UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

Ana Paula Fernandes Ribeiro

O exercício físico isolado ou combinado com o ômega-3 modula a periodontite apical induzida em ratos

Araçatuba
2024

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

Ana Paula Fernandes Ribeiro

O exercício físico isolado ou combinado com o ômega-3 modula a periodontite apical induzida em ratos

Tese apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do título de Doutora em Endodontia.

Orientador: Prof. Ass. Dr. Rogério de Castilho Jacinto

Coorientador: Prof. Ass. Dr. Luciano Tavares Ângelo Cintra

Araçatuba
2024

Catalogação na Publicação (CIP)

Diretoria Técnica de Biblioteca e Documentação – FOA / UNESP

R484e

Ribeiro, Ana Paula Fernandes.
O exercício físico isolado ou combinado com o ômega-3 modula a periodontite apical induzida em ratos / Ana Paula Fernandes Ribeiro. - Araçatuba, 2024
39 f. : il. ; tab.

Tese (Doutorado) – Universidade Estadual Paulista (UNESP), Faculdade de Odontologia, Araçatuba
Orientador: Prof. Rogério de Castilho Jacinto
Coorientador: Prof. Luciano Tavares Angelo Cintra

1. Periodontite periapical 2. Natação 3. Exercício físico 4. Endodontia 5. Ácidos graxos ômega-3 I. T.

Black D24
CDD 617.67

Claudio Hideo Matsumoto – CRB-8/5550

Dedico essa tese à minha mãe, **Deolinda Fernandes Tomazini**.

Mãe, você sempre lutou para que eu pudesse realizar os meus sonhos e sempre me deu asas para que eu pudesse voar cada vez mais alto. Nunca mediu esforços por mim e sempre apoiou e incentivou todos os meus sonhos e meus passos. Muito obrigada por TUDO!

Sua fé e amor me sustentam. Sua força, garra e dedicação me inspiram. Obrigada por ser esse exemplo de ser humano. Obrigada por ter um coração enorme e um caráter intocável. Sem dúvidas, é a melhor mãe que Deus poderia me dar, te escolheria mil vezes! Você tem o meu amor, respeito e gratidão, eternamente! Espero que um dia eu possa ser, pelo menos, metade do ser humano maravilhoso que você é, e que eu consiga retribuir tudo o que já fez por mim. Que Deus te abençoe! Eu te amo demais!

Agradecimentos

A Deus

Que me sustenta, me ama e nunca me abandona!

Obrigada meu Pai, por ter preparado que eu viesse para a Unesp-FOA, aqui fiz meu mestrado, minha especialização, tive minha primeira oportunidade como docente e agora chego ao fim de mais um sonho, meu doutorado. Obrigada por me capacitar e fortalecer todos os dias, por me proteger nas estradas, me amparar e por colocar pessoas tão maravilhosas na minha vida.

Sou infinitamente grata pois, o Senhor providencia tudo! Foram muitas lutas e muitas perdas durante esse processo, mas o Senhor sempre providenciou bênçãos e mais bênçãos, sempre me deu assas quando faltou o chão. Por fim, obrigada por ter planejado uma vida linda para mim, uma vida muito melhor do que a que eu planejei.

À Nossa Senhora

Obrigada minha Mãezinha do céu, por sempre passar à frente de todas as situações na minha vida! Obrigada por me cobrir com seu mato de amor e por me proteger. Com seu amor e poder de Mãe, sei que as coisas sempre terminarão bem. Obrigada também, por interceder por mim diante do Pai e por todas as graças e bênçãos já recebidas.

Aos Familiares

Quero agradecer meus pais, **Deolinda Fernandes Tomazini** e **Paulo Roberto Ribeiro da Silva**, por me apoiarem e me amarem tanto. Obrigada por terem me passado tantos valores. Com os pequenos exemplos do dia a dia, aprendi a ser uma pessoa correta e dar valor a tudo e todos que estão à minha volta. Obrigada pelos ensinamentos e principalmente, por me ensinarem a amar. Tento, todos os dias, ser a melhor filha, pessoa e profissional possível, porque tenho pais maravilhosos, que merecem todo amor, carinho e respeito em retribuição! Eu amo vocês! Um agradecimento especial à minha mãe, que cuida de mim nos pequenos e grandes detalhes e que, mesmo com tanta dificuldade me manteve onde sempre sonhei. Eu te amo minha guerreira!

Ao Orientador

Prof. Rogério de Castilho Jacinto, ao senhor, minha eterna gratidão, por toda orientação no mestrado, na especialização e no doutorado. Foi e sempre será uma honra fazer parte da sua equipe de pesquisa e tê-lo como orientador. Obrigada por estar sempre disponível, por sempre me receber com muita educação e paciência. Obrigada por compartilhar comigo todo seu conhecimento, o senhor é uma inspiração para mim. Que Deus sempre o abençoe!

Aos Professores

Minha gratidão a todos os professores do departamento, **prof. Gustavo Sivieri de Araújo**, **prof. Elói Dezan Junior**, **prof. João Eduardo Gomes Filho**, **prof. Luciano Tavares Ângelo Cintra** e **prof. Rogério de Castilho Jacinto**, vocês são inspirações e exemplos. Obrigada pela dedicação, respeito e conhecimento, de todos os dias. É um privilégio conviver e aprender com vocês, grandes nomes da endodontia brasileira.

Um agradecimento especial ao **prof. Luciano Tavares Ângelo Cintra,** meu coorientador. Obrigada por compartilhar seu tempo, sua experiência, seus conhecimentos e seu trabalho, o senhor foi uma peça fundamental e indispensável para o desenvolvimento desse projeto. Que honra ter trabalhado ao seu lado. Eu aprendi muito com o senhor!

A Banca

Prof. Edilson Ervolino, meu eterno agradecimento por ceder seu laboratório para que a análise imuno-histoquímica fosse realizada. Obrigada por toda atenção prestada, por sempre nos receber com muito carinho e por ter aceitado compor a banca do exame geral de qualificação. Agradeço suas considerações para este trabalho, pois elas contribuíram muito para aperfeiçoá-lo.

Ao **prof. Caio Sampaio,** obrigada por ter aceitado compor a banca do exame geral de qualificação, esse é um momento muito importante para mim e para o enriquecimento do trabalho. Agradeço também por todo o convívio

durante a pós-graduação, por sempre ser muito educado e solicito. É uma inspiração para mim.

Agradeço ao **prof. Elói Dezan Junior**, que durante minha toda minha jornada na Unesp, me incentivou a ser docente, a desenvolver minhas habilidades clínicas e me impulsionou a realizar o curso de especialização. O senhor enxergou algo em mim, quando nem eu mesma tinha visto ainda e seu apoio e confiança em mim, me fizeram dar esse passo. Hoje me sinto mais completa e pronta para a vida acadêmica e clínica. Sua presença como parte da banca da minha defesa de tese, era indispensável!! Obrigada por fazer parte de mais essa etapa da minha vida. Jamais esquecerei do senhor.

Ao **prof. prof. Marco Antonio Hungaro Duarte,** obrigada por ter aceitado compor a banca da minha defesa de tese, seu nome sempre esteve na minha mente para esse momento. O senhor tem minha admiração! Além de ser um excelente professor e pesquisador, que é referência na área, é uma pessoa humilde e educada. Obrigada por fazer parte desse momento tão importante da minha vida.

A **profa. Francine Benetti,** obrigada Fran por aceitar tão prontamente o convite para fazer parte da banca da minha defesa de tese. Você é uma inspiração para mim! Lembro quando entrei na pós e te conheci, pensei: quero ser como ela. Sempre batalhadora, inteligente, educada e um amor de pessoa. Que bom ter conhecido você e mais ainda, que bom poder ter você aqui de novo, nesse momento tão importante para mim. Tudo de melhor na sua vida Fran, você merece!!

Ao **prof. João Miguel Marques dos Santos,** é uma honra ter o senhor participando da minha defesa de tese. Sei que nessa visita a universidade, o senhor está com a agenda cheia de compromissos e ainda assim aceitou participar desse momento. Obrigada por dedicar um tempo para corrigir meu trabalho e por ter aceitado o convite.

Aos Amigos

Aos amigos **Natália Machado, Ana Cláudia, Gladiston, Henrique, Flavio, Cristiane, Pedro, Carol Loureiro, Júlia, Laura, Bharbara, Rômulo, Julissa, Rafaela, Larissa, Ana Maria, Carol Barros, Sabrina** obrigada por todas as conversas, risadas, por todo conhecimento e experiência trocados. Levarei vocês eternamente em meu coração!!

A equipe Castilho **Carol Loureiro, Júlia, Gladiston, Laura, Sabrina e Yuri**, obrigada por toda ajuda, todo carinho e todos os trabalhos juntos. Os dias no laboratório foram mais leves e felizes porque vocês estavam lá. Eu jamais me esquecerei do que vivemos!!

Minha eterna gratidão a minha segunda equipe de pesquisa, **Cris, Nati, Pedro, Carol e Michely**. Sem vocês tudo seria muito mais difícil!! Obrigada pelos dias no biotério, por toda ajuda e ensinamentos desde o manejo com os animais até os procedimentos experimentais. Vocês têm um lugar muito especial no meu coração.

Agradeço especialmente a **Michely de Lima Rodrigues**, aluna dedicadíssima de dois projetos de iniciação científica FAPESP associados a esta tese. Mi, você foi meu braço direito! Como sou grata por você ter aparecido e ter abraçado esse projeto comigo. Muito obrigada por sua dedicação, por seu trabalho árduo e pela nossa convivência! Eu torço muito por você. Você merece voar alto. Conte comigo sempre!

Não posso me esquecer de quem sempre andou ao meu lado, acompanhou minha história para chegar até aqui e dividiu comigo a alegria por mais essa conquista. A vocês meus amigos, **Mirela**, **Guilherme, Meghy**, **Marcos, Amanda**, **Kátia e Letícia,** o meu muito obrigada! Cada apoio, palavra e carinho de vocês foi fundamental em minha vida. Eu amo vocês!

