1374	7,099 ±0,6922	7,031 ±0,2861	7,209 ±0,3989	6,748 ±0,4011 ^{**\$}	6,963 ±0,4723 ^{**\$}
Hemoglobin g/dL	13,28 ±0,3801	12,86 ±0,6023	12,95 ±0,3891	11,93 ±1,423*	11,52 ±0,4353 ^{**\$}	11,74 ±0,5833 ^{**\$}	11,29 ±0,4755 ^{**\$}	11,63 ±0,6442 ^{**\$}
Hematocrit (%)	47,72 ±1,515	47,72 ±1,515	47,37 ±1,598	44,10 ±5,415	42,58 ±1,519 ^{**\$}	42,88 ±2,436 ^{**\$}	39,56 ±2,068 ^{**%}	41,60 ±2,918 ^{**\$}
Mean Corpuscular Volume (fL)	62,19 ±2,283	61,82 ±1,666	60,75 ±1,768	62,11 ±3,548	60,67 ±2,630	59,57 ±1,179	58,74 ±1,667	59,88 ±2,927
Mean corpuscular hemoglobin concentration g/dL	27,77 ±0,3082	27,51 ±0,5036	27,49 ±0,9418	27,00 ±0,7399	27,04 ±0,2351 ^{&}	27,34 ±0,5593 ^{&}	28,50 ±0,9354 [°]	27,97 ±1,057
Platelets (x10 ⁹ /L)	814,8 ±90,29	817,1 ±92,29	781,9 ±79,48	802,3 ±132,0	837,6 ±135,6	938,3 ±175,7	788,8 ±132,4	917,4 ±220,3
Total leukocytes /μL	4.200 ±1.277	5.200 ±1.188	5.600 ±1,092	5.600 ±1.092	4.900 ±0.5268	4.100 ±1.028	4.800 ±1.063	4.700 ±0.9501

Tabela A1 - Material Suplementar do Manuscrito

(Continuação)

Assessed variable	SAL (n=9)	ET (n=8)	UA (n=8)	ET +UA (n=8)	LPS (n=8)	LPS+ET (n=8)	LPS+UA (n=8)	LPS+Et+UA (n=8)
Bands / μ L	0	0	0	0	0	0	0	0
Neutrophils (%)	20,38 $\pm 7,249$	16,22 $\pm 4,206$	9,333 $\pm 4,3559^*$	9,333 $\pm 4,359^*$	16,67 $\pm 12,00$	9,667 $\pm 4,822^*$	8,500 $\pm 3,780^*$	13,44 $\pm 9,098$
Neutrophils / μ L	974,8 $\pm 510,4$	790,4 $\pm 309,1$	417,1 $\pm 294,1$	417,1 $\pm 294,1$	711,8 $\pm 541,0$	358,8 $\pm 133,8^*$	348,5 $\pm 132,7$	553,8 $\pm 354,1$
Lymphocytes (%)	74,00 $\pm 8,912$	78,89 $\pm 4,729$	9,333 $\pm 4,3559^*$	87,89 $\pm 4,595^*$	80,11 $\pm 11,22$	83,44 $\pm 5,364$	83,44 $\pm 6,821$	77,89 $\pm 9,597$
Lymphocytes / μ L	3.187 ± 1.306	3.758 $\pm 839,0$	3755 \pm 1657	3.755 ± 1.657	3.380 $\pm 567,3$	3.411 ± 1.066	3.319 ± 1.015	3.416 $\pm 977,7$
Monocytes (%)	3,778 $\pm 2,991$	2,556 $\pm 1,509$	1,667 $\pm 1,323$	1,667 $\pm 1,323$	2,444 $\pm 1,810$	8,444 $\pm 2,128^{*}\$$	5,444 $\pm 1,424^{*}\$$	7,333 $\pm 3,162^{*}\$$
Monocytes / μ L	167,0 $\pm 97,18$	120,4 $\pm 81,11$	62,67 $\pm 64,77$	62,67 $\pm 64,77$	107,8 $\pm 89,75$	332,1 $\pm 84,18^{*}\$$	207,1 $\pm 58,73$	330,6 $\pm 172,2^{*}\$$
Eosinophils (%)	1,444 $\pm 1,424$	2,000 $\pm 1,414$	0,5556 $\pm 0,8819$	0,5556 $\pm 0,8819$	0,7788 $\pm 0,6667$	1,111 $\pm 1,014$	1,222 $\pm 1,054$	0,6667 $\pm 0,6667$

