UNIVERSIDADE FEDERAL DO PARANÁ

PRISCILA ALVES TEIXEIRA RIBAS

IMPACTO DO EXERCÍCIO FÍSICO SOBRE O REPARO PERIODONTAL – ESTUDO *IN VIVO* SOBRE O MOMENTO DA ATIVIDADE NO TRATAMENTO PERIODONTAL

CURITIBA

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Tese apresentada ao Programa de Pós Graduação em Odontologia, Setor de Ciências da Saúde, Universidade Federal do Paraná, como requisito à obtenção do título de Doutor em Odontologia.

Orientador: Prof. Dr. João Paulo Steffens

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ODONTOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **PRISCILA ALVES TEIXEIRA RIBAS**, intitulada: **Impacto do exercício físico sobre o reparo periodontal - Estudo in vivo sobre o momento da atividade no tratamento periodontal**, sob orientação do Prof. Dr. JOÃO PAULO STEFFENS, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 10 de Abril de 2025.

JOÃO PAULO STEFFENS
Presidente da Banca Examinadora

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RESUMO

A periodontite é um problema de saúde pública, pela sua alta prevalência e associação com outras doenças não transmissíveis. A inatividade física é descrita como fator de risco, mas os atuais guias de prática clínica para tratamento da periodontite não sugerem ou recomendam o exercício. Assim, o objetivo deste estudo foi avaliar, em humanos e animais, o impacto do exercício físico no tratamento/reparo periodontal e o momento ideal para prescrevê-lo. No estudo em humanos, vinte e quatro pacientes foram alocados aleatoriamente em 2 grupos e avaliados em 3 períodos de tempo (T0-linha de base, T1-45d, T2-90d). O grupo 1 (n=10) recebeu instrumentação subgengival (IS) em T0; reavaliação e exercício em T1; e reavaliação final em T2. O grupo 2 (n=14) começou a se exercitar em T0; recebeu reavaliação e IS em T1; e reavaliação final em T2. Os parâmetros clínicos incluíram profundidade de sondagem (PS), nível clínico de inserção (NIC), sangramento à sondagem (SS) e índice de placa. O fluido crevicular foi analisado por imunoensaio multiplex. O exercício físico proposto durava 7 minutos e foi realizado 3 vezes/semana usando um aplicativo. Para o estudo em animais, quarenta e oito ratos Wistar machos foram alocados aleatoriamente em 6 grupos: O grupo Reparo (Rep) (n = 16) teve a ligadura removida no início do estudo (linha de base), e os ratos foram eutanasiados após 28 ou 56 dias. O grupo Reparo e Exercício (Rep + Ex) (n = 16) teve a ligadura removida e começou a se exercitar na linha de base, e os ratos foram eutanasiados após 28 ou 56 dias. O grupo Exercício (Ex) (n = 16) começou a se exercitar sem a remoção da ligadura na linha de base. Após 28 dias, oito ratos foram eutanasiados, e os oito ratos restantes tiveram as ligaduras removidas (tornando-se o grupo Ex + Rep), sendo eutanasiados em 56 dias. O treinamento envolveu natação de 30min, 5d/semana. Mandíbulas, sangue e espécimes gengivais foram coletados para análise radiográfica, histológica e do perfil inflamatório. Nos humanos, todos os parâmetros clínicos melhoraram em T2. O grupo 2, mas não o grupo 1, mostrou uma melhora significativa em SS e NIC em T1. Não houve interação significativa ou diferenças intergrupo para nenhum parâmetro clínico. Para sítios inicialmente com PS≥4mm, ambos os grupos mostraram reduções significativas em PS e NIC, em ambos os pontos de tempo. Apenas IL-1β e IFNγ foram significativamente reduzidos para ambos os grupos em T2. No estudo em animais, em 56d, ambos os grupos de exercícios mostraram significativamente menor perda óssea em comparação com Rep sozinho ($p<0,05$). A análise histológica revelou que o grupo Rep+Ex também exibiu um aumento em fibroblastos e vasos sanguíneos ($p<0,01$ e $p<0,001$, respectivamente). A análise do perfil inflamatório em 56 d mostrou que os grupos Ex+Rep tinham níveis séricos de VEGF estatisticamente maiores do que Rep ou Rep+Ex ($p=0,01$). Na gengiva, o EGF foi significativamente maior nos grupos de exercício em comparação com Rep ($p=0,01$). Concluiu-se que ambos os protocolos de exercício demonstraram melhorias nos parâmetros periodontais após 90 dias em humanos e o exercício tardio ou precoce após a remoção da ligadura em ratos, melhorou o reparo ósseo, possivelmente por meio da modulação do fator de crescimento.

Palavras-chave: exercício; reparo; ligadura.

ABSTRACT

Periodontitis is a public health problem due to its high prevalence and association with other non-communicable diseases. Physical inactivity is described as a risk factor, but current clinical practice guidelines for the treatment of periodontitis do not suggest or recommend exercise. Therefore, the aim of this study was to evaluate, in humans and animals, the impact of physical exercise on periodontal treatment/repair and the ideal time to prescribe it. In the human study, twenty-four patients were randomly allocated into 2 groups and evaluated in 3 time periods (T0-baseline, T1-45d, T2-90d). Group 1 (n=10) received subgingival instrumentation (SI) at T0; reassessment and exercise at T1; and final reassessment at T2. Group 2 (n=14) started exercising at T0; received reassessment and SI at T1; and final reassessment at T2. Clinical parameters included probing depth (PD), clinical attachment level (CAL), bleeding on probing (BoP), and plaque index. Crevicular fluid was analyzed by multiplex immunoassay. The proposed physical exercise lasted 7 minutes and was performed 3 times/week using an app. For the animal study, forty-eight male Wistar rats were randomly allocated into 6 groups: The Repair (Rep) group (n = 16) had the ligature removed at the beginning of the study (baseline), and the rats were euthanized after 28 or 56 days. The Repair and Exercise (Rep + Ex) group (n = 16) had the ligature removed and started exercising at baseline, and the rats were euthanized after 28 or 56 days. The Exercise (Ex) group (n = 16) started exercising without ligature removal at baseline. After 28 days, eight rats were euthanized, and the remaining eight rats had their ligatures removed (becoming the Ex + Rep group) and were euthanized at 56 days. Training involved swimming for 30 min, 5 d/wk. Mandibles, blood, and gingival specimens were collected for radiographic, histological, and inflammatory profile analysis. In humans, all clinical parameters improved at T2. Group 2, but not group 1, showed a significant improvement in BoP and CAL at T1. There were no significant interactions or intergroup differences for any clinical parameter. For sites with initial PD ≥4 mm, both groups showed significant reductions in PD and CAL at both time points. Only IL-1β and IFNγ were significantly reduced for both groups at T2. In the animal study, at 56 d, both exercise groups showed significantly less bone loss compared with Rep alone ($p<0.05$). Histological analysis revealed that the Rep+Ex group also exhibited an increase in fibroblasts and blood vessels ($p<0.01$ and $p<0.001$, respectively). Analysis of the inflammatory profile at 56 d showed that the Ex+Rep groups had statistically higher serum VEGF levels than either Rep or Rep+Ex ($p=0.01$). In the gingiva, EGF was significantly higher in the exercise groups compared with Rep ($p=0.01$). It was concluded that both exercise protocols demonstrated improvements in periodontal parameters after 90 days in humans, and that exercise, either late or early after ligature removal in rats, improved bone repair, possibly through growth factor modulation.

Keywords: exercise; repair; ligature.

SUMÁRIO

INTRODUÇÃO

A periodontite é uma doença inflamatória crônica, não transmissível, caracterizada pela destruição progressiva dos tecidos de suporte dos dentes, resultado de uma resposta imunoinflamatória exacerbada frente a um biofilme microbiano disbiótico. Quando não tratada adequadamente, a doença pode culminar na perda dentária (Papapanou et al., 2018). Trata-se da segunda condição oral mais prevalente na população humana, afetando aproximadamente 50% da população mundial (Billings et al., 2018). Especificamente, sua forma grave é considerada a sexta doença não transmissível (DNT) mais prevalente globalmente (Kassebaum et al., 2014).

Do ponto de vista epidemiológico, a periodontite representa um expressivo desafio à saúde pública (Jin et al., 2013; Tonetti et al., 2017). No Brasil, dados regionais apontam que, na cidade de Curitiba, mais de 66% dos indivíduos com idade superior a 18 anos apresentam algum grau de comprometimento periodontal (Dos Anjos et al., 2024). Em função da alta prevalência e da cronicidade da condição, os encaminhamentos aos Centros de Especialidades Odontológicas têm sido limitados a casos complexos, frequentemente relacionados à recidiva da doença ou a falhas terapêuticas (Ministério da Saúde do Brasil, 2018).