Aos Funcionários

Obrigada **Autran Santiago, Jorge Trevelim**, **Carlos Kazu** e **Andressa**, funcionários do Departamento, pois vocês sempre me ajudam de alguma forma. Obrigada pelo acolhimento, pelo respeito e pela amizade. Vocês contribuem muito para o bom funcionamento do departamento. Que Deus os abençoe!

As funcionárias da seção de Pós-Graduação, **Valéria de Queiroz M. Zagatto**, **Cristiane Regina Lui Matos** e **Lilian Sayuri Mada,** obrigada por toda atenção, disposição e ajuda.

Aos Animais

Os ratos *wistar,* fornecidos pelo biotério central da Universidade, foram fundamentais para o desenvolvimento desse projeto, sem eles não seria possível estudar a relação entre atividade física e periodontite apical.

À Faculdade de Odontologia de Araçatuba

Na pessoa do Diretor **Prof. Tit. Alberto Carlos Botazzo Delbem** e do Vice-Diretor **Prof. Luciano Tavares Angelo Cintra**, por proporcionarem a realização desta pesquisa.

A coordenação do Curso de Pós-Graduação em Ciência Odontológica, na pessoa do **Prof. Assoc. Juliano Pelim Pessan** e o Vice Coordenador

Prof. Assoc. Rogério de Castilho Jacinto. Ao Chefe de Departamento **Prof. Assoc. Ronald Jefferson Martins** e o Vice-Chefe **Prof. Assoc. Eloi Dezan Júnior,** obrigada por toda dedicação e trabalho.

Às Agências de Fomento

Obrigada a **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001**, processo nº 88887.644332/2021-00 por ter fomentado minha bolsa de doutorado e a Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), processos nº 2020/13089-3 e nº 2022/04884-0, por terem apoiado dois projetos de iniciação científica vinculados a esta tese.

"Conheça todas as teorias, domine todas as técnicas, mas ao tocar uma alma humana, seja apenas outra alma humana."

Carl Jung

RIBEIRO, A.P.F. **O exercício físico isolado ou combinado com o ômega-3 modula a periodontite apical induzida em ratos.** 2024. Tese (Doutorado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2024.

Resumo

A periodontite apical (PA) é resultado da contaminação bacteriana no canal radicular e sua progressão é altamente dependente da resposta imune do hospedeiro. O objetivo desse trabalho foi investigar o impacto do exercício físico moderado, isoladamente ou em combinação com a suplementação de ômega 3, na PA induzida em ratos, analisando o perfil imuno inflamatório, a presença de bactérias no canal radicular e região apical, a perda óssea e as fibras colágenas. Trinta ratos *Wistar* foram divididos em 3 grupos: Controle; Exercício físico e Exercício físico + ômega 3. O experimento iniciou com a suplementação do ômega 3, que durou 60 dias e foi administrada por gavagem. O protocolo de natação ocorreu em duas etapas, adaptação ao ambiente aquático e treino de natação. A PA foi induzida no 30º dia e os ratos foram eutanasiados no 60º dia. Os molares superiores foram processados e corados com Hematoxilina e Eosina (H&E), Brown Brenn (BB) e Picrosirius Red (PSR) e imuno-histoquímica de interleucina 17 (IL-17), fator de necrose tumoral alfa (TNF-α) e fosfatase ácida resistente ao tartarato (TRAP) e para análise micro tomográfica. As análises de H&E, BB, IL-14 e TNF- α foram realizadas por meio da atribuição de escores. As análises foram realizadas por meio da atribuição de escores que foram submetidos aos testes de Kruskal-Wallis, Tukey Shapiro-Wilk, Mann-Whitney e Análise de Variância One Way, com significância de 5%. A intensidade do infiltrado inflamatório foi maior no grupo controle ($p<0,05$). A atividade física limitou a disseminação de bactérias ($p<0,05$) e quando combinada com o ômega-3, apresentou maior percentual de fibras imaturas ($p<0,05$). O grupo controle apresentou maior número de células positivas para TRAP ($p<0,05$). A natação por si só, reduziu a imunomarcação de TNF-α e quando combinada com ômega 3, reduziu a imunomarcação de IL-17. A análise microtomográfica mostrou que os animais que realizaram a natação apresentaram menor perda de volume ósseo alveolar ($p< 0,05$). A atividade física isolada aprimorou os mecanismos de defesa, reduzindo a inflamação por meio da modulação do TNF-α e controlando a contaminação bacteriana, enquanto sua combinação com ômega 3 melhorou ainda mais a modulação inflamatória por meio do controle da IL-17, reduziu a perda de tecido ósseo e estimulou a produção de colágeno para limitar a inflamação e diminuir a atividade osteoclástica.

Palavras-chave: Periodontite Apical, natação, exercício, endodontia, ácido graxo ômega 3.

RIBEIRO, A.P.F. **Physical exercise alone or combined with omega-3 modulates apical periodontitis induced in rats.** 2024. Tese (Doutorado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2024.

Abstract

Apical periodontitis (AP) is the result of bacterial contamination of the pulp tissue and its progression is highly dependent on the host's immune response. The objective of this work was to investigate the impact of moderate physical exercise, alone or in combination with omega 3 supplementation, on AP induced in rats, analyzing the immuno-inflammatory profile, the presence of bacteria in the root canal and apical region, bone loss and collagen fibers. Thirty Wistar rats were divided into 3 groups: Control; Physical exercise and Physical exercise + omega 3. Omega 3 supplementation was administered by gavage for 60 days. The swimming protocol occurred in two stages, acclimatization to the aquatic environment and swimming training. AP was induced on the 30th day and the rats were euthanized on the 60th day. The upper molars were processed and stained with Hematoxylin and Eosin, Brown Brenn and Picrosirius Red and immunohistochemistry for IL-17, TNF-α and TRAP and for micro tomographic analysis. The analyzes were carried out by assigning scores that were submitted to the Kruskal-Wallis, Tukey Shapiro-Wilk, Mann-Whitney and One Way Analysis of Variance tests, with a significance of 5%. The intensity of the inflammatory infiltrate was greater in the control group ($p<0.05$). Physical activity alone reduced immunostaining for TNF-α and limited the spread of bacteria ($p<0.05$). Furthermore, when combined with omega 3, it reduced immunostaining for IL-17 and presented a higher percentage of birefringent structures of immature fibers ($p<0.05$). Microtomographic analysis showed that animals that performed the physical activity of swimming had a smaller area of alveolar bone loss ($p< 0.05$). The control group showed a greater number of TRAP-positive cells ($p<0.05$). Physical activity alone enhanced defense mechanisms, reducing inflammation by modulating TNF-α and controlling bacterial contamination, while combining it with omega-3 further improved inflammatory modulation through IL-17 control, reduced bone tissue loss, and stimulated collagen production to limit inflammation and decrease osteoclastic activity.

Keywords: Apical periodontitis, swimming, exercise, endodontics, omega 3 fatty acid.

LISTA DE TABELAS

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

H&E - Hematoxilina e Eosina

BB - Brown Brenn

PSR - Picrosirius Red

TNF-α- tumor necrosis factor-alpha

IL-17 - interleukin 17

TRAP - tartrate-resistant acid phosphatase

EPA - eicosapentaenoic acid

DHA - docosahexaenoic acid

SUMÁRIO

Anexos

1- Introduction

Apical periodontitis (AP) arises from the infiltration of microorganisms into the root canal system (Nair, 2004). As the infectious process and associated inflammatory response reach the apex of the tooth, a periapical lesion is developed. In an attempt to suppress invading microorganisms, the host's inflammatory response intensifies the process, leading to the recruitment of defense cells, the release of inflammatory mediators and the resorption of bone tissue (Samuel et al., 2021; Azuma et al., 2021; Barreiros et al., 2018). The amplification and progression of this process are highly dependent on the host's immune response (Samuel et al., 2021, Cantiga-Silva et al., 2021).

Failure of the immune response to suppress bacteria can lead to an overactive response from defense cells (Howait et al., 2019). Macrophages, T lymphocytes and other cells release chemical mediators, such as tumor necrosis factor-alpha (TNF-α), interleukin 17 (IL-17), prostaglandins and bradykinins, which, when present at high levels, indirectly trigger osteoclastogenesis (Cintra et al., 2018). Furthermore, IL-17 is capable of mediating host defense against bacterial infections or may contribute to chronic inflammation, while TNF-α is associated with bone loss and stimulates the synthesis of prostaglandins and proteases (Borgo et al., 2023, Almeida-Junior et al., 2023).

Physical exercise is one of the strategies established to promote immunomodulation to increase the host's immune response (Andrade et al., 2017), being among the most influential factors in regulating the defense system, and can deeply sensitize it, making the organism less susceptible to inflammation and infections (Shimojo et al., 2019, Andrade et al., 2017). Regular, moderate-intensity activities can strengthen the immune system throughout life, thereby suppressing the abnormal production of inflammatory cytokines, facilitating the development of anti-infective and antioxidant agents, increasing immune cell counts, and reducing tissue inflammation (Lombardi et al., 2019, Guo et al., 2020; Simpson et al., 2015).

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs) have been widely accepted as dietary supplementation adjuvant to the treatment of chronic inflammatory diseases due to their remarkable therapeutic effects (Deore et al., 2017, Azuma et al., 2017). ω-3 PUFAs, represented by eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are capable of reducing inflammation, inhibiting bone resorption, suppressing the synthesis of lipid mediators, reducing arachidonic acid levels, altering the functions of polymorphonuclear leukocytes, modulate lymphocyte proliferation and the production of pro-inflammatory cytokines, stimulating bone regeneration and increasing the host's antioxidant capacity (Kesavalu et al., 2006, Iwasaki et al., 2010, Azuma et al., 2021) . Furthermore, ω-3 PUFAs act by preventing the resorption of bone tissue (Azuma et al., 2018). Specifically in dentistry, ω-3 PUFAs are being used in the treatment of gingivitis, periodontal disease, AP and stomatitis (Azuma et al., 2022).

As there is still no study analyzing the effects of physical activity on AP the objective of this study was to analyze, in Wistar rats, the impact that moderate intensity physical activity, alone or combined with fatty acid, can have on infectious and inflammatory oral diseases of endodontic origin. The null hypothesis tested was that

physical activity alone or combined with omega-3 will promote less bone loss and less inflammation.