Tabela A1 - Material Suplementar do Manuscrito

(Continuação)

Assessed variable	SAL (n=9)	ET (n=8)	UA (n=8)	ET +UA (n=8)	LPS (n=8)	LPS+ET (n=8)	LPS+UA (n=8)	LPS+Et+U A (n=8)
Eosinophils / μ L	66,78 \pm 63,03	103,9 \pm 91,24	18,22 \pm 42,33	31,81 \pm 37,43	22,00 \pm 27,43	46,33 \pm 37,68	50,56 \pm 47,61	37,44 \pm 22,35
Basophils / μ L	0	0	0	0	0	0	0	0
Plasma proteins g/dL	6,889 \pm 0,4014	6,644 \pm 0,5981	6,467 \pm 0,2236	6,275 \pm 0,2605	6,067 \pm 0,3000*	5,933 \pm 0,4472*	6,067 \pm 0,3873*	6,267 \pm 0,3464
Irisin (Median [p25, p75])	3516 [3476, 4519]	3712 [3630, 5170]	3591 [3580, 4104]	4813 [3788, 5798]	2058 [1163, 2144]	3678 [2825, 3749]	2862 [2806, 2893]	3361 [3305, 4342]
P2X1 (Mean \pm sd)	10195 \pm 662,7	10391 \pm 528,4	10470 \pm 694,6	10155 \pm 426,7	10678 \pm 467,9	10532 \pm 482,5	10455 \pm 458,2	10413 \pm 468,4
P2X2 (Mean \pm sd)	10345 \pm 506,6	10489 \pm 380,8	10323 \pm 436,1	10432 \pm 475,3	10543 \pm 329,8	10743 \pm 329,8	10422 \pm 261	10394 \pm 423,8
P2X3 (Mean \pm sd)	10464 \pm 472,4	10557 \pm 383,9	10388 \pm 395,7	10539 \pm 360,1	10579 \pm 360,1	10645 \pm 307,1	10383 \pm 261,1	10488 \pm 414,2

Tabela A1 - Material Suplementar do Manuscrito

(Continuação)

Assessed variable	SAL (n=9)	ET (n=8)	UA (n=8)	ET +UA (n=8)	LPS (n=8)	LPS+ET (n=8)	LPS+UA (n=8)	LPS+Et+UA (n=8)
P2X4 (Mean ± sd)	12903 ± 1566 ^{a, b, e}	25300 ± 4332 ^{a, c, d, e, f}	9771 ± 2394 ^{a, b, e, g, h}	13298 ± 1768 ^{a, b, e}	48933 ± 2399 ^{b, c, d, e, f, g, h}	19498 ± 2538 ^{a, e, f}	35973 ± 13015 ^{a, b, c, d, f, g, h}	18217 ± 1532 ^{a, e, f}
P2X5 (Mean ± sd)	10401 ± 434,1	10517 ± 378,5	10350 ± 398,9	10473 ± 360,1	10520 ± 479,6	10608 ± 292,9	10308 ± 262,9	10420 ± 427,9
P2X6 (Median [p25, p75])	14716 [1609, 39124]	9514 [3254, 28037]	1477 [830,3, 2745] ^{a, h}	10085 [2291, 11754]	15985 [2253, 39124] ^f	15963 [8451, 28037]	11363 [6282, 14355]	20258 [11404, 26647] ^f
P2X7 (Median [p25, p75])	2.730.035 [1.988.797, 3.016.791] ^a	2.537.994 [1.802.275, 3.583.338] ^{a, e}	3.049.203 [2.530.713, 4.511.415]	2.409.220 [1.757.621, 2.753.824] ^{a, e}	7.654.931 [4.583.646, 9.500.244] ^{b, c, d}	5.462.494 [2.337.017, 7.058.713]	5.462.494 [3.837.018, 7.058.713] ^{b, c}	3.349.203 [2.747.712, 5.289.984]
P2Y1 (Mean ± sd)	6730 ± 2630 ^a	4616 ± 3337 ^{a, e}	5148 ± 2892 ^a	4943 ± 4219 ^{a, e}	12537 ± 2380 ^{b, c, d, f, g, h}	7306 ± 2732 ^a	9385 ± 4312 ^{b, c}	6536 ± 2210 ^a
P2Y2 (Mean ± sd)	1909 ± 614,1	1609 ± 455 ^a	1822 ± 486,4 ^a	1842 ± 799	3131 ± 1456 ^{b, f}	2387 ± 979,8	2711 ± 1020	2287 ± 726,3