Adicionalmente, destaca-se que a periodontite compartilha fatores de risco com outras DNTs, como doenças cardiovasculares, diabetes mellitus e complicações gestacionais, com as quais mantém associações independentes e clinicamente relevantes (Sanz et al., 2013; Sanz et al., 2018; Sanz et al., 2020). Fatores comportamentais e ambientais, tais como obesidade, dieta inadequada, tabagismo e inatividade física, também têm sido implicados na patogênese da periodontite (Chapple et al., 2017). Apesar da relevância desses determinantes, observa-se uma limitada integração entre os diferentes núcleos da atenção primária à saúde para o manejo interdisciplinar da doença (Duarte et al., 2024).

Nesse contexto, diversos estudos observacionais têm sugerido uma associação inversa entre a prática de atividade física — especialmente no domínio do lazer, ou seja, o exercício físico estruturado — e a prevalência de periodontite (Merchant et al., 2003; Al-Zahrani et al., 2005; Bawadi et al., 2011). Estudos longitudinais de intervenção corroboram esses achados, evidenciando efeitos positivos do exercício físico sobre a saúde periodontal (Omori et al., 2018; Sudhanshu et al., 2017). Uma meta-análise recente apontou que a prática regular de exercícios, com frequência entre três e cinco vezes por semana, está associada a menor prevalência da doença em comparação com indivíduos sedentários

(Ferreira et al., 2019).

Especificamente, a atividade física de lazer tem sido identificada como um fator protetor para a periodontite, sendo o único domínio da atividade física claramente associado a benefícios periodontais (Marruganti et al., 2023; Anjos et al., 2023). Indivíduos fisicamente inativos apresentam risco até dez vezes maior de desenvolver formas graves da doença (Marruganti et al., 2022). As principais hipóteses que sustentam essa associação incluem a redução da inflamação sistêmica e a facilitação de mudanças comportamentais positivas por parte dos pacientes (Ferreira et al., 2019; Church et al., 2011).

Entretanto, o estilo de vida contemporâneo tem se caracterizado por níveis crescentes de sedentarismo, agravados pelo acesso limitado a espaços adequados para a prática de exercícios (Church et al., 2011; Schmidt et al., 2016). Diante desse cenário, o treinamento em circuito se apresenta como uma alternativa viável, prática e acessível, pois pode ser realizado em diferentes ambientes, sem necessidade de equipamentos especializados, com baixo custo e tempo reduzido (Klika et al., 2013).

As diretrizes clínicas atuais para o manejo de diversas DNTs incluem o exercício físico como componente essencial da abordagem terapêutica (American Diabetes Association Professional Practice Committee, 2024; Virani et al., 2023; Garvey et al., 2016). No entanto, no que se refere à periodontite, as diretrizes vigentes mencionam apenas a possibilidade de que o exercício físico possa influenciar positivamente os desfechos clínicos, sem evidências conclusivas (Sanz et al., 2020). Ademais, até o presente momento, nenhum estudo clínico avaliou diretamente o impacto do exercício físico sobre o reparo periodontal após a remoção da ligadura em modelos animais.

Em estudos experimentais com roedores, têm sido descritos diversos efeitos benéficos da atividade física no contexto da periodontite, como a redução de comportamentos ansiosos (Andrade et al., 2017; Bertolini et al., 2022), melhora do controle glicêmico e da resposta inflamatória em animais diabéticos (Andrade et al., 2018), e a diminuição da progressão da doença periodontal induzida (Andrade et al., 2017; Souza e Baptista, 2023).

Notadamente, o exercício aquático demonstrou efeito protetor sobre os tecidos periodontais, por meio da redução da inflamação e estímulo à regeneração óssea (Bortolini et al., 2019). Da mesma forma, o treinamento intervalado de alta intensidade mostrou-se eficaz na atenuação da progressão da periodontite, com benefícios adicionais relacionados à melhora do comportamento e redução da ansiedade e inflamação sistêmica nos animais (Pereira et al., 2024). Outros achados experimentais sugerem que o exercício físico pode modular positivamente a resposta inflamatória, promover a produção de colágeno e reduzir a atividade osteoclástica, favorecendo, assim, o reparo periodontal (Ribeiro et al., 2025). Tais evidências reforçam o potencial terapêutico da atividade física, particularmente em modelos experimentais.

Diante do exposto, o presente estudo teve como objetivo principal comparar dois protocolos de prescrição de exercício físico como intervenção adjuvante ao tratamento periodontal não cirúrgico, realizados antes ou após a instrumentação subgengival, utilizando um aplicativo de treinamento em circuito para smartphones. Com o intuito de avaliar a plausibilidade biológica dessa intervenção, bem como os possíveis efeitos relacionados à modificação comportamental (efeito Hawthorne), foi conduzido um estudo experimental paralelo em ratos, com indução de periodontite por ligadura e intervenção com exercício aquático em diferentes tempos de acompanhamento.

CAPÍTULO 1 – ARTIGO:

“App-Guided Exercise Improves Periodontal Status in Periodontitis Treatment - A Pilot Randomized Clinical Trial”

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ABSTRACT

Objective: Periodontitis is a non-communicable disease that affects the supporting structures of the teeth. This study aimed to compare two protocols for adjunctive exercise prescription– before or after subgingival instrumentation (SI)- during periodontal therapy. **Methods:** Twenty-four patients were randomly allocated into 2 groups and evaluated at 3 time points (T0-baseline, T1-45d, T2-90d). Group 1 (n=10) received SI at T0; re-evaluation and exercise at T1; and final re-evaluation at T2. Group 2 (n=14) started exercising at T0; received re-evaluation and SI at T1; and final reevaluation at T2. Clinical parameters included probing depth (PD), clinical attachment loss (CAL), bleeding on probing (BoP) and plaque index. Crevicular fluid was analyzed by multiplex immunoassay. The exercise lasted 7 minutes and was performed 3-times/week using an app. **Results:** Twenty-four patients completed the study. All clinical parameters improved at T2. Group 2, but not Group 1, showed a significant improvement in BoP and CAL at T1. There was no significant interaction or intergroup differences for any clinical parameter. For initial PD≥4mm sites, both groups showed significant reductions in PD and CAL at both time points. Only IL-1β and IFN-γ were significantly reduced for both groups at T2. **Conclusion:** Both exercise/SI protocols demonstrated improvements in periodontal parameters after 90 days.

Descriptors: Periodontal diseases, Exercise, Periodontics.

INTRODUCTION

Periodontitis is the second most prevalent oral disease in humans, affecting 50% of the world's population [1]. It is characterized by progressive destruction of the tooth-supporting apparatus and may lead to tooth loss if untreated [2]. Additionally, severe periodontitis is the sixth most common non-communicable disease (NCD) in humans [3]. As a major public health problem [4,5], periodontitis shares common risk factors with other NCDs and may also impact negatively upon systemic health – being independently associated with cardiovascular diseases [6], diabetes [7] and adverse pregnancy outcomes [8]. Moreover, obesity, poor nutrition, tobacco smoking and physical inactivity have been associated with an increase in periodontitis risk [9], although multidisciplinary treatment within primary health care is generally underperformed [10].

Physical exercise is a planned, structured and repetitive activity aiming to improve or maintain one or more components of physical fitness, performance or health [11,12]. When individuals transit from inactivity to an active lifestyle, the general effects of exercise are more significant. Physical inactivity has been associated with the development of 40 chronic diseases. Individuals who are active have lower mortality rates, cardiovascular and metabolic diseases, depression, colon and breast cancer [13]. Moreover, exercise may protect against the development of diabetes mellitus, osteoporosis, arterial hypertension, obesity, anxiety and stress [14], in addition to being associated with a lower systemic inflammatory profile [15].

Some observational studies have shown that exercise may be associated with a reduced prevalence of periodontitis [14,16,17]. Longitudinal interventional studies have demonstrated potentially beneficial effects of physical exercise on the periodontium [18,19]. In addition, a systematic review and meta-analysis demonstrated that the frequency of physical exercise - a routine of exercises between 3 and 5 times a week – was associated with a lower prevalence of periodontitis compared to inactive individuals [20]. Two potential explanations for this association are due to: 1) a reduced systemic inflammatory burden and; 2) via a positive patient attitude to behavior change in general [20,21].

The modern world is characterized by an increasingly sedentary lifestyle, with limited access to exercises facilities [21,22]. Circuit training has been proposed as a quick way of improving levels of fitness, since it can be undertaken anywhere and without specialist equipment [23]. One regime for circuit interval training consists of performing as many repetitions as possible in 30 seconds, followed by a 10 to 15-second recovery [24]. Such training protocol is also reported to maximize fitness outcomes with minimal investment [23].