2- Methodology

2.1. Experimental Design

The experimental protocol was approved by the Animal Ethics Committee (CEUA 0062-2021) of the São Paulo State University (UNESP) School of Dentistry, Araçatuba São Paulo, Brazil, and the entire study was conducted in accordance with ARRIVE guidelines.

Thirty male Wistar rats, aged 6 weeks and with body weight approximately 200g, were included in the study. The rats were housed in a temperature-controlled room (25ºC) with a 12-hour light/dark cycle and received water and rodent food (ad libitum). The exclusion criterion adopted was to remove from the study rats that were unable to perform 60 minutes of swimming at the end of the week of adaptation to the aquatic environment.

The sample size was determined based on previous studies (Conti et al., 2020), the difference of 1 in the scores was considered significant, the alpha error was determined at 5% and the power at 95%. The minimum number obtained in the sample calculation was 7 rats per group, but for safety reasons, 3 more rats were added to each group, due to the risk of losing rats during the experiments. The rats were randomly distributed into 3 groups (n= 10): Control (C)- rats with AP, without swimming training and without omega-3; Physical Exercise (PE)- rats with AP, who performed moderate physical exercise (swimming) and did not receive omega-3; Physical exercise + omega 3 (PEO)- rats with AP, who underwent moderate physical exercise (swimming) and received supplementation with omega 3.

2.2. Omega 3 supplementation

The rats in the PEO group received omega 3 (Ômega-3 Catarinense; Laboratório Catarinense SA, Joinville, SC, Brazil) once a day, by gavage, at a dosage of 40 mg/kg (60% EPA and 40% DHA), diluted in 1 ml of distilled water for 60 days (Azuma et al., 2021). The rats were weighed twice a week to adjust the omega 3 dose. Rats in groups control and PE received distilled water as a control for the same period.

2.3. Training protocol

Swimming training was performed in polypropylene tanks [100 cm (L) × 80 cm (W) × 90 cm (H)], with water at a temperature between 32-34ºC, where the rats were continuously supervised. PVC pipes were placed inside the tanks, to serve as lanes, to separate the rats during swimming, thus, 5 rats were placed per tank. All training sessions were consistently scheduled at the same time of day. The exercise protocol occurred in two stages: acclimatization to the aquatic environment and subsequent swimming training (Plecevic et al., 2018, Cheng et al., 2018).

The acclimatization period lasted 6 days. On the first day, the rats from the PE and PEO groups initiated their exercise with a 10-minute session. Thereafter, the exercise duration increased by10 minutes/day, until on the sixth day the rats were performing the swimming for 60 minutes. All rats were dried with cloth towels immediately every time after they finish swimming. The seventh day of the adaptation week was a rest day for the rats. All rats were able to perform 60 minutes of swimming on the sixth day of the adaptation week.

In the second stage, the swimming training protocol remained constant as follows: the rats swam 60 minutes/day, five days a week, for 6 weeks, with two days of weekly rest. During the training sessions, the rats swam without adding overload. Group C rats were placed in tanks with shallow water, for 1 minute a day, five days a week, for 6 weeks, to undergo the same water stimulus as the trained rats. After water activities, all animals were dried with towels.

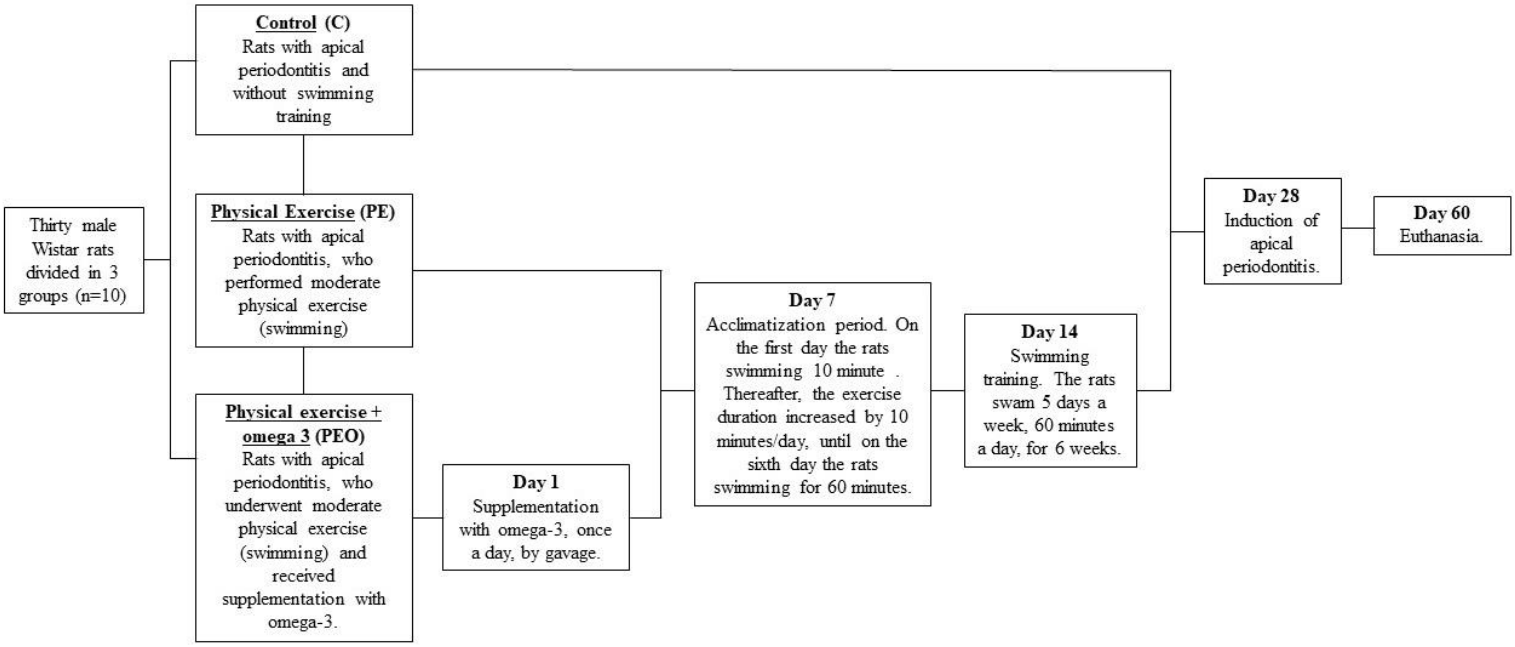
2.4. Induction of apical periodontitis

The rats were anesthetized by administering an intramuscular injection containing 87 mg/kg^{-1} of ketamine (Vetaset; Fort Dodge Animal Health Ltd, São Paulo, Brazil) and 13 mg/kg^{-1} of xylazine (Coopazine; Coopers Ltd Brasil, São Paulo, Brazil). The AP was induced by exposing the pulp on the occlusal surface of the upper and lower right first and second molars using round surgical burs (LN Long Neck Drill; Maillefer, Dentsply Ind e Com Ltda, Petrópolis, RJ, Brazil). The pulps were exposed for 30 days, resulting in the formation of a periapical lesion (Cintra et al., 2016).

2.5. Euthanasia

On the 60th day, the rats were euthanized with an overdose of sodium thiopental anesthetic (240 mg/kg – Thiopentax, Cristalia Produtos Químicos Farmacêuticos Ltda.). The flowchart in figure 1 shows the entire experimental design of the study.

Figure 1: Flowchart representing the study design.



2.6. Sample processing

The right maxillae were collected and fixed in a buffered solution with 4% formaldehyde, at neutral pH, for the first 22 h. Subsequently, the samples were washed in running water for 12 hours. After washing, the maxillae were decalcified in 10% EDTA (Sigma-Aldrich) for 3 months. After the demineralization process, the pieces were washed again in running water, dehydrated in alcohol, clarified in xylene, and embedded in paraffin. After inclusion, 5 µm thick serial sections of the upper first molar were prepared using a microtome (Leica - RM 2045). The sections were stained using either the Hematoxylin and Eosin (H&E), the Brown Brenn (BB) techniques and Picrosirius Red (PSR).

Immunohistochemical analysis was conducted using the indirect immunoperoxidase technique, for the marker's TNF-α, IL-17 and TRAP. Histological sections from the maxillary molars were deparaffinized in xylene and then hydrated in ethanol. Antigen retrieval was performed by inserting the slides in a citrate buffer solution (Antigen Retrieval Buffer; Spring Bioscience, Pleasanton, CA, USA) in a pressurized chamber (Decloaking Chamber; Biocare Medical, Concord, CA, USA) at 95° C for 10 min. The slides were washed with phosphate-buffered saline at the end of each phase of the immunohistochemical reaction. The blade were placed in a 3% H_2O_2 solution for 1 hour and 20 min and in 1% bovine serum albumin for 12 hours to block the activity of endogenous peroxidase and nonspecific sites. The histological slides were incubated with one of the primary antibodies' antis– TNF-α and anti– IL-17 (Santa Cruz Biotechnology, Santa Cruz, CA), and placed in a chamber for 24 hours. Slides were incubated with a biotinylated secondary antibody for 1 hour and 30 min and treated with streptavidin-horseradish peroxidase for 1 hour and 30 min (Dako Labeled Streptavidin-Biotin Universal Kit; Dako Laboratories). The slides were washed with phosphate-buffered saline and the reaction was developed using the chromogen 3,3'-diaminobenzidine

tetrahydrochloride (DAB Chromogen kit; Dako Laboratories) and was counterstained with Harris hematoxylin (Benetti et al., 2019).

2.7. Histologic and Immunohistochemical analysis

The intensity of periapical inflammation was analyzed in groups C, PE, and PEO, by H&E staining, assigning the following scores: no inflammation (score 1: 0 > 10 cells), mild inflammation (score 2: > 10 and < 25 inflammatory cells), moderate inflammation (score 3: 25 – 125 inflammatory cells) and severe inflammation (score 4: > 125 inflammatory cells) (Cintra et al., 2016).