Tabela A1 - Material Suplementar do Manuscrito

(Continuação)

Assessed variable	SAL (n=9)	ET (n=8)	UA (n=8)	ET +UA (n=8)	LPS (n=8)	LPS+ET (n=8)	LPS+UA (n=8)	LPS+Et+UA (n=8)
P2Y4 (Mean ± sd)	4112 ± 1476	3684 ± 819,7	4352 ± 1029	3958 ± 966,3	6445 ± 4047	3763 ± 776,2	6241 ± 2711	5736 ± 3297
P2Y6 (Mean ± sd)	372,3 ± 157,1	338 ± 157,1	323,9 ± 187,4	321,1 ± 153,0	439 ± 153,0	396,9 ± 246,8	423,9 ± 309,6	398,8 ± 160,2
P2Y12 (Median [p25, p75])	1894 [1436, 2915]	1444 [1020, 1874] a	1933 [1165, 2900]	1933 [1399, 2734]	4221 [2865, 7394] b, g	1645 [1146, 1874] a	2042 [1426, 2564]	2024 [1623, 2884]
P2Y13 (Mean ± sd)	3919 ± 918,8	3543 ± 2495	3803 ± 1922	3251 ± 1566 a	6163 ± 639,1 c, h	3765 ± 512,9	5359 ± 2813	3473 ± 1294 a
A1 (Mean ± sd)	8007 ± 78,33	8006 ± 74,94	7975 ± 95,13	8042 ± 104,3	7997 ± 124,4	8040 ± 98,00	7959 ± 98,00	8057 ± 73,13
A2B (Mean ± sd)	10825 ± 561,9	10747 ± 561,9	10874 ± 466,7	10947 ± 466,7	11033 ± 519,6	11128 ± 324,2	10817 ± 301,2	10918 ± 420,7
A2A (Median [p25, p75])	10706 [10209, 11295] a	10804 [10554, 11222] a	10585 [10306, 11020]	11041 [10632, 11359] a	7994 [7955, 8070] b, c, d, g, h	11140 [10999, 11993] a	10500 [10250, 10800]	10601 [10362, 11046] a

Tabela A1 - Material Suplementar do Manuscrito

(Continuação)

Assessed variable	SAL (n=9)	ET (n=8)	UA (n=8)	ET +UA (n=8)	LPS (n=8)	LPS+ET (n=8)	LPS+UA (n=8)	LPS+Et+U A (n=8)
NTPDase1 (Median [p25, p75])	5194 [5078, 5340]	5022 [4061, 5263] ^a	5109 [5012, 5194] ^a	5066 [4027, 5190] ^a	7456 [6939, 9768] ^{b, c, f, h}	5755 [4004, 6825]	5778 [4812, 6678]	5083 [3903, 6825] ^a
CD73 (Median [p25, p75])	4896 [4487, 5301] ^a	4721 [4389, 5101] ^a	4799 [4345, 5278] ^a	4467 [4203, 5018] ^a	6934 [6861, 7094] ^{b, c, d, f}	6806 [4664, 6973]	6712 [5465, 6870]	5138 [3927, 6919]
ADA (Mean ± sd)	7834 ± 2171	7149 ± 2430 ^a	7723 ± 1862	6984 ± 1950 ^a	9668 ± 2100 ^{b, c}	7911 ± 515,7	7619 ± 727,9	7810 ± 548,9
IL-2 (Median [p25, p75])	320,5 [239,2, 434,3]	329,4 [287,3, 384,7]	316,8 [242,1, 387,7]	316,0 [253,2, 399,7]	312,1 [245,9, 406,7]	328,5 [293,6, 386,5]	359,8 [268,5, 411,5]	302,0 [265,1, 341,1]
IL-6 (Mean ± sd)	936,4 ± 411,2	921,4 ± 331,8	829,1 ± 308,8	926,1 ± 375,8	1273 ± 710,7	911,9 ± 324,9	933,5 ± 377,4	929,6 ± 369
IL-4 (Mean ± sd)	84,83 ± 35,48	94,67 ± 31,13	91,38 ± 34,39	93,72 ± 35,34	92,46 ± 36,16	98,59 ± 26,37	99,78 ± 30,85	91,73 ± 34,03