The treatment of several chronic inflammatory diseases, such as diabetes, hypertension and obesity, needs to embrace non-pharmacological self-care interventions for a healthier lifestyle, which include physical exercise [25]. The current guidelines for the treatment of Stage I-III periodontitis states that 'maybe' physical exercise should be prescribed during the initial phase of periodontal treatment [26]. Therefore, the aim of this randomized pilot clinical trial was to compare two protocols for the prescription of adjunctive physical exercise prior to, or following subgingival instrumentation, using a smartphone circuit training app to guide exercise training during non-surgical periodontal therapy.

METHODOLOGY

This randomized parallel-arm pilot clinical trial was approved by the Institutional Ethics Committee under the protocol #02621018.4.0000.0102 and registered at the Brazilian Registry of Clinical Trials (ReBEC #RBR-5jxh6c). It was performed according to the declaration of Helsinki as revised in 2013 and reported according to CONSORT guidelines [27].

Population and Eligibility Criteria

All participants were selected at the Federal University of Paraná Dental Clinics, Curitiba, Brazil, and signed an informed consent. Inclusion criteria were: 1) having at least 20 teeth, excluding third molars, with at least 2 non-adjacent teeth with interproximal sites showing probing depth (PD) ≥4mm, bleeding on probing (BoP) and clinical attachment loss (CAL); 2) 30-50 years old; 3) non-practitioners of exercise for at least one year; 4) no contraindication to exercise; 5) body mass index (BMI) ranging from 18.5 to 29.9 (kg/m²); and 6) having a smartphone. Exclusion criteria: 1) history of periodontal therapy within the previous 6 months; 2) smokers; 3) having a systemic NCD; 4) taking any medication that may influence the inflammatory, immunological, microbiological and clinical condition of the periodontium.

Clinical Examination

All patients underwent full periodontal examination at each time point using same-batch standardized manual probes (CPUNC 15, Hu-Friedy, Chicago, IL, US). Two previously trained and calibrated researchers (GG and PR, Kappa ± 1mm>0.9) performed a full mouth clinical examination by assessing O'Leary's plaque index (PI) at 4 sites per tooth and PD, BoP and CAL at 6 sites per tooth. All patients received oral hygiene instruction (OHI). Repeated clinical examinations took place after 45 days (T1) and 90 days (T2), by the same baseline examiners, who were blinded to treatment allocation.

Experimental Design and Treatment Protocol

Following the initial clinical examination at T0, participants were randomly assigned into two groups - using computer-generated random numbers - and followed for a total of 3 months. Group 1 (G1) received, at baseline (T0): subgingival instrumentation (SI); at T1: periodontal re-evaluation, repeated SI and started exercising; and at T2: final periodontal re-evaluation. Group 2 (G2), at T0: started exercising; at T1: received periodontal re-evaluation and SI; and at T2: received final periodontal re-evaluation. Allocation sequence was generated and implemented by a researcher who was not the examiner (RF, HV, NG, MB or HT).

Subgingival Instrumentation (SI)

SI was performed quadrant-wise using hand curettes under local anesthesia (Mepivacaine 2% with 1:100,000 epinephrine) within one week. At the first round of SI (T0 for G1 and T1 for G2), all sites with PD>3mm were instrumented. Repeated SI was restricted to sites with no closed pockets (defined as PD≥4mm and no BoP if PD=4mm) [26].

Exercise Training Protocol

Patients were asked to complete a seven-minute circuit training workout using only bodyweight resistance, 3 times/week [23]. A free smartphone application (Seven, 2018, Perigee AB, Malmö, Sweden) was used to guide the volunteers through the exercises. The exercise circuit included two sequential rounds of pushups, abdominal crunches, squats, plank, high knees, lunges and side planks. Exercises were performed for 30 seconds followed by a 10-second rest [28]. All participants received a video tutorial to ensure proper performance and compliance was assessed through weekly screenshots of the app’s calendar interface that records each training session.

Inflammatory Profile Analysis

Gingival crevicular fluid samples were collected from the initially deepest PD site showing attachment loss in each individual at all time points, as previously described [29,30]. Briefly, one paper point was inserted in each collection site for 10s, then removed and stored in 200mL phosphate-buffered saline (PBS) supplemented with protease inhibitor. Blood-contaminated samples were discarded. After three minutes, the procedure was repeated until a total of 4 paper points were collected from the same site, representing 40s of exposition, as previously reported. Samples were stored at -80^{o}C until analysis using a multiplex immunoassay (HCYTOMAG-60K, Merck KGaA, Darmstadt, Germany), to determine the concentration of Interferon (IFN)-γ, Interleukin (IL)-1 receptor antagonist (-1ra), IL-1β, IL-4, IL-6, IL-10, Tumor Necrosis Factor (TNF) and Vascular Endothelial Growth Factor (VEGF). All procedures were conducted according to the manufacturer's instructions.

Sample Size and Statistical analysis.

The primary outcome was defined as mean PD reduction in sites with PD≥4mm. Since this pilot trial intended to validate an innovative proposed exercise training protocol, we anticipated a minimum final sample size of 10 individuals per group. Statistical analysis was performed using JAMOVI (https://jamovi.org). For baseline intergroup comparisons of continuous variables, unpaired t-test was used after confirming normal distribution and homogeneity of variances; and frequency distribution was assessed using Fisher's exact test. Mixed analysis of variance (mixed ANOVA) was used for all numerical clinical and immunological parameters. Chi-square test was used to test the frequency distribution of closed pockets at T1 and T2. To rule out the possibility of local behavior modification (resulting in lower plaque levels) influencing the results in G2, we tested the association between PI and BoP / PI and PD using linear regression. For all analyses, statistical significance was set at $p<0.05$.

RESULTS

A total of 157 individuals were screened from 2019 to 2023. After invitation to participate and application of inclusion and exclusion criteria, 33 patients were enrolled in the study and randomized. Eight participants (5 from G1 and 3 from G2) dropped out from the study during follow-up, and 1 (G1) was excluded due to an allergic reaction to local anesthesia. Twenty-four individuals were included in the final analysis (G1: n=10; G2: n=14) (Figure 1). None of the participants reported any side effect related to physical exercise.

The mean age of the participants was 40.6±7 years in G1 and 41.9±7 in G2, with mostly white female participants in both groups. There were no significant differences in baseline characteristics or clinical parameters between the groups (Table 1).

Overall, subgingival instrumentation at T0 (G1) improved all clinical parameters, with a statistically significant reduction in mean PD and plaque at T1; and showed a further statistically significant reduction at T2, after physical exercise was added ($p<0.001$). In G2, physical exercise without SI significantly reduced all clinical parameters at T1; and that reduction was even greater in T2, after SI was delivered (except for mean CAL). There was no statistically significant interaction between time and grouping, or differences between G1 and G2 for any full mouth clinical parameter (Table 2).

The associations between PI and PD or BoP improvement (T0-T1) in group 2 were not statistically significant (r^2= 0.08, p=0.33 and r^2= 0.14, p=0.19, respectively) (Figure 2).

A statistically significant reduction in mean PD and CAL of initially moderate to deep pockets through different time points was observed in both groups ($p<0.05$), except for mean CAL between T1 and T2 in Group 1. Although CAL reduction was statistically significant with exercise (G2; $p<0.001$), an inter-group statistically significant difference for mean CAL at T1 could still be observed ($p<0.05$; Figure 3).

The percentage of closed pockets was approximately 60% at T1 and 80% at T2, with no statistically significant difference between groups at either time points (0.71 and 0.25, respectively) (Table 3).

All inflammatory markers analyzed showed great intra-group variability. When the impact of either SI (G1) or exercise (G2) alone was considered, by analyzing from T0 to T1, divergent trends are observed for IL-4 (decreased by exercise and increased after SI) and VEGF (increased by exercise and decreased by SI). However, those differences were not statistically significant. At T2, only IL-1ß and IFN-γ were significantly reduced ($p<0.05$). IL-6 levels were below the detection limit of the assay (Figure 4).

DISCUSSION

Physical exercise has been recommended as an intervention in the management of several inflammatory diseases, and emerging data suggest that could also be the case for periodontitis [18,19]. This pilot randomized clinical trial aimed to evaluate the optimal timing for the prescription of physical exercise as an adjunct to non-surgical periodontal therapy. Overall, our results demonstrated a beneficial impact of physical exercise on all periodontal clinical parameters, as well as a significant reduction in mean PD of initially moderate to deep pockets (>3mm); and no difference between the timing of exercise prescription on periodontal clinical parameters after 3 months.