The presence of microorganisms was assessed by BB staining, by optical microscopy with oil immersion, analyzing the thirds of the root canal and periapical region, where Gram-positive bacteria were dark blue and Gram-negative bacteria were red (Brown & Brenn, 1931). The modified scoring system of Samuel et al. (2019) was used to detect bacterial penetration from the coronary third to the periapical region: score 0: absence of bacteria; score 1: presence of bacteria in the cervical third of the canal; score 2: bacteria in the middle third; score 3: bacteria in the apical third without involvement of the foramen; score 4: presence of bacteria in the apical third with involvement of the foramen; score 5: presence of bacteria in the periapical lesion.

The maturation levels of collagen fibers were evaluated through Picrosirius Red (PSR) staining under polarized light microscopy. Greenish-yellow fibers were considered immature and thin, while yellowish-red fibers were classified as mature and thick. After color selection, the software automatically calculated the marked area of each type of collagen fiber (Leica QWin V3, Leica Microsystems) (Benetti et al., 2020).

For immunohistochemical analyses, positive TNF-α and IL-17 immunolabeling were defined by the brown color present in the cell cytoplasm and extracellular matrix. The following scores were defined according to the number of immunoreactive cells and intensity of immunostaining of the extracellular matrix: 0- complete absence of immunoreactive cells; 1- extremely low immunoreactivity; 2- low immunoreactivity; 3- moderate immunoreactivity; 4- high immunoreactivity; 5- extremely high immunoreactivity (Cosme-Silva et al., 2019). Immunohistochemical analysis was performed using the indirect immunoperoxidase technique (Benetti et al., 2018), for the TRAP marker.

For immunohistochemical analyses, positive immunoreactivity was defined by the brown color present in the cell cytoplasm and extracellular matrix. TRAP analyze were performed considering the perimeter of bone resorption suffered by AP, followed by counting TRAP-positive multinucleated cells. Finally, the ratio between positive cells and perimeter size was determined (Azuma et al., 2017).

The analysis of inflammatory and immunological scores, as well as the assessment of the presence of bacteria, was carried out by a blind calibrated researcher, so that there was no influence on the interpretation of the data.

2.8. Micro tomographic analysis

The right jaws were scanned using the µCT system (Bruker SkyScan 1272, Aartselaar, Belgium). The specimens were placed in a vial and scanned individually, always in the same position, incisor facing upwards. The region of interest (ROI) was composed of the empty space of periapical bone resorption. Alveolar bone volume (BV) was measured using CTAn software (Skyscan) (Cosme-Silva et al., 2020).

2.9. Statistical analysis

The results were tabulated for each group. Non-parametric data were analyzed using multiple comparisons with the Kruskal-Wallis, Tukey, Shapiro-Wilk, Mann-Whitney tests and One Way Analysis of Variance, with a significance level of 5% ($p<0.05$).

Results

The histological analysis of the teeth of the rats in all groups is represented in Figure 2. The specimens of all groups showed the presence of pulp necrosis and periapical lesion formation 30 days after pulp exposure. The intensity of the inflammatory infiltrate was significantly higher in group C, compared to the PE and PEO groups ($p< 0.05$) (Table 1).

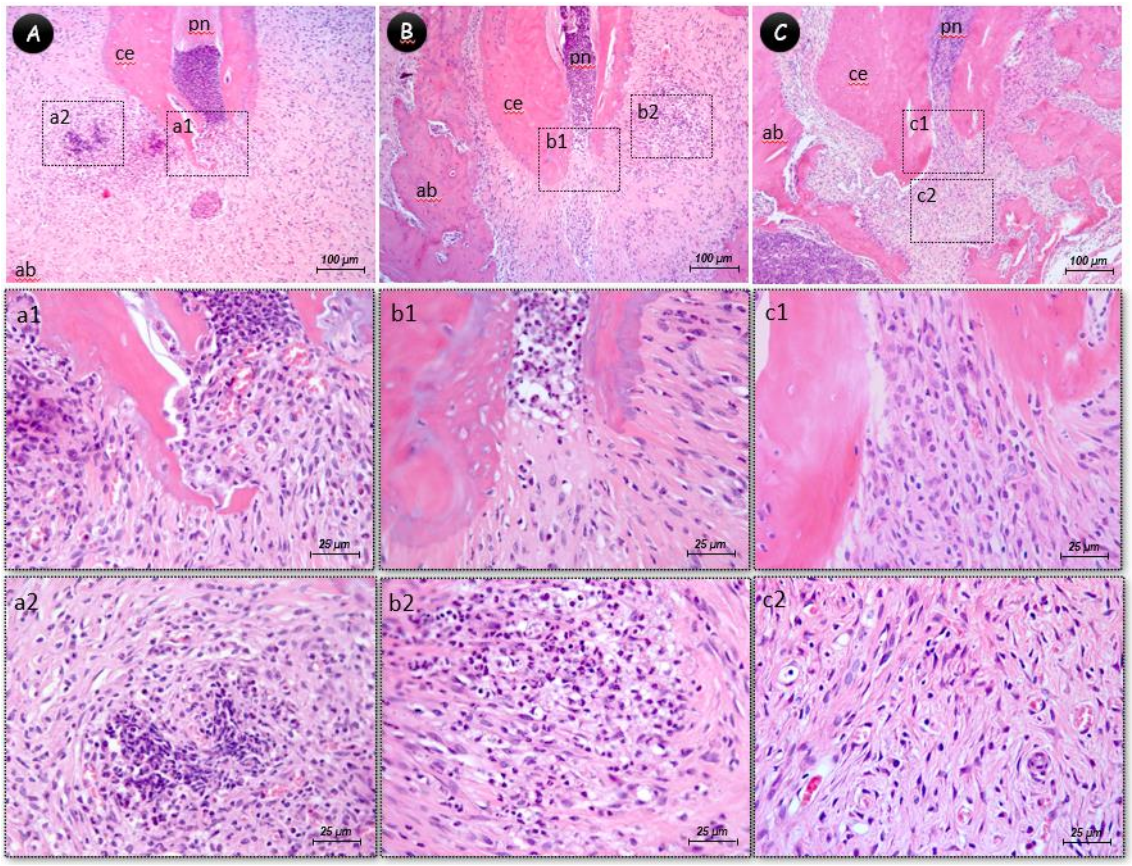


Figure 2. Representative images of histological analysis of the periapical region after 30 days of pulp exposure. Group C (A, a1, a2) presented a moderate to intense inflammatory infiltrate, in addition to an extensive area of periapical bone resorption; group PE (B, b1, b2) exhibited a mild to moderate inflammatory infiltrate and a smaller area of bone resorption. The PEO group (C, c1, c2) exhibited a mild inflammatory infiltrate and little area of apical bone resorption. ab, alveolar bone; ce, cementum; pn, pulp necrosis; (hematoxylin-eosin stain, original magnification ×100 and ×400).

Table 1. Scores, median and *P* values of histologic findings in rats from all groups

Histologic criteria	**Scores**	**Groups**			***P value***
		Control	**PE**	**PEO**	
Inflammatory infiltrate	1	0/10	0/10	0/10	*p* = .002
	2	3/10	7/10	10/10	
	3	4/10	3/10	0/10	
	4	3/10	0/10	0/10	
	Median*	3^a	2^b	2^b	

*Different superscript letters represent statistically significant differences, p <0.05.

Immunohistochemical analysis showed moderate levels of the cytokine's IL-17 and TNF- α in group C (p< 0.05). The PE group showed few immunoreactive cells for the cytokine TNF-α and moderate immunostaining of IL-17 (p< 0.05). In the PEO group, there was low IL-17 and TNF-α immunoreactivity, with two rats showing extremely low immunoreactivity for TNF-α (p< 0.05) (Table 2; Figure 3).

Table 2. Scores, median and *P* values of immunohistochemical findings in rats from all groups

Inflammatory mediators	**Scores**	**Groups**			***P value***
		Control	**PE**	**PEO**	
IL-17	0	0/10	0/10	0/10	p < .001
	1	0/10	0/10	0/10	
	2	1/10	2/10	9/10	
	3	7/10	8/10	1/10	
	4	1/10	0/10	0/10	
	5	1/10	0/10	0/10	
	Median*	3^a	3^a	2^b	
TNF-α	0	0/10	0/10	0/10	p < 0.01
	1	0/10	0/10	2/10	
	2	2/10	9/10	6/10	
	3	7/10	1/10	2/10	
	4	1/10	0/10	0/10	
	5	0/10	0/10	0/10	
	Median*	3^a	2^b	2^b	

*Different superscript letters represent statistically significant differences, p <0.05.

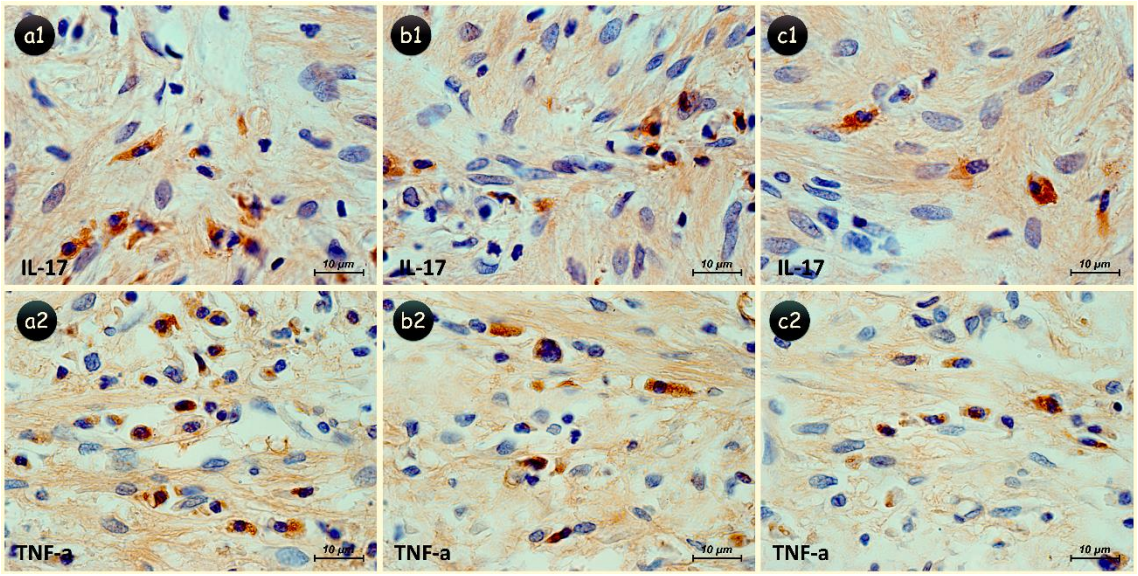


Figure 3. Representative images of IL-17 immunoreaction between groups: (a1) group C; (b1) PE group; (c1) PEO group. Representative histological sections of TNF-α immunoreaction between groups: (a2) group C; (b2) PE group; (c2) PEO group. (Immunohistochemistry of IL-17 and TNF-α, magnification×1000).