Tabela A1 - Material Suplementar do Manuscrito

Assessed variable	(Conclusão)							
	SAL (n=9)	ET (n=8)	UA (n=8)	ET +UA (n=8)	LPS (n=8)	LPS+ET (n=8)	LPS+UA (n=8)	LPS+Et+UA (n=8)
IFN γ (Mean \pm sd)	587,3 \pm 306,9	471,9 \pm 138,7	523,2 \pm 189,6	481,0 \pm 163,1	670,3 \pm 431,8	469,2 \pm 137,9	484,1 \pm 159,1	476,3 \pm 162,6
TNF α (Median [p25, p75])	701,6 [566,8, 879] ^a	673,9 [509,4, 780,5] ^{a,e}	679,5 [415,7, 803,6] ^{a,e}	655,3 [487,6, 811,8] ^{a,e}	2956 [2828, 3057] ^{b,c,d,f,g}	701,8 [487,6, 1642] ^a	2090 [2002, 2157] ^{b,c,e}	889,5 [737,6, 1845]
IL-17a (Mean \pm sd)	204,0 \pm 81,52	219,1 \pm 71,45	218,1 \pm 82,34	226,8 \pm 87,67	1713 \pm 110,3	679,5 \pm 477,1	1181 \pm 49,1	1031 \pm 52,03
IL-10 (Mean \pm sd)	258,2 \pm 99,72	277,4 \pm 78,08	258,1 \pm 91,2	281,0 \pm 91,04	197,7 \pm 68,06	283,1 \pm 96,41	295,6 \pm 97,93	287,0 \pm 96,56
Irisin (Median [p25, p75])	3516 [3476, 4519] ^a	3712 [3630, 5170] ^{a,e}	3591 [3580, 4104] ^a	4813 [3788, 5798] ^{a,e}	2058 [1163, 2144] ^{b,c,d,f,g}	3678 [2825, 3749] ^a	2862 [2806, 2893] ^{b,c}	3361 [3305, 4342]
Creatinine (Mean \pm sd)	0,488 \pm 0,073 ^{a,g,h}	0,464 \pm 0,066 ^{a,e,g}	0,501 \pm 0,067 ^{a,e,g}	0,477 \pm 0,0646 ^{a,e,g}	1,374 \pm 0,348 ^{b,c,d,e,f,g,h}	0,910 \pm 0,143 ^{a,b,c,d,f,h}	0,968 \pm 0,099 ^{a,b,c,d,f,h}	0,537 \pm 0,068 ^{a,e,g}

Data are presented as means \pm SD for parametric variables or as median [interquartile range, IQR] for non-parametric variables. Sample size: n = 8–9 per group. Statistical significance was determined by One-Way ANOVA followed by Tukey's post-hoc test (parametric) or the Kruskal-Wallis test followed by Dunn's post-hoc test (non-parametric).

Statistical Significance (Inter-group comparisons): Lowercase letters denote significant differences ($p < 0.05$) compared to a specific group, as follows: a vs. LPS; b vs. ET; c vs. ET+UA; d vs. SAL; e vs. LPS+UA; f vs. UA; g vs. LPS+ET; h vs. LPS+ET+UA.

Statistical Significance (Intra-group temporal comparisons): For body weight and longitudinal data, symbols denote significant differences ($p < 0.05$) within the same group at different time points: **** vs. Mid-study weight; # vs. Initial weight; & vs. Final weight.

Abbreviations: SAL, saline; ET, exercise training; UA, ursolic acid; LPS, lipopolysaccharide; RBC, red blood cells; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cells; TNF- α , tumor necrosis factor alpha; IL, interleukin; IFN- γ , interferon gamma; NTPDase1, ectonucleoside triphosphate diphosphohydrolase-1; CD73, 5'-nucleotidase; ADA, adenosine deaminase.