Physical exercise (G2) promoted a significant reduction in probing depth (PD), bleeding on probing (BoP) and plaque percentage after 45 days (T1). However, no statistically significant associations were observed between the improvement in PD and BoP, nor between the reduction in PD and plaque index (PI) — despite the decrease in PI in both groups. These findings suggest that the modulation of the inflammatory response promoted by physical exercise may occur by biological mechanisms independent of the reduction in plaque levels, reinforcing the hypothesis that exercise plays a direct role in decreasing inflammation, as previously described [15,20]. Previous studies have demonstrated that physical exercise is potentially protective against gingival bleeding

[15,18]. Furthermore, starting a physical exercise routine produced similar results on periodontal parameters to SI after 45 days. This is in accordance with Omori et al. [18], who presumed that a routine of appropriate exercises had a similar effect to nonsurgical periodontal treatment in people with obesity.

When SI was associated with a physical exercise routine (G1, at T2), PD reduction in moderate to deep sites was higher than in SI alone (G1, at T1). These findings are consistent with those of Sudhanshu et al. [19], who showed that a routine of yoga exercises adjunctive to periodontal treatment yielded better results for PI, PD reduction and CAL gain compared to periodontal treatment alone [19].

A training protocol including exercising three times a week is in accordance with previously reported methods for certain health benefits [21]. Moreover, it has been shown that 2 consecutive days of an extreme conditioning program training led to a significant decrease in anti-inflammatory cytokines and could suppress the immune system [31]. Our physical exercise model was based on circuit training using only bodyweight, which is a recognized alternative for maintaining physical fitness [22] and has become popular due to its time-effectiveness. Additionally, this training approach combines aerobic and resistance training into a single exercise bout lasting 7 minutes [23]. Previous studies have shown that both aerobic and resistance exercises could modulate systemic inflammation, but aerobic exercise is more effective in modulating inflammatory markers, such as IL-2, IL-4, IL-6 and TNF than resistance training [32,33]. In our study, the great intra-group variability precluded definite conclusions on the regulation of inflammatory markers, but only IL-1ß and IFN-γ were significantly reduced at the end of the experiment ($p<0.05$). Additionally, we used time for standardizing the results, instead of total crevicular fluid volume, due to structural limitations in our laboratory, in accordance with previous studies [29,30].

The use of a cost-free smartphone application to assist the practice of physical exercise routine seems to be simple and widely applicable. Such technologies represent promising tools for motivation and improvement in patients' knowledge and compliance [34,35]. In addition, a systematic review with meta-analysis showed that smartphone applications seem to be effective in increasing physical exercise in short periods of time (up to 3 months) [36], which is in accordance with our study design.

The World Health Organization recommends 150 minutes of moderate-intensity physical activity per week to deliver health benefits [13]. Another study showed that 90 minutes a week of moderate-intensity exercise might have health benefits compared to inactive people [37]. However, a previous study from our group showed that even 1 to 75 min/week leisure exercise may improve the odds for better self-perception of oral health by

20% [38]. In this pilot study, we prescribed a routine of 21 min/week exercise and demonstrated positive results on periodontal treatment. These findings suggest that any level of increased physical activity may be beneficial for individuals, and oral health professionals should encourage their patients to increase their time with physical exercise [14,26]. It remains debatable, however, if our findings are solely related to physical exercise or are influenced by confounders, such as stress management and control, or improved self-care (especially oral health care following OHI) due to enrollment in research (Hawthorne effect) [39], which had to be performed due to ethical issues.

Our study was specifically designed to address two central questions: 1) what is the optimal timing for exercise prescription during the active phase of periodontal treatment? and 2) what is the impact of exercise alone when compared to a positive control (SI) on clinical periodontal parameters? A negative control was not included for ethical reasons. Despite having positive effects, exercising alone is not intended to replace periodontal treatment but rather help improve periodontal parameters in the short and longer terms. One possible advantage of starting an exercise routine earlier in periodontal treatment (Step 1) could be the reduced need for subgingival instrumentation or even periodontal surgery. In our study, G1 received two possible subgingival interventions (if endpoints of periodontal therapy were not achieved at the site level) during the 90 days, while G2 received only one.

Although consistent evidence from other studies supports the benefits of physical exercise interventions in periodontal treatment [18,19,40], this is one of the few longitudinal studies that confirms these findings. There are limitations to this pilot trial: in a definitive study the aim would be to achieve adequate power; for ethical reasons both groups received baseline OHI, and this is a confounding factor for the improvements seen for G2 in PD and CAL at T1 - there would be merit in a definitive study following G1 and G2 having OHI at T0, but G2 only undertaking the exercise for 3-months; recruiting participants who were willing to start an exercise routine was extremely difficult; control of other cofounders was limited, since individuals who start training may also improve self-care through diet and oral hygiene, for example. However, overall, this study suggests there may be a beneficial impact of a circuit training protocol on periodontal parameters as part of step 1 and step 2 of periodontal care. Moreover, not allowing behavior modification would also limit the extrapolation of these findings to real patients in daily clinical practice. In vivo studies in pre-clinical models could assist in ruling out biases related to behavioral modification in human clinical trials.

CONCLUSION

Both protocols of exercise prescription, either before or after subgingival instrumentation, significantly improved periodontal parameters after 90 days. Additionally, physical exercise alongside oral hygiene instruction significantly reduced probing depth in initially moderate to deep pockets, suggesting that early prescription could facilitate periodontal treatment.

AUTHOR´S CONTRIBUTIONS

GG, JS and TD designed the study. GG and PR performed all clinical examinations. RF, HV, NG, MB and HT served as dental assistants during clinical appointments. HT monitored patients' compliance. IC collaborated on data analysis. TD and JS performed statistical analyses. GG drafted and PR edited the manuscript. JS supervised all experimental procedures. JS and IC edited the final manuscript. All authors approved and agreed to be responsible for all aspects of this research.

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CONFLICT OF INTEREST

There were no conflicts of interest.

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DATA AVAILABILITY

Supporting data is available through the corresponding author upon reasonable request.

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FIGURE LEGENDS

Figure 1: Study flowchart

Figure 2: Associations between A) Bleeding on probing (BoP, %) and B) Probing Depth (PD, mm) improvement and plaque reduction (%) in T1 compared to T0 in Group 2.
Linear Regression; $p>0.05$.

Figure 3: Mean probing depth (PD, mm) and clinical attachment loss (CAL, mm) in initially moderate to deep sites (PD>3mm) at the patient level for group 1 (G1) and group 2 (G2).
* $p<0.05$; ** $p<0.01$; *** $p<0.001$ intragroup comparison to previous time point, unless otherwise connected
# $p<0.05$ intergroup comparison

Figure 4: Mean (SD) concentration of inflammation-related markers in gingival crevicular fluid according to time point (T0, T1 or T2) and Group (1 and 2).
* $p<0.05$ compared to baseline

Table 1: Baseline sociodemographic and periodontal characteristics of participants.

Variable	G1 (N=10)	G2 (N=14)	p-value*
Sex (Male, %)	40%	35.7%	1.00 NS
Skin color (White, %)	80%	91%	0.59 NS
Age (Years)	40.6±6.62	41.9±6.67	0.63 NS
Height (m)	1.65±0.10	1.65±0.08	0.96 NS
Weight (kg)	68.5±12.10	62.6±10.26	0.24 NS
Probing Depth (mm)	2.36±0.31	2.27±0.28	0.48 NS
Clinical Attachment Loss (mm)	2.35±0.56	2.60±0.68	0.34 NS
Bleeding on Probing (Yes, %)	17.3±5.93	19.2±9.01	0.56 NS
Plaque (Yes, %)	57.4±16.6	47.0±15.7	0.13 NS

* t-test, except for sex and skin color (Fisher's exact test).

Table 2: Periodontal clinical parameters of patients from Group 1 and Group 2 at different time points (T0, T1 and T2).

Variable	Group	Baseline (T0)	45 days (T1)	90 days (T2)	p-value*
PD (mm)	1	2.36±0.31$_{A}$	2.17±0.26$_{B}$	1.93±0.20$_{C}$	<0.001 (Time)
	2	2.27±0.28$_{A}$	2.11±0.34$_{B}$	1.91±0.30$_{C}$	0.62 (Group) 0.66 (Int)
CAL (mm)	1	2.35±0.56$_{A}$	2.19±0.38$_{A,B}$	2.05±0.34$_{B}$	<0.001 (Time)
	2	2.60±0.68$_{A}$	2.34±0.73$_{B}$	2.17±0.65$_{B}$	0.48 (Group) 0.38 (Int)
BoP (%)	1	17.26±5.93$_{A}$	10.14±4.30$_{A}$	3.27±2.25$_{B}$	<0.001 (Time)
	2	19.20±9.01$_{A}$	10.49±7.83$_{B}$	4.11±4.38$_{C}$	0.59 (Group) 0.87 (Int)
Plaque (%)	1	57.37±16.65$_{A}$	35.91±13.85$_{B}$	22.57±8.04$_{C}$	<0.001 (Time)
	2	47.00±15.70$_{A}$	30.47±12.36$_{B}$	18.64±8.47$_{C}$	0.07 (Group) 0.63 (Int)

* Mixed ANOVA.
Abbreviations: PD, probing depth. CAL, clinical attachment loss. BoP, bleeding on probing. Int, interaction.
Different capital letters in each row indicate statistically significant differences in time points.
There was no statistically significant interaction or intergroup difference for any parameter.