Data of bacteriological findings in thirds of the root and statistical differences among the groups are showed in Table 3. Figure 4 represents the scores defined according to the presence of bacteria in the root canal and periapical region.

Table 3. Scores, median and *P* values of bacteriological findings in thirds of the root canal and periapical region.

Histologic criteria	**Scores**	**Groups**			***P value***
		Control	**PE**	**PEO**	
Presence of Bacteria	1	0/10	0/10	0/10	$p < .001$
	2	0/10	0/10	4/10	
	3	0/10	7/10	6/10	
	4	8/10	2/10	0/10	
	5	2/10	1/10	0/10	
	Median*	4^a	3^b	3^b	

*Different superscript letters represent statistically significant differences, $p < 0.05$.

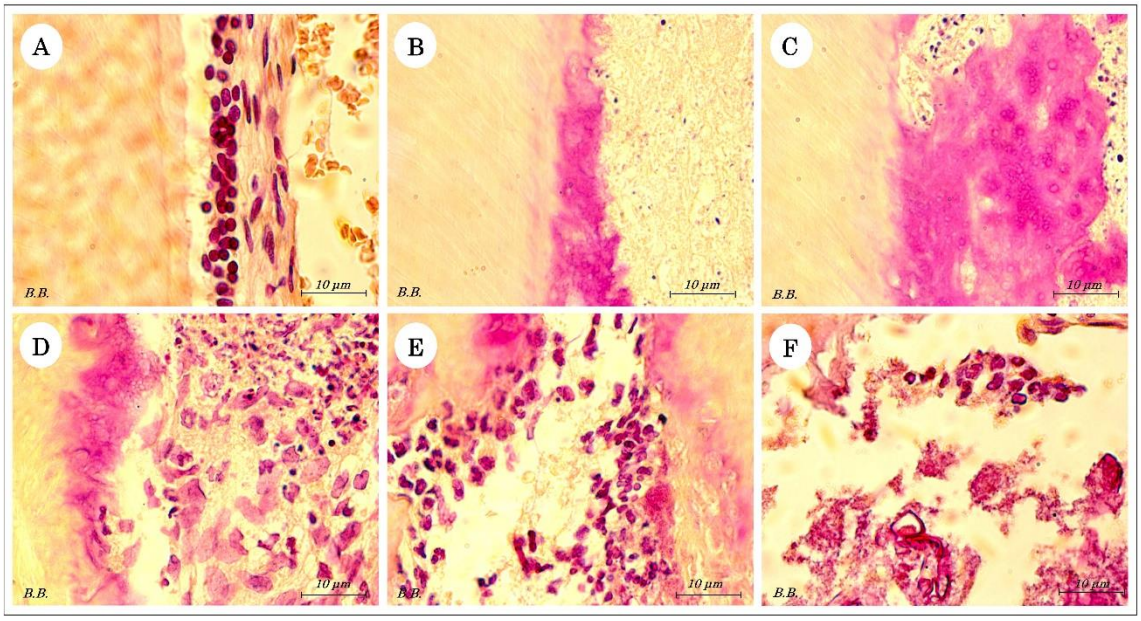


Figure 4. Representative images of the scores attributed to the presence of bacteria in the thirds of the root canal and periapical region. (A) score 0; (B) score 1; (C) score 2; (D) score 3; (E) score 4; (F) score 5. (BB stain, original magnification×1000).

When evaluating the predominance of collagen fibers (figure 1), statistical analysis of immature fibers revealed that the PEO group presented a higher percentage of birefringent structures, significantly different from groups C and PE ($p < 0.05$). (Table 4).

Table 4. Percentage of immature and mature collagen fibers by group.

Histologic criteria	**Scores**	**Groups**			***P value***
		C	**PE**	**PEO**	
PSR	Green fibers*	0.28 ± 0.10^{a}	0.33 ± 0.12^{a}	0.64 ± 0.13^{b}	$p < .001$
	Red fibers*	56.31 ± 3.99^{a}	54.22 ± 7.54^{a}	42.62 ± 7.32^{b}	$p = .002$

Different superscript letters represent statistically significant differences, $p < 0.05$.

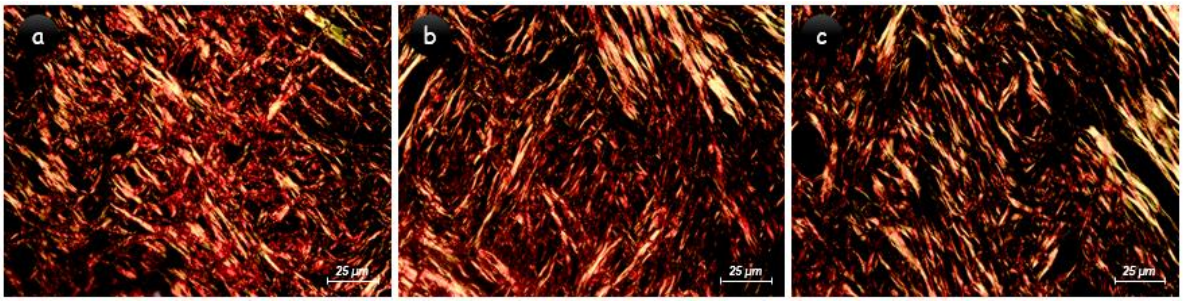


Figure 5. Representative images of mature and immature collagen fibers, under conventional light and polarized light. Groups C (a) and PE (b) had a higher percentage of red fibers. While in the PEO group (c) there was a higher percentage of green fibers.

The microtomographic analysis (figure 6) showed that the animals that performed the physical activity of swimming had less loss of alveolar bone volume compared to

group C ($p< 0.05$). In animals that received omega-3 supplementation, the volume of bone structure loss was even smaller ($p< 0.05$) (table 5).

Table 5: Mean, standard deviation and p-value of micro tomographic findings.

Criteria	**Groups**			***P value***
	C	**PE**	**PEO**	
Volume - µCT (mm^3)	194.5 ± 6.6^a	165.5 ± 2.8^b	143.7 ± 6.0^c	$p < .001$

Different superscript letters represent statistically significant differences, $p <0.05$.

Figure 7 represents the immunohistochemical analysis of each group. It is noted that the groups that performed physical activity had fewer cells immunolabeled for TRAP (osteoclasts), with even better results when omega 3 was associated (Table 6) ($p<0.05$).

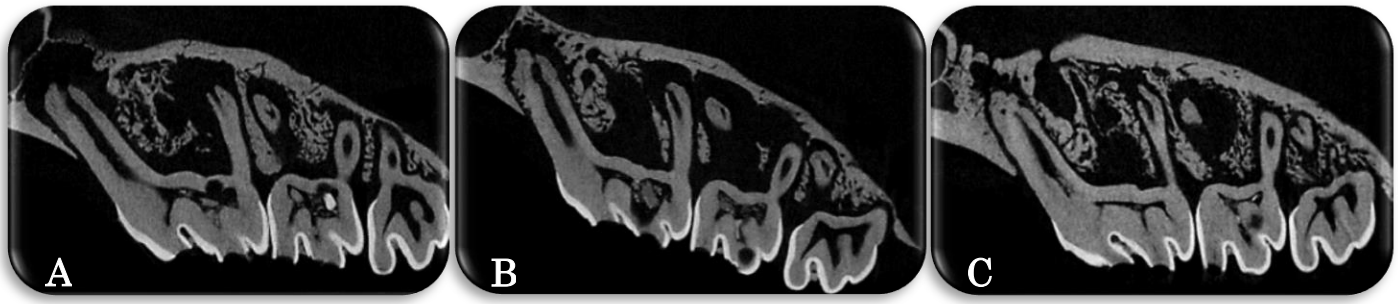


Figure 6. Micro tomographic images of the upper first molar, representative of each group. (A) C Group. (B) PE Group. (C) PEO Group.

Table 6: Mean, standard deviation and p-value of TRAP analysis.

Histologic criteria	**Groups**			***P value***
	C	**PE**	**PEO**	
TRAP	42.1 ± 15.34^a	31.9 ± 6.17^b	20.8 ± 1.47^c	$p < .001$

Different superscript letters represent statistically significant differences, $p <0.05$.