Table 3: Number (n) and frequency (%) of closed pockets.

Closed pockets (n,%)	45 days (T1)	90 days (T2)
G1 (n=144)	87 (60.4%)	121 (84.0%)
G2 (n=151)	88 (58.3%)	119 (78.8%)
P-value (χ^2)	0.71	0.25

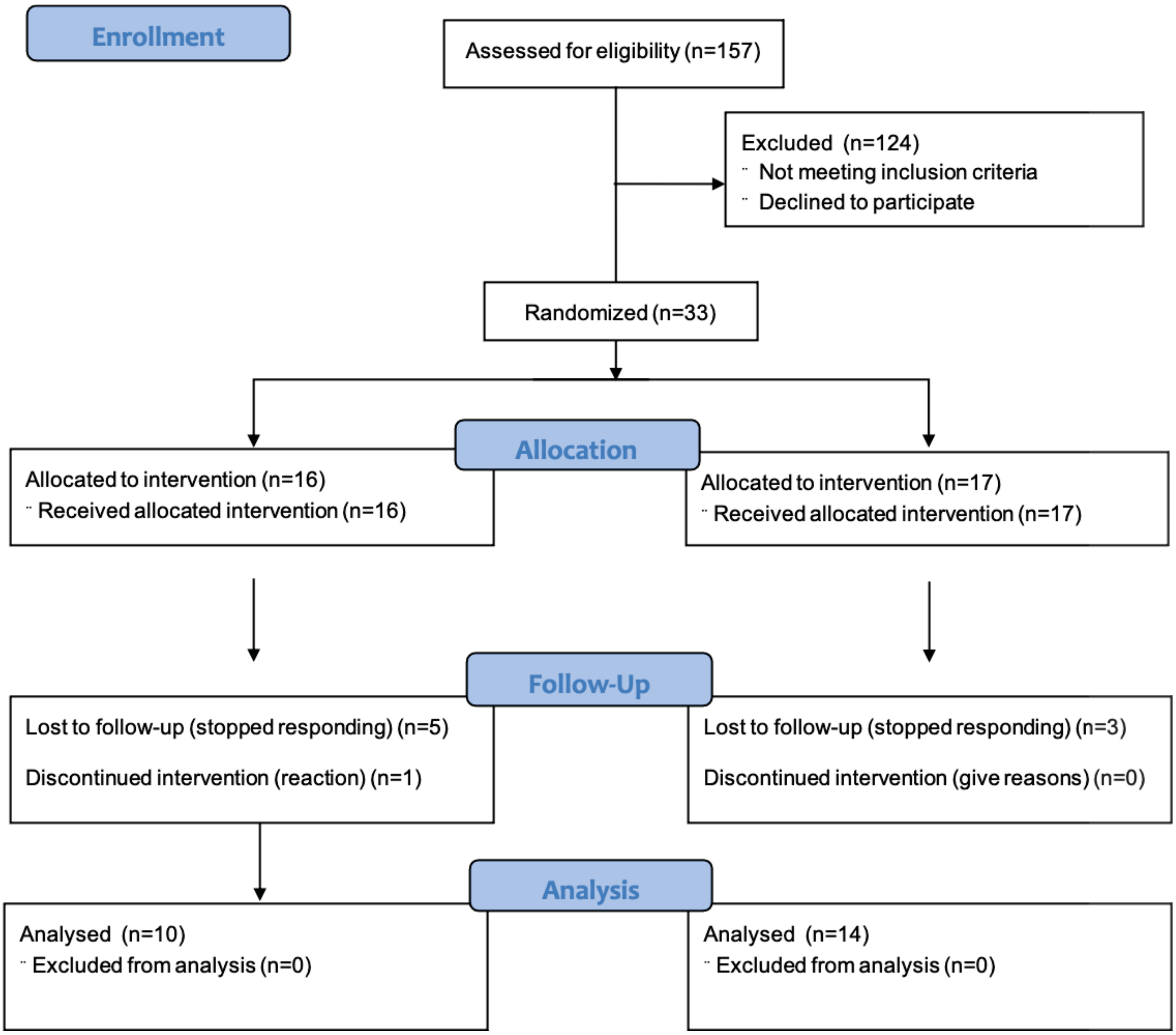
Enrollment
Assessed for eligibility (n=157)
Excluded (n=124)
Not meeting inclusion criteria
Declined to participate
Randomized (n=33)
Allocation
Allocated to intervention (n=16)
Received allocated intervention (n=16)
Allocated to intervention (n=17)
Received allocated intervention (n=17)
Follow-Up
Lost to follow-up (stopped responding) (n=5)
Discontinued intervention (reaction) (n=1)
Lost to follow-up (stopped responding) (n=3)
Discontinued intervention (give reasons) (n=0)
Analysis
Analysed (n=10)
Excluded from analysis (n=0)
Analysed (n=14)
Excluded from analysis (n=0)

Figure 1

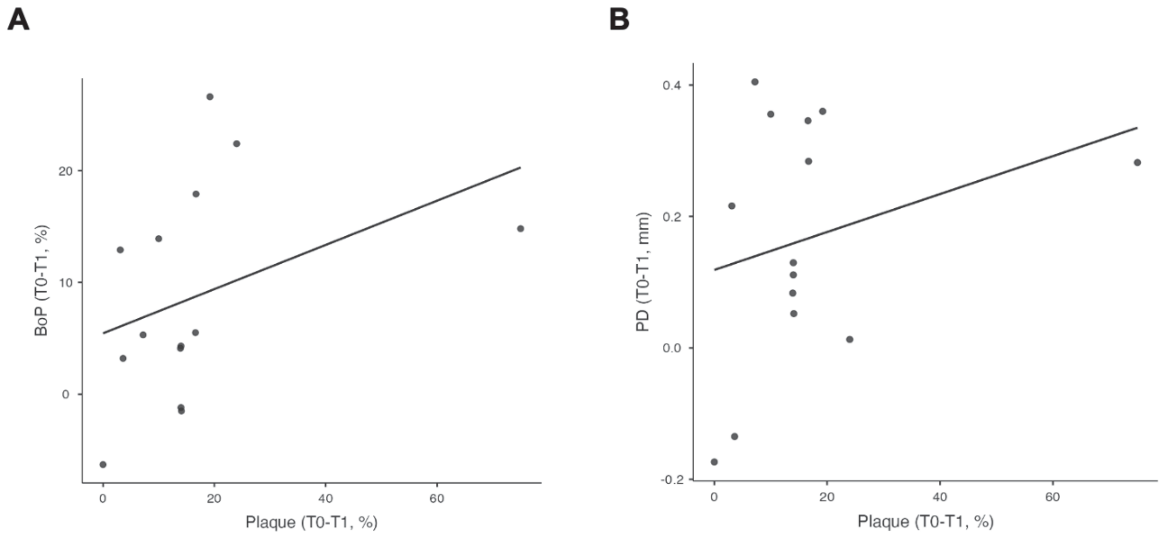

Figure 2

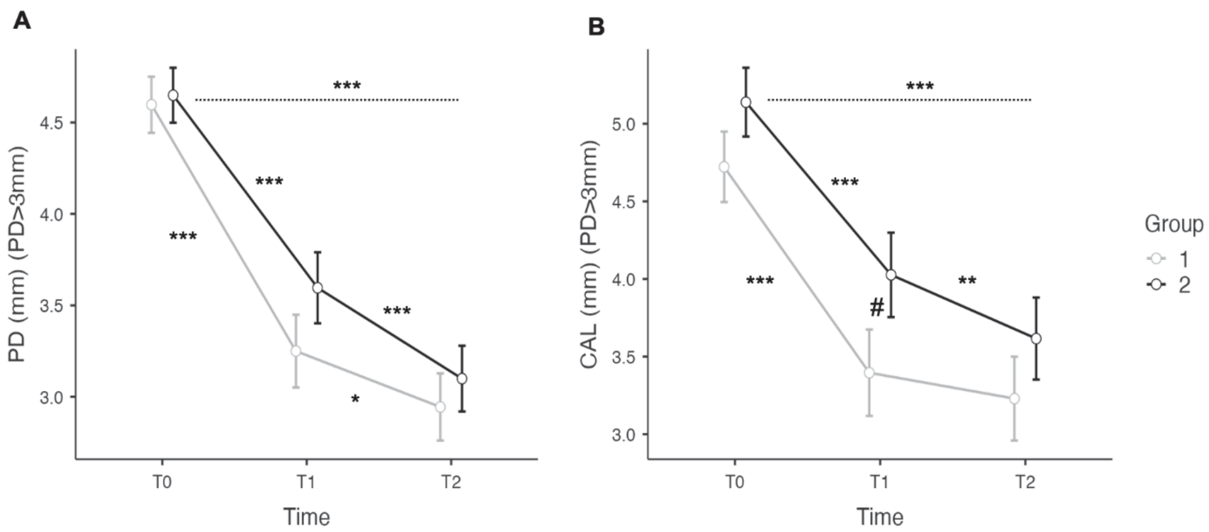
A
PD (mm) (PD>3mm)
4.5
4.0
3.5
3.0
***
***
***
***
*
T0
T1
T2
Time
B
CAL (mm) (PD>3mm)
5.0
4.5
4.0
3.5
3.0
***
***
***
#
**
T0
T1
T2
Time
Group
1
2