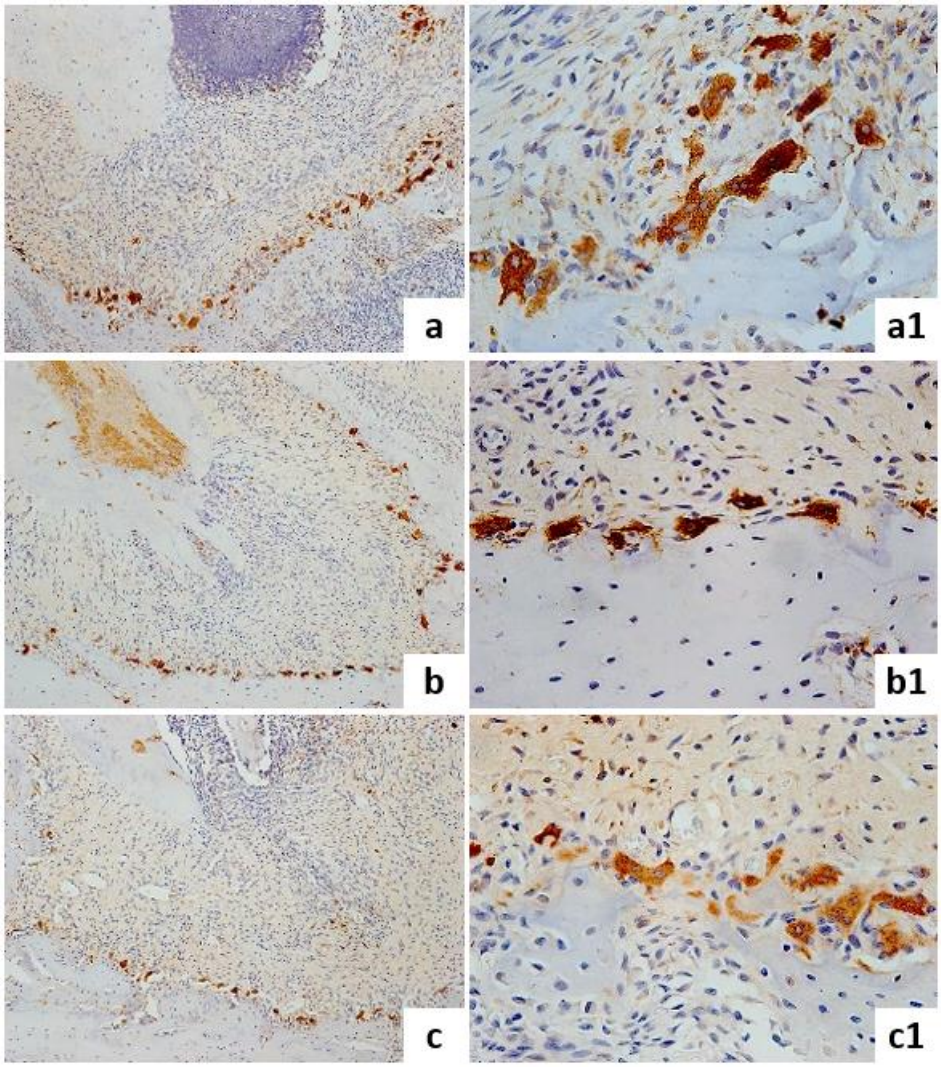


Figure 7. TRAP-positive multinucleated cells in alveolar bone surface. Immunohistochemistry images of C group (a–a1); Images of PE group (b–b1). Images of PEO group (c–c1). (H&E staining, ×100 and ×400 original magnification).

Discussion

This study analyzed the impact of moderate intensity physical exercise, alone or in combination with omega 3 supplementation, on the inflammatory and immunohistochemical profile, on the location and distribution of bacteria after the induction of AP, and on the production of collagen fibers. As physical exercise alone influenced the progression of the periapical lesion, the immunostaining of TNF-α and TRAP and the apical bone loss, the first null hypothesis of the study was partially discarded. The practice of moderate physical exercise associated with supplementation showed significant results in terms of improving the inflammatory condition produced by AP, containing bacterial advancement, loss of bone tissue, immunostaining of pro-inflammatory cytokines and stimulation of fibroblasts, therefore the second null hypothesis was rejected.

Regularly practicing moderate-intensity physical exercise controls the immune system, improving the body's defense against infections (Scheffer & Latini, 2020). Several studies have shown the role of physical exercise in periodontal disease, reducing the prevalence of the disease, attenuating the progression of the lesion, and reducing the inflammatory process and alveolar bone loss (Andrade et al., 2017, Ferreira et al., 2019, Uchida et al., 2021). The physical exercise method used in this study was a gradually increased moderate swimming with no weight loading, consistent with previous research (Cheng et al., 2018, Plecevic et al., 2018]. The use of swimming rats as a model of exercise presents advantages over treadmill running, since swimming is a natural ability of the rat and is a low-impact exercise that minimizes stress on the joints and musculoskeletal system (Cheng et al., 2018). Additionally, swimming in rats has demonstrated similarities in adaptations to human exercise, suggesting its relevance as a translational model (Voltarelli et al., 2002, Azuma et al., 2018). The present work showed the effects of physical exercise on AP, thus showing that swimming caused modulation of the immune system, enhancing the body's capabilities against microbial infections.

The inflammatory profile showed how moderate physical exercise significantly influenced the inflammatory response associated with AP since the group of rats without physical exercise presented a much more intense inflammatory process with greater apical bone resorption. Our results are in line with Andrade and collaborators (Andrade et al., 2018), where swimming reduced inflammation caused by periodontal disease in diabetic rats.

The effects of omega 3 on AP have been discussed in the literature (Azuma et al., 2017, Gobatto et al., 2021, Azuma et al., 2021). PUFAs act as inflammatory modulators, antioxidant agents, and activators of the inflammation resolution process (Djuricic & Calder, 2021). Due to the promising results of previous studies and the fact that the use of omega 3 is increasing among patients, this study associated the systemic use of the fatty acid with physical exercise, showing significant results.

Interleukins-17 are capable of exacerbating the inflammatory process within periapical lesions and stimulating the release of other pro-inflammatory cytokines (Henriques et al., 2011). The fact that IL-17 showed low immunoreactivity in the rats that performed swimming and received omega 3 indicates that the inflammatory process of AP was lower in this group, in agreement with the other analyses. In the PE group there may have been a moderate immunostaining of IL-17 due to the greater presence of bacteria in the apical and periapical region. In fact, it has been shown that the release of inflammatory mediators can be stimulated by the presence of bacteria, especially in the tooth apical region (Samuel et al., 2019).

The cytokine TNF-α is related to bone loss and is also associated with inflammatory processes (Almeida-Junior et al., 2023). Balducci et al (2010) proved that physical exercise improves serum levels of some inflammatory cytokines, including TNF-α. The rats that performed the physical exercise showed low immunoreactivity of this cytokine, indicating reduced bone tissue loss and a less inflammatory process. These findings suggest that physical exercise also reduced local levels of TNF-α.

Models using rats are well standardized for studies on AP and swimming (Cintra et al., 2016; Cheng et al., 2018). This study was limited by the use of male rats to evaluate

the influence of swimming on the immunoinflammatory response of AP. A swimming study with male and female rats showed that swimming behavior was lower in females and that there are important differences when females are in different hormonal phases (Barros & Ferigolo, 1998). Furthermore, differences in cytokine expression were observed between male and female animals subjected to swimming (Subbotina et al., 2023). This study model can be used in future research to study the inflammatory response of AP in female rats subjected to swimming.

Our results provide evidence of the influence of physical exercise on microorganisms. The group PEO showed some rats presenting bacterial colonies restricted to the middle third of the canal, and although the majority of rats presented the presence of bacteria in the apical third, it can be highlighted that the combination of fatty acid with physical exercise provided better systemic conditions and acted more effectively in containing bacteria, as in this group there was no presence of bacteria in the apical foramen and the periapical tissues. This containment of the advancement of microorganisms can be explained by the antimicrobial activity of omega 3 polyunsaturated fatty acids, as both eicosapentaenoic acid and docosahexaenoic acid have a broad-spectrum inhibitory effect against several Gram-positive and Gram-negative bacteria (Ribeiro-Vidal et al., 2020).

The PSR technique is a specific staining method for detecting collagen fibers in tissues. The different colors presented occur as a result of the packaging of the fibers. The greenish-yellow color represents deposition of immature collagen, while the yellowish-red color characterizes the maturation of the fibers (Cintra et al., 2017). This study observed that the PEO group had a higher percentage of green fibers and a lower percentage of red fibers, that is, it was the group where there was a greater quantity of immature collagen fibers, due to tissue repair. This may have occurred as a result of PUFA resolution activity. EPA and DHA are precursors for the synthesis of new specialized mediators in solution, thus activating the process of inflammatory resolution (Djuricic and Calder, 2021). The PE group, despite having a greater quantity of immature fibers and fewer mature collagen fibers, compared to group C, did not present statistically significant results to affirm that isolated physical activity was capable of causing the stimulation of fibroblasts to limit inflammation.

TRAP is a specific immunological marker for osteoclasts (Sarıtekin et al., 2019) and it was observed that group C exhibited a greater number of osteoclast cells, characterizing greater bone tissue resorption activity. Our results are in line with those obtained by Sanbe and colleagues (2009), where the authors observed that the increase in the number of TRAP-positive osteoclasts in periodontal tissue caused greater resorption of alveolar bone tissue. The groups PE and PEO showed, in the microtomographic analysis, a significant reduction in the size of the periapical lesion, characterizing less loss of bone tissue and less activity of osteoclasts. It is also worth mentioning that the area of bone loss was much smaller in animals supplemented with omega-3. This can be explained because ω-3 PUFAs not only interrupt bone resorption but also promote the formation of new bone tissue in the periapical region (Azuma et al., 2017).

Conclusion

Physical activity alone enhanced defense mechanisms, reducing inflammation by modulating TNF-α and controlling bacterial contamination, while combining it with omega-3 further improved inflammatory modulation through IL-17 control, reduced bone tissue loss, and stimulated collagen production to limit inflammation and decrease osteoclastic activity.

References

Almeida-Junior, L.A., de Carvalho, M.S., Almeida, L.K.Y., Silva-Sousa, A.C., Sousa-Neto, M.D., Silva, R.A.B., Silva, L.A.B., Paula-Silva, F.W.G. (2023). TNF-α-TNFR1 Signaling Mediates Inflammation and Bone Resorption in Apical Periodontitis. J Endod, 49(10), 1319-1328.e2. https://doi.org/10.1016/j.joen.2023.07.013

Andrade, E.F., Orlando, D.R., Gomes, J.A.S., Foureaux, R.C., Costa, R.C., Varaschin, M.S., Rogatto, G.P., de Moura, R.F., Pereira, L.J. (2017). Exercise attenuates alveolar bone loss and anxiety-like behaviour in rats with periodontitis. J Clin Periodontol, 44(11), 1153-1163. https://doi.org/10.1111/jcpe.12794

Andrade, E.F., Silva, V.O., Moura, N.O., Foureaux, R.C., Orlando, D.R., Moura, R.F., Pereira, L.J. (2018). Physical Exercise Improves Glycemic and Inflammatory Profile and Attenuates Progression of Periodontitis in Diabetic Rats (HFD/STZ). Nutrients, 10(11), 1702. https://doi.org/10.3390/nu10111702

Azuma, M.M., Gomes-Filho, J.E., Ervolino, E., Cardoso, C.B.M., Pipa, C.B., Kawai, T., Conti, L.C., Cintra, L.T.A. (2018). Omega 3 Fatty Acids Reduce Inflammation in Rat Apical Periodontitis. J Endod, 44(4), 604-608. https://doi.org/10.1016/j.joen.2017.12.008

Azuma, M.M., Gomes-Filho, J.E., Ervolino, E., Pipa, C.B., Cardoso, C.B.M., Andrada, A.C., Kawai, T., Cintra, L.T.A. (2017). Omega 3 Fatty Acids Reduce Bone Resorption While Promoting Bone Generation in Rat Apical Periodontitis. J Endod, 43(6), 970-976. https://doi.org/10.1016/j.joen.2017.01.006

Azuma, M.M., Cardoso, C.B.M., Samuel, R.O., Pipa, C.B., Bomfim, S.R.M., Narciso, L.G., Gomes-Filho, J.E., Cintra, L.T.A. (2021). Omega 3 Fatty Acids Alter Systemic Inflammatory Mediators Caused by Apical Periodontitis. J Endod, 47(2), 272-277. https://doi.org/10.1016/j.joen.2020.11.015

Azuma MM, Cardoso CBM, da Silva CC, de Oliveira PHC, Jacinto RC, Andrada AC, Cintra LTA. The use of omega-3 fatty acids in the treatment of oral diseases. Oral Dis. 2022 Mar;28(2):264-274. doi: 10.1111/odi.13667.

Balducci, S., Zanuso, S., Nicolucci, A., Fernando, F., Cavallo, S., Cardelli, P., Fallucca, S., Alessi, E., Letizia, C., Jimenez, A., Fallucca, F., Pugliese, G. (2010). Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss. Nutr Metab Cardiovasc Dis, 20(8), 608-17. https://doi.org/10.1016/j.numecd.2009.04.015

Balto HA, Alabdulaaly L, Bahammam S, Al-Ekrish AA. Comparative analysis of prevalence of apical periodontitis in smokers and non-smokers using cone-beam computed tomography. Saudi Dent J. 2019 Jan;31(1):52-57. doi: 10.1016/j.sdentj.2018.09.006

Barreiros D, Nelson P Filho, Paula-Silva FWG, Oliveira KMH, Lucisano MP, Rossi A, Silva LAB, Küchler EC, Silva RAB. MMP2 and MMP9 are Associated with Apical Periodontitis Progression and Might be Modulated by TLR2 and MyD88. Braz Dent J. 2018 Jan-Feb;29(1):43-47. doi: 10.1590/0103-6440201801731

Barros H.M., Ferigolo M. (1998). Ethopharmacology of imipramine in the forced-swimming test: gender differences. Neurosci Biobehav Rev, 23(2), 279-86. doi: 10.1016/s0149-7634(98)00029-3

Benetti, F., Briso, A.L.F., Carminatti, M., de Araújo Lopes, J.M., Barbosa, J.G., Ervolino, E., Gomes-Filho, J.E., Cintra, L.T.A. (2019). The presence of osteocalcin, osteopontin and reactive oxygen species-positive cells in pulp tissue after dental bleaching. Int Endod J, 52(5), 665-675. 665–675. https://doi.org/10.1111/iej.13049

Benetti F, Bueno CRE, Reis-Prado AHD, Souza MT, Goto J, Camargo JMP, Duarte MAH, Dezan-Júnior E, Zanotto ED, Cintra LTA. Biocompatibility, Biomineralization, and Maturation of Collagen by RTR®, Bioglass and DM Bone® Materials. Braz Dent J. 2020 Sep-Oct;31(5):477-484. doi: 10.1590/0103-6440202003660

Benetti F, Gomes-Filho JE, Ferreira LL, Sivieri-Araújo G, Ervolino E, Briso ALF, Cintra LTA. Concentration-dependent effect of bleaching agents on the immunolabelling of interleukin-6, interleukin-17 and CD5-positive cells in the dental pulp. Int Endod J. 2018 Jul;51(7):789-799. doi: 10.1111/iej.12891

Borgo Sarmento, E., Helena Teixeira de Lima Ribeiro Andrade, R., Cristina Gomes, C., Ramôa Pires, F., Bastos Valente, M.I., Mafra Moreno, A., Orsini, M., Carvalho Ferreira, D., Souza Gonçalves, L., Armada, L. (2023). Proinflammatory Cytokine Expression in Apical Periodontitis from Diabetic Patients. Int J Dent, 10, 4961827. https://doi.org/10.1155/2023/4961827

Brown, J.H., Brenn, L. (1931). A method for the differential staining of Gram-positive and Gram-negative bacteria in tissue sections. Bull Johns Hopkins Hosp, 48, 69–73.

Cantiga-Silva, C., Estrela, C., Segura-Egea, J.J., Azevedo, J.P., de Oliveira, P.H.C., Cardoso, C.B.M., Pinheiro, T.N., Ervolino, E., Sivieri-Araújo, G., Cintra, L.T.A. (2021). Inflammatory profile of apical periodontitis associated with liver fibrosis in rats: histological and immunohistochemical analysis. Int Endod J. 54(8), 1353-1361. https://doi.org/10.1111/iej.13519

Cheng, M., Cong, J., Wu, Y., Xie, J., Wang, S., Zhao, Y., Zang, X. (2018). Chronic Swimming Exercise Ameliorates Low-Soybean-Oil Diet-Induced Spatial Memory Impairment by Enhancing BDNF-Mediated Synaptic Potentiation in Developing Spontaneously Hypertensive Rats. Neurochem Res, 43(5), 1047-1057. https://doi.org/10.1007/s11064-018-2515-x

Cintra, L.T.A., Estrela, C., Azuma, M.M., Queiroz, Í.O.A., Kawai, T., Gomes-Filho, J.E. (2018). Endodontic medicine: interrelationships among apical periodontitis, systemic disorders, and tissue responses of dental materials. Braz Oral Res, 18, 32(suppl 1):e68. https://doi.org/10.1590/1807-3107bor-2018.vol32.0068

Cintra, L.T., Samuel, R.O., Azuma, M.M., de Queiróz, A.O., Ervolino, E., Sumida, D.H., de Lima, V.M., Gomes-Filho, J.E. (2016). Multiple Apical Periodontitis Influences Serum Levels of Cytokines and Nitric Oxide. J Endod, 42(5), 747-51. https://doi.org/10.1016/j.joen.2016.01.022

Cintra LTA, Ferreira LL, Benetti F, Gastélum AA, Gomes-Filho JE, Ervolino E, Briso ALF. The effect of dental bleaching on pulpal tissue response in a diabetic animal model. Int Endod J. 2017 Aug;50(8):790-798. doi: 10.1111/iej.12692

Conti, L.C., Segura-Egea, J.J., Cardoso, C.B.M., Benetti, F., Azuma, M.M., Oliveira, P.H.C., Bomfim, S.R.M., Cintra, L.T.A. (2020). Relationship between apical periodontitis and atherosclerosis in rats: lipid profile and histological study. Int Endod J, 53(10), 1387-1397. https://doi.org/10.1111/iej.13350

Cosme-Silva, L., Dal-Fabbro, R., Cintra, L.T.A., Dos Santos, V.R., Duque, C., Ervolino, E., Mogami Bomfim, S., Gomes-Filho, J.E. (2019). Systemic administration of probiotics reduces the severity of apical periodontitis. Int Endod J, 52(12), 1738-1749. https://doi.org/10.1111/iej.13192

Cosme-Silva L, Dal-Fabbro R, Cintra LTA, Ervolino E, Plazza F, Mogami Bomfim S, Duarte PCT, Junior VEDS, Gomes-Filho JE. Reduced bone resorption and inflammation in apical periodontitis evoked by dietary supplementation with probiotics in rats. Int Endod J. 2020 Aug;53(8):1084-1092. doi: 10.1111/iej.13311

Deore, G.D., Gurav, A.N., Patil, R., Shete, A.R., Naiktari, R.S., Inamdar, S.P. (2014). Omega 3 fatty acids as a host modulator in chronic periodontitis patients: a randomised, double-blind, palcebo-controlled, clinical trial. J Periodontal Implant Sci, 44(1), 25-32. https://doi.org/10.5051/jpis.2014.44.1.25

Djuricic, I., Calder, P.C. (2021). Beneficial Outcomes of Omega-6 and Omega 3 Polyunsaturated Fatty Acids on Human Health: An Update for 2021. Nutrients, 13(7), 2421. https://doi.org/10.3390/nu13072421

Ferioli M, Zauli G, Maiorano P, Milani D, Mirandola P, Neri LM. Role of physical exercise in the regulation of epigenetic mechanisms in inflammation, cancer, neurodegenerative diseases, and aging process. J Cell Physiol. 2019 Sep;234(9):14852-14864. doi: 10.1002/jcp.28304

Ferreira, R.O., Corrêa, M.G., Magno, M.B., Almeida, A.P.C.P.S.C., Fagundes, N.C.F., Rosing, C.K., Maia, L.C., Lima, R.R. (2019). Physical Activity Reduces the Prevalence of Periodontal Disease: Systematic Review and Meta-Analysis. Front Physiol, 10, 234. https://doi.org/10.3389/fphys.2019.00234

Freire MS, Oliveira NG, Lima SMF, Porto WF, Martins DCM, Silva ON, Chaves SB, Sousa MV, Ricart CAO, Castro MS, Fontes W, Franco OL, Rezende TMB. IL-4 absence triggers distinct pathways in apical periodontitis development. J Proteomics. 2021 Feb 20;233:104080. doi: 10.1016/j.jprot.2020.104080

Gobatto, C.A., de Mello, M.A., Sibuya, C.Y., de Azevedo, J.R., dos Santos, L.A., Kokubun, E. (2001). Maximal lactate steady state in rats submitted to swimming exercise. Comp Biochem Physiol A Mol Integr Physiol, 130(1), 21-7. https://doi.org/10.1016/s1095-6433(01)00362-2

Guo, S., Huang, Y., Zhang, Y., Huang, H., Hong, S., Liu, T. (2020). Impacts of exercise interventions on different diseases and organ functions in mice. J Sport Health Sci, 9(1), 53-73. https://doi.org/10.1016/j.jshs.2019.07.004

Häkkinen L, Uitto VJ, Larjava H. Cell biology of gingival wound healing. Periodontol 2000. 2000 Oct;24:127-52. PMID: 11276865.