Figure 3

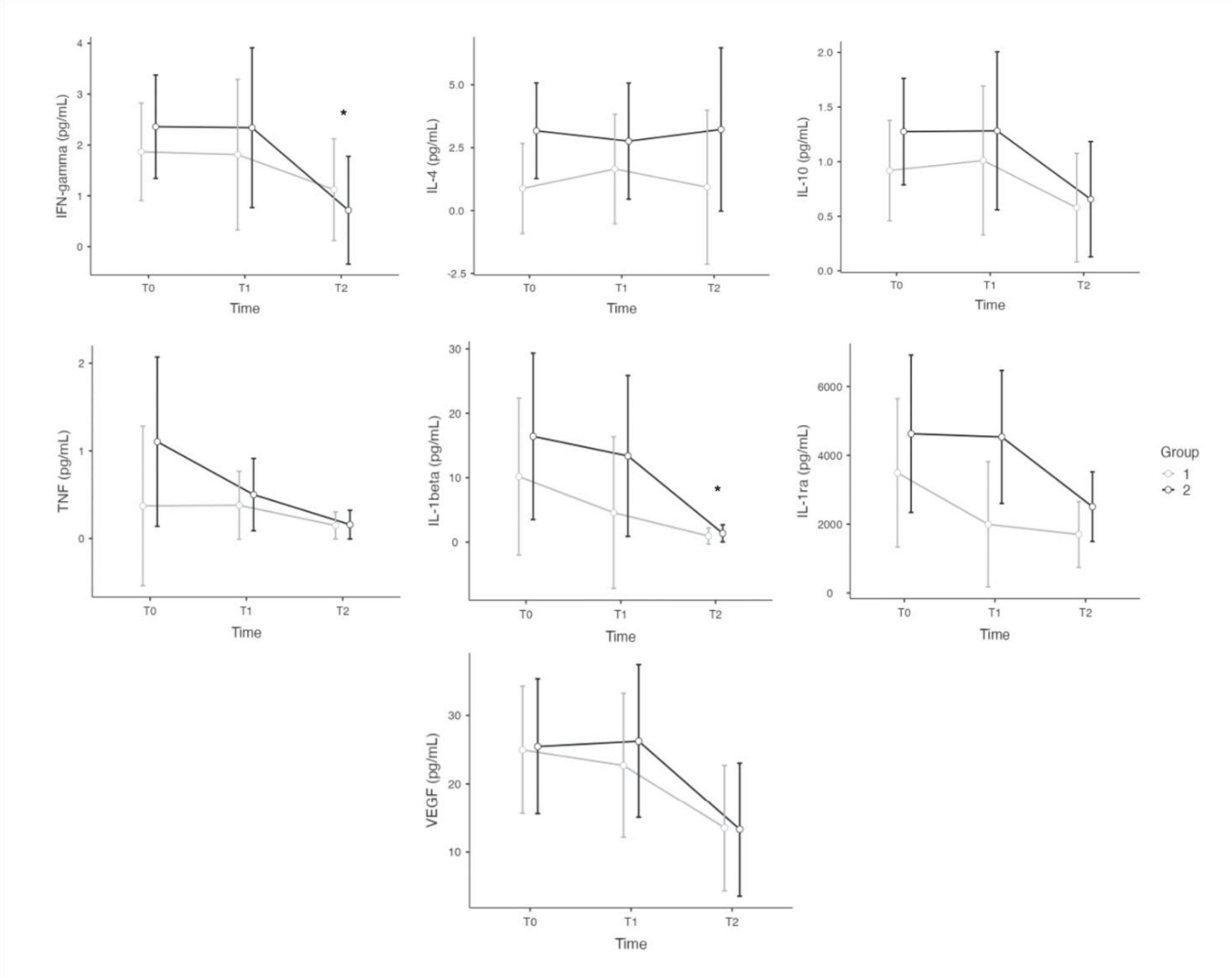
IFN-gamma (pg/mL)
IL-4 (pg/mL)
IL-10 (pg/mL)
TNF (pg/mL)
IL-1beta (pg/mL)
IL-1ra (pg/mL)
VEGF (pg/mL)
Time
T0
T1
T2
*
Group
1
2

Figure 4

CAPÍTULO 2 – ARTIGO:

“Exercise improves periodontal repair following ligature-induced periodontitis in rats”**

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Abstract

Periodontitis is a chronic inflammatory condition that involves bone resorption and leads to tooth loss, if untreated. This study aimed to evaluate the biological plausibility of exercise's potential impact on periodontal repair in rats over different time periods. Forty-eight male Wistar rats were randomly assigned to 6 groups using computer-generated numbers (n = 8/group): The Repair (Rep) group (n = 16) had the ligature removed at the beginning of the study (baseline), and the rats were euthanized after 28 or 56 days (n = 8/group). The Repair and Exercise (Rep + Ex) group (n = 16) had the ligature removed and began exercising at baseline, and the rats were euthanized after 28 or 56 days (n = 8/group). The Exercise (Ex) group (n = 16) began exercising without ligature removal at baseline. After 28 days, eight rats were euthanized, and the remaining eight rats had the ligatures removed (becoming the Ex + Rep group) and were euthanized at 56 days. Training involved 30min swimming, 5d/week. At 28d, half of the animals in the Ex group were sacrificed, and the remaining animals had their ligatures removed (Ex+Rep). All groups were evaluated at 28d and 56d. Mandibles, blood and gingival specimens were collected for radiographic, histological and inflammatory profile analysis. At 56d, both exercise groups showed significantly lower bone loss compared to Repair alone. Histological analysis revealed the Repair+Exercise group exhibited increased percentages of fibroblasts and blood vessels. The Exercise+Repair groups had statistically higher serum VEGF levels than Repair or Repair+Exercise. In the gingiva, EGF was significantly higher in the exercise groups compared to Repair. In conclusion, late or early exercise improved bone repair in rats, possibly through growth factor modulation.

Keywords: Alveolar Bone Loss; Inflammation; Swimming; Models, Animal.

Introduction

Periodontitis is a noncommunicable disease associated with a dysbiotic biofilm and characterized by progressive degradation of the tooth-supporting structure, including bone loss [1]. Levels of pro-inflammatory cytokines, both systemic and local, are elevated in individuals with periodontitis [2], as well as in other chronic diseases such as diabetes mellitus, cardiovascular diseases and obesity [3 – 5]. Current guidelines for such diseases include physical exercise as a component of their treatment protocol [6 – 8], while the current guidelines for periodontitis treatment states that exercise “maybe” has an impact [9].

Several human studies have suggested that physical activity, and particularly its leisure component (i.e., exercise), may be linked to a lower prevalence of periodontitis [10 – 12]. A systematic review and meta-analysis also support this association, indicating that there is a potential reduction in the occurrence of periodontal disease with regular physical activity [13]. Leisure-time physical activity was shown to be a protective indicator for periodontitis [14], and the only domain with the potential to reflect the benefits of physical activity on oral health [15]. In fact, individuals who lack regular exercise were 10 times more likely to have severe forms of periodontitis [16].

Exercise has been shown to attenuate anxious behavior during the progression of periodontitis in rats and mice [17,18], improve the glycemic and inflammatory profiles of rats with diabetes [19], and attenuate or have no influence on the progression of experimental periodontitis [17,20]. However, no previous study has evaluated the impact of exercise on periodontal repair (i.e., after the removal of ligatures that mimic periodontitis inflammation) in an animal model. Therefore, in order to assess the biological plausibility of this relationship and given the potential behavioral modification of participants in clinical studies, the aim of this study was to evaluate the potential impact of exercise on periodontal repair in rats over different time periods.

Materials and methods

Animal study

Forty-eight Wistar male rats (200-250g) were kept under standardized temperature (23±2°C), with 12-hour light cycles, and food/water provided ad libitum. All experimental protocols were approved by the local Institutional Ethics Committee for Animal Experimentation and carried out following the Brazilian Society of Science in Laboratory Animals (SBCAL) and the National Council for the Control of Animal Experimentation (CONCEA). This study was developed and reported following the ARRIVE (Animal Research: Reporting of In Vivo Experiments) standards.

Acclimatization

All animals were acclimatized to the aquatic environment to control for intergroup differences in stress. They spent 2 hours/day for five days in a polyethylene tank with five centimeters of water at a temperature of 32±2°C so that they could touch their paws to the bottom of the tank (adapted from Rambo et al., 2009) [21]. At the end of all sessions, they were dried and returned to the same cages.

Induction of periodontitis

To induce periodontitis in day -7 (seven days before baseline), animals were anesthetized with ketamine (0.08 ml/100 g b.w.) and xylazine (0.04 ml/100 g b.w.) and received a cotton ligature #30 around the lower first molar teeth, bilaterally. Ligatures were kept for seven days and removed to promote periodontal repair, as previously described [22].

Groups

Animals were randomly assigned to one of 6 groups using computed-generated numbers (n=8/group): The Repair (Rep) group (n=16) had the ligature removed at the beginning of the study (baseline), and rats were euthanized after 28 or 56 days (n=8/group). The Repair and Exercise (Rep+Ex) group (n=16) had the ligature removed and began exercising at baseline, and rats were euthanized after 28 or 56 days (n=8/group). The Exercise (Ex) group (n=16) started exercising without ligature removal at baseline. After 28 days, eight rats were euthanized, and the remaining eight rats had the ligatures removed (becoming the Ex+Rep group), being euthanized at 56 days (Figure 1).

Swimming

Swimming sessions were performed with progressive increase in time. Tanks were filled with fifty centimeters of water at a temperature of 32±2°C to avoid resting from touching tails to the bottom. Animals started with 10 minutes on the first day and added 10 minutes each day until they completed 60 minutes at the end of the first week (adapted from Bezerra et al., 2013) [23]. From the second week onward, animals swam 60 minutes/day, five days/week.

Euthanasia and sample collection

After the experiment, all animals were euthanized by anesthetic overdose and cardiac puncture. Blood samples were centrifuged for 10min at 3,000 rpm to obtain serum for the analyses of inflammatory markers and acid phosphatase. Mucogingival tissues from the left first molars were removed and kept at -80 °C for subsequent analysis of inflammatory markers, while anatomical specimens of the right side were stored in 10% formalin for 48h, then washed in running tap water and kept in 70% ethanol for radiographic analysis and, subsequently, received further histological processing.

Radiographic analysis of bone loss

The xDent x70 device (XDENT Dental Equipment's, Ribeirão Preto, SP, Brazil) was used to perform the radiographic shots, with distance, exposure time, and positioning standardization, according to the device's pre-settings for digital sensor for infants using periapical technique. Exposure was set at 190 ms and the distance between the tubehead and the sensor was of 7cm, which was equivalent to the height of the sensor holder used. The Saevo Digital Image (Software version 2.0.0.20, Ribeirão Preto, SP, Brazil) was used to conduct the analyses. Measurements taken from the cementoenamel junction (CEJ) to the alveolar bone crest (ABC) were taken in the mesial root of the first molars in millimeters [22] and performed by a single trained examiner who was blinded to the treatment group. All measurements were performed three times under similar environmental conditions, and the mean result was used for analysis [24]. An intraclass correlation coefficient was calculated a posteriori and resulted in 0.9445 (95%CI: 0.9194-0.963).

Histological analysis

After radiographic analysis, five samples from each group were decalcified in a solution containing 300 ml 85% formic acid, 300 ml 10% formalin, and 900 ml distilled water solution for 30 days. The processing was done in semi-serial sections of 5 µm-thick samples, which were then stained with Hematoxylin and Eosin. Images were captured using the Cell F software (Olympus Soft Imaging Solutions GmbH, Ishikawa-machi, Tokyo, Japan) and then analyzed by a previously trained evaluator who was blinded to the treatment group. For stereometric analysis, a 1,000µm x 500µm grid was positioned vertically in the connective tissue of the images in a 20x image, the bottom part of the grid in contact with the bone tissue, and the left edge in contact with the tooth— a 19x9 grid totaling 200 points of interest. Repair-related structures were identified and counted: fibroblasts, blood vessels and extracellular matrix. Areas outside the area of interest were excluded, and the remaining points were considered the total number of possible points (100%) [24].

Inflammatory profile analysis

Gingival tissue was macerated for protein extraction in T-PER (Thermo Fischer, Meridian Road Rockford, Illinois, USA) containing protease inhibitor (SIGMAFAST, Sigma-Aldrich Co. LLC, Hesse, Darmstadt, Germany). Serum and gingival samples were submitted to a multiplex assay according to the manufacturer's instructions (MILIPLEX Rat Cytokine/ Chemokine Magnetic Bead Panel, Merck KGaA, Hesse, Darmstadt, Germany). Tumor necrosis factor-alpha (TNFα), interleukin (IL) 1-beta (IL-1β), IL-4, IL-6, IL-10, vascular endothelial growth factor (VEGF) and interferon-gamma (IFN-γ) were analyzed in both serum and gingiva, whilst epidermal growth factor (EGF) was only analyzed in gingiva (due to a problem with the manufacturer in one of the kits). In the gingival tissue, total protein was quantified using the DC Protein Assay Method (Bio-Rad Laboratories, Hercules, California, USA) to normalize the results, expressed as pg/mg total protein.

Acid Phosphatase

For serum samples, acid phosphatase (ACP) was analyzed according to the manufacturer's instructions (MAK446, Sigma-Aldrich, Missouri, Saint Louis, USA) and absorbance was measured at 405 nm. The result was expressed in U/L.

Statistical analysis

The primary outcome was radiographic bone loss. The sample size calculation was carried out to detect an intergroup difference of ten percent with a standard deviation of seven [22]; eight animals per group were needed, establishing alpha at 5% and a power of 80%.

Homogeneity of variances and normal distribution were checked using Levene and Shapiro-Wilk tests, respectively. If both criteria were met, one-way ANOVA and post-hoc Tukey tests were performed. Otherwise, the non-parametric Kruskal–Wallis and Dunn's post-test were performed. Values were expressed as mean and standard error of the mean. All tests were performed using free software (Jamovi version 2.4.11.0 for Windows 10, https://www.jamovi.org). The significance level was set at $p<0.05$.

Results

No animals or ligatures were lost during the experiment. At 28 days, bone loss analysis showed no significant differences among groups ($p>0.05$). On the other hand, at 56 days, the Rep+Ex and Ex+Rep groups showed significantly lower bone loss than the Rep group ($p<0.05$) (Figure 2).

In the histological analysis, Ex group had significantly lower percentages of fibroblasts and blood vessels compared to Rep ($p<0.01$). Rep+Ex presented a significantly lower percentage of fibroblasts and a higher percentage of extracellular matrix compared to the Rep group ($p<0.05$) (Figure 3).

At 56 days, both fibroblasts and blood vessels percentages for Rep+Ex were significantly higher in Rep+Ex group than in Rep or Ex+Rep groups ($p<0.01$), while extracellular matrix was lower ($p<0.001$). Ex+Rep showed a significantly lower percentage of fibroblasts when compared to Rep group (Figure 4).

Inflammatory profile analysis at 28 days showed an overall higher serum IL-4 levels and gingival VEGF in exercise groups, with significant difference only between Ex and Rep groups ($p=0.02$). Gingival IL-1β was significantly increased in the Ex group compared to Rep ($p=0.01$). At 56 days, serum IL-4 levels in exercise groups were undetectable ($p=0.02$ compared to Rep), and Ex+Rep group showed statistically higher serum VEGF levels than Rep or Rep+Ex ($p=0.01$). In gingiva, IL-4 was only detectable in Rep+Ex groups ($p=0.03$ compared to Rep and Ex+Rep groups), and EGF was significantly higher in exercise groups compared to Rep ($p=0.01$). There were no significant differences in ACP concentration in serum samples among groups. IL-6 and IFN-γ were not detected in any sample (Table 1).

Discussion

In this study, we analyzed the biological plausibility and associated profile of physical exercise influencing periodontal repair after ligature-induced periodontitis in rats, through radiographic, histological and immunological analyses. Our findings suggest that both exercise protocols (Rep+Ex and Ex+Rep) showed similar improvements in bone height compared to the control group at 56d.

Notably, physical exercise alone (i.e., without ligature removal; Group Ex) showed no significantly higher bone loss than the control group (Rep) at 28d, despite higher levels of factors associated with periodontitis and non-resolving inflammation (such as gingival IL-1β and VEGF). This finding suggests that exercise alone arrests further disease progression.

In human studies, participants tend to develop healthier habits when observed, introducing bias due to the Hawthorne effect [25]. Therefore, we used rats to test the biological plausibility of this association, providing greater control over external and competing factors, and bringing translational research with the goal of producing more applicable and meaningful results for human health [26].