Henriques, L.C., de Brito, L.C., Tavares, W.L., Vieira, L.Q., Ribeiro Sobrinho, A.P. (2011). Cytokine analysis in lesions refractory to endodontic treatment. J Endod, 37(12), 1659-62. https://doi.org/10.1016/j.joen.2011.08.007

Hong CY, Lin SK, Wang HW, Shun CT, Yang CN, Lai EH, Cheng SJ, Chen MH, Yang H, Lin HY, Wu FY, Kok SH. Metformin Reduces Bone Resorption in Apical Periodontitis Through Regulation of Osteoblast and Osteoclast Differentiation. J Endod. 2023 Sep;49(9):1129-1137. doi: 10.1016/j.joen.2023.07.005

Howait, M., Albassam, A., Yamada, C., Sasaki, H., Bahammam, L., Azuma, M.M., Cintra, L.T.A., Satoskar, A.R., Yamada, S., White, R., Kawai, T., Movila, A. (2019). Elevated Expression of Macrophage Migration Inhibitory Factor Promotes Inflammatory Bone Resorption Induced in a Mouse Model of Periradicular Periodontitis. J Immunol, 202(7), 2035-2043. https://doi.org/10.4049/jimmunol.1801161

Iwasaki, M., Yoshihara, A., Moynihan, P., Watanabe, R., Taylor, G.W., Miyazaki, H. (2010). Longitudinal relationship between dietary ω-3 fatty acids and periodontal disease. Nutrition, 26(11-12), 1105-9. https://doi.org/10.1016/j.nut.2009.09.010

Jakovljevic A, Miletic M, Nikolic N, Beljic-Ivanovic K, Andric M, Milasin J. Notch signaling pathway mediates alveolar bone resorption in apical periodontitis. Med Hypotheses. 2019 Mar;124:87-90. doi: 10.1016/j.mehy.2019.02.018

Kesavalu, L., Vasudevan, B., Raghu, B., Browning, E., Dawson, D., Novak, J.M., Correll, M.C., Steffen, M.J., Bhattacharya, A., Fernandes, G., Ebersole, J.L. (2006). Omega 3 fatty acid effect on alveolar bone loss in rats. J Dent Res, 85(7), 648-52. https://doi.org/10.1177/154405910608500713

Kim JS, Cho IH, Kim KH, Hwang YS. Identification of galectin-10 as a biomarker for periodontitis based on proteomic analysis of gingival crevicular fluid. Mol Med Rep. 2021 Feb;23(2):123. doi: 10.3892/mmr.2020.11762.

Lombardi, G., Ziemann, E., Banfi, G. (2019). Physical Activity and Bone Health: What Is the Role of Immune System? A Narrative Review of the Third Way. Front Endocrinol (Lausanne), 7;10, 60. https://doi.org/10.3389/fendo.2019.00060

Nair, P.N. (2004). Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med, 15(6), 348-381. https://doi.org/10.1177/154411130401500604

Nandakumar R, Madayiputhiya N, Fouad AF. Proteomic analysis of endodontic infections by liquid chromatography-tandem mass spectrometry. Oral Microbiol Immunol. 2009 Aug;24(4):347-52. doi: 10.1111/j.1399-302X.2009.00520.x.

Petean IBF, Küchler EC, Soares IMV, Segato RAB, Silva LABD, Antunes LAA, Salles AG, Antunes LS, Sousa-Neto MD. Genetic Polymorphisms in RANK and RANKL are Associated with Persistent Apical Periodontitis. J Endod. 2019 May;45(5):526-531. doi: 10.1016/j.joen.2018.10.022.

Plecevic, S., Jakovljevic, B., Savic, M., Zivkovic, V., Nikolic, T., Jeremic, J., Milosavljevic, I., Srejovic, I., Tasic, N., Djuric, D., Jakovljevic, V. (2018). Comparison of short-term and medium-term swimming training on cardiodynamics and coronary flow in high salt-induced hypertensive and normotensive rats. Mol Cell Biochem, 447(1-2), 33-45. https://doi.org/10.1007/s11010-018-3291-2

Pomini KT, Buchaim DV, Bighetti ACC, Andreo JC, Rosso MPO, Escudero JSB, Della Coletta BB, Alcalde MP, Duarte MAH, Pitol DL, Issa JPM, Ervolino E, Moscatel MBM, Bellini MZ, Souza AT, Soares WC, Buchaim RL. Use of Photobiomodulation Combined with Fibrin Sealant and Bone Substitute Improving the Bone Repair of Critical Defects. Polymers (Basel). 2022 Oct 4;14(19):4170. doi: 10.3390/polym14194170

Ribeiro-Vidal, H., Sánchez, M.C., Alonso-Español, A., Figuero, E., Ciudad, M.J., Collado, L., Herrera, D., Sanz, M. (2020). Antimicrobial Activity of EPA and DHA against Oral Pathogenic Bacteria Using an In Vitro Multi-Species Subgingival Biofilm Model. Nutrients, 12(9), 2812. https://doi.org/10.3390/nu12092812

Samuel, R.O., Ervolino, E., de Azevedo Queiroz, Í.O., Azuma, M.M., Ferreira, G.T., Cintra, L.T.A. (2019). Th1/Th2/Th17/Treg Balance in Apical Periodontitis of Normoglycemic and Diabetic Rats. J Endod, 45(8), 1009-1015. https://doi.org/10.1016/j.joen.2019.05.003

Sanbe T, Tomofuji T, Ekuni D, Azuma T, Irie K, Tamaki N, Yamamoto T, Morita M. Vitamin C intake inhibits serum lipid peroxidation and osteoclast differentiation on alveolar bone in rats fed on a high-cholesterol diet. Arch Oral Biol. 2009 Mar;54(3):235-40. doi: 10.1016/j.archoralbio.2008.11.001

Sarıtekin E, Üreyen Kaya B, Aşcı H, Özmen Ö. Anti-inflammatory and antiresorptive functions of melatonin on experimentally induced periapical lesions. Int Endod J. 2019 Oct;52(10):1466-1478. doi: 10.1111/iej.13138

Scheffer, D.D.L., Latini, A. Exercise-induced immune system response: Anti-inflammatory status on peripheral and central organs (2020) Biochim Biophys Acta Mol Basis Dis, 1866(10), 165823. https://doi.org/10.1016/j.bbadis.2020.165823

Shimojo, G., Joseph, B., Shah, R., Consolim-Colombo, F.M., De Angelis, K., Ulloa, L. (2019). Exercise activates vagal induction of dopamine and attenuates systemic inflammation. Brain Behav Immun, 75, 181-191. https://doi.org/10.1016/j.bbi.2018.10.005

Simpson RJ, Kunz H, Agha N, Graff R. Exercise and the Regulation of Immune Functions. Prog Mol Biol Transl Sci. 2015;135:355-80. doi: 10.1016/bs.pmbts.2015.08.001.

Subbotina, A. Y., Martyusheva, A. S., Ratmirov, A. M., Abramova, A. Y., Alekseeva, I. V., & Pertsov, S. S. (2023). Age and Sex Characteristics of the Blood Cytokine Profile in Rats Subjected to Prenatal Stress. Bulletin of experimental biology and medicine, 174(3), 299–303. https://doi.org/10.1007/s10517-023-05695-4

Uchida, F., Oh, S., Shida, T., Suzuki, H., Yamagata, K., Mizokami, Y., Bukawa, H., Tanaka, K., Shoda, J. (2021). Effects of Exercise on the Oral Microbiota and Saliva of

Patients with Non-Alcoholic Fatty Liver Disease. Int J Environ Res Public Health, 18(7), 3470. https://doi.org/10.3390/ijerph18073470

Voltarelli, F.A., Gobatto, C.A., de Mello, M.A. (2002). Determination of anaerobic threshold in rats using the lactate minimum test. Braz J Med Biol Res, 35(11), 1389-94. https://doi.org/10.1590/s0100-879x2002001100018

Anexo I- Comitê de Ética de Uso de Animais

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Certificamos que o Projeto de Pesquisa intitulado **"Efeitos do exercício físico de natação na periodontite apical, análise dos tecidos pulpar, ósseo e periodontal"**, Processo FOA nº 0062-2021, sob responsabilidade de Rogério de Castilho Jacinto apresenta um protocolo experimental de acordo com os Princípios Éticos da Experimentação Animal e sua execução foi aprovada pela CEUA em 04 de Março de 2021.

VALIDADE DESTE CERTIFICADO: 25 de Março de 2023.
DATA DA SUBMISSÃO DO RELATÓRIO FINAL: até 25 de Abril de 2023.

CERTIFICATE

We certify that the study entitled **"Effects of swimming exercise on apical periodontitis, analysis of the tissue of pulp, bone and periodontal"**, Protocol FOA nº 0062-2021, under the supervision of Rogério de Castilho Jacinto presents an experimental protocol in accordance with the Ethical Principles of Animal Experimentation and its implementation was approved by CEUA on March 04, 2021.

VALIDITY OF THIS CERTIFICATE: March 25, 2023.
DATE OF SUBMISSION OF THE FINAL REPORT: April 25, 2023.

Prof. Associado João Carlos Callera
Coordenador da CEUA
CEUA Coordinator

CEUA - Comissão de Ética no Uso de Animais
Faculdade de Odontologia de Araçatuba
Faculdade de Medicina Veterinária de Araçatuba
Rua José Bonifácio, 1193 – Vila Mendonça - CEP: 16015-050 – ARAÇATUBA – SP
Fone (18) 3636-3234 Email CEUA: ceua.foa@unesp.br

Anexo II – Materiais utilizados para o protocolo de natação

Tambores plásticos

Canos de PVC formando raias

Termostato elétrico