At 56 days, the Rep+Ex and Ex+Rep groups presented a statistically significantly lower bone loss than the Rep group. Although we used a unique repair model not previously tested with exercise, it is possible to assume that this is in agreement with a previous study that found that physical exercise in rats had a protective effect on bone height [27]. Similarly, in our study, we observed no statistically significant differences in serum or gingival TNF-α or IL-10 during periodontal repair. This is in contrast with studies on the progression of periodontitis, where animals that performed physical exercise and had periodontal disease presented lower expression of TNF-α, while IL-10 was increased [17,19].

Histologically, the Rep+Ex group showed better repair-related responses, such as an increase in the percentage of fibroblasts and blood vessels, showing that there was greater synthesis of connective tissue fibers and angiogenesis through which repair was possibly improved. Vascularization of periodontal tissue can improve the supply of nutrients and oxygen to repair tissues, and the promotion of an antioxidant microenvironment more favorable to regeneration [28]. Interestingly, the Ex+Rep showed, at 56d, repair-related responses not significantly different from the control group, despite the improved bone height and higher gingival levels of EGF.

Ligature is a well-established method for inducing experimental periodontitis, allowing controlled analysis of disease progression and treatment. We used a 7-day period for periodontitis induction, as this interval is sufficient to establish bone loss and a characteristic inflammatory response, allowing us to evaluate the initial biological mechanisms of periodontal repair [29,30].

Periodontal repair is expected to occur after ligature removal, resulting in bone gain [22], as the ligature contains the irritants (including bacteria) that lead to periodontitis. In humans, conventional treatment for periodontitis includes the use of instruments that remove biofilm and calculus subgingivally (i.e., scaling and root planning). Ligature removal in rats (our repair model) is not significantly different from scaling and root planning alone, as previously described [31], although this additional step could add trauma due to the size of the hand instruments compared to the animals.

We were able to observe that, after 56 days, there was a significant radiographic improvement in exercise groups, showing that over time, exercise makes a positive difference. By using rats in research, we want to be able to bring the responses and expectations of treatments to humans. And these findings are in line with previous work that also reported relevant benefits of exercise training on the host response to periodontal treatment [32 − 35].

Various types of physical activity, such as aerobic exercise, resistance (strength) training, and high-intensity exercise, have shown positive impacts on different aspects of animal health. In rodents, particularly rats and mice, freewheel training has been shown to improve cardiovascular function [36], while swimming has been shown to decrease ligature-induced periodontal bone loss [17,19]. Furthermore, a study demonstrated that when comparing three exercise models in rats (voluntary wheel running, motorized treadmills and swimming), swimming was the most beneficial option due to its safe and controlled intensity levels, without impacting the inflammatory system [37]. However, water can bring additional stress to the experiment. Therefore, we acclimatized all rats, including the control group, in water [21].

Previous studies measuring the effect of chronic swimming on bone modeling involved control groups, swimming with load weights (1% of body weight) and swimming without extra load (body weight only). Morphometric parameters, bone density, histomorphometry, and biochemical analysis of minerals were significantly better in swimming rats, regardless of the load, compared to controls, indicating that swimming exercise has a positive effect on bone growth and development in young rats [38]. One study suggested that 6-week heavy load training induces immune dysfunction and neuroendocrine responses, which may be one of the underlying mechanisms of immune dysfunction in overtraining [39]. Due to the possibility of heavier training protocols impact overall periodontal response as it seems to be the case in humans [40], we chose not to use extra load during the swimming sessions to avoid interference in the responses. Therefore, swimming was performed under the supervision of at least one of the authors, who would gently poke animals if they started floating.

Our study is the first to identify the biological plausibility and the mediators involved in the periodontal repair response to exercise. However, some limitations can be observed. For example, our study was performed only in male rats. In addition, as in any animal study, translation to humans is limited. An ideal rat model for periodontal treatment is debatable and some may advocate in favor of mixed models with physical and biological agents to induce periodontitis; and mechanical and/or chemical agents to mimic treatment. Also, our primary outcome was based on a bidimensional analysis of bone loss, whilst periodontal defects may have other components. On the other hand, linear analysis in ligature models can be more sensitive than microCT, as the latter may analyze greater areas and dilute the results [24]. Also, it is important to state that our aim is not to propose that exercise could replace periodontal treatment, but it could reduce the need for more surgical and invasive procedures, improving periodontal and inflammatory parameters in general, as a result of a protective and supportive effect.

In conclusion, the implementation of physical exercise as an adjuvant intervention to periodontal treatment has demonstrated beneficial effects on periodontal tissue repair, evidenced by the reduction of gingival inflammation, decreased production of pro-inflammatory cytokines, and preservation of bone integrity. However, the analysis of the timing of exercise prescription did not reveal statistically significant differences between the groups evaluated.

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Table 1 – Mean (SE) of serum and gingival inflammatory profile at 28 and 56 days in each group.

Analyte	28 days				56 days			
	Rep	Rep+Ex	Ex	p-value*	Rep	Rep+Ex	Ex+Rep	p-value*
Serum								
IL-1β	35.2(11.2)	32.3(6.5)	22.3(1.0)	0.19	13.4(4.5)	50.3(9.3)	37.7(13.9)	0.06
TNF-α	3.3(1.1)	7.0(1.5)	6.8(1.5)	0.14	3.7(0.8)	2.5(0.7)	1.3(0.7)	0.19
IL-4	0.0(2.1)$_{A}$	75.3(18.2)$_{AB}$	53.1(21.5)$_{B}$	**0.02**	9.9(4.9)$_{A}$	N/D$_{B}$	N/D$_{B}$	**0.02**
IL-10	115.7(13.0)	115.7(23.5)	95.7(5.0)	0.78	52.3(7.2)	138.2(36.1)	89.2(34.6)	0.17
VEGF	103.9(23.2)	103.9(6.6)	99.6 (1.3)	0.23	88.0(5.5)$_{AB}$	79.3(6.2)$_{A}$	171.0(27.9)$_{B}$	**0.01**
ACP	31.8(9.8)	29.0(3.0)	22.4(3.0)	0.47	21.7(3.2)	23.2(1.8)	42.2(9.5)	0.06
Gingiva								
IL-1β	7.5(0.7)$_{A}$	9.8(2.5)$_{AB}$	18.9(4.4)$_{B}$	**0.01**	8.2(0.9)	8.3(4.2)	5.4(0.5)	0.31
TNF-α	1.6(0.2)	1.6(0.2)	1.5(0.5)	0.94	1.0(0.2)	0.6(0.2)	0.6(0.2)	0.49
IL-4	0.0(1.3)	N/D	0.0(0.2)	0.26	N/D$_{A}$	0.1(0.9)$_{B}$	N/D$_{A}$	**0.03**
IL-10	1.4(0.7)	1.5(0.2)	3.5(0.8)	0.11	0.7(0.1)	1.1(0.4)	0.5(0.1)	0.33
EGF	46.4(5.0)	50.6(13.4)	68.4(8.4)	0.23	24.2(4.7)$_{A}$	56.0(7.5)$_{B}$	63.8(36.2)$_{C}$	**0.01**
VEGF	19.4(2.8)$_{A}$	34.5(13.8)$_{AB}$	81.2(20.5)$_{B}$	**0.02**	19.0(2.9)	23.9(7.3)	26.9(4.8)	0.72

* Kruskal-Wallis with Dunn's post-test; N/D: not detected (below lower limit of the assay); IFN-γ and IL-6 not consistently detected in serum or gingiva.
Serum levels expressed as pg/ml. ACP level expressed as U/L. Gingival levels expressed as pg/mg.

Figure legends

Figure 1: Study timeline and experimental procedures.

Figure 2: Linear measurement (mm) between cement–enamel junction (CEJ) and alveolar bone crest (ABC) in the mesial surface of the first molar tooth in each experimental group.

*ANOVA; $p<0.05$.

Figure 3: Histologic and stereometric analysis of periodontal tissues at 28 days: A) representative histologic images stained with hematoxylin and eosin (magnification 20x); Mean (SE) percentage of B) fibroblasts; C) blood vessels; D) extracellular matrix in the connective tissue in each experimental group. OE— oral epithelium; JE—junctional epithelium; CT—connective tissue; T—tooth.

ANOVA; *$p<0.05$; **$p<0.01$ and ***$p<0.001$.

Figure 4: Histologic and stereometric analysis of periodontal tissues at 56 days: A) representative histologic images stained with hematoxylin and eosin (magnification 20x); Mean (SE) percentage of B) fibroblasts; C) blood vessels; D) extracellular matrix in the connective tissue in each experimental group. OE— oral epithelium; JE—junctional epithelium; CT—connective tissue; T—tooth.

ANOVA; **$p<0.01$ and ***$p<0.001$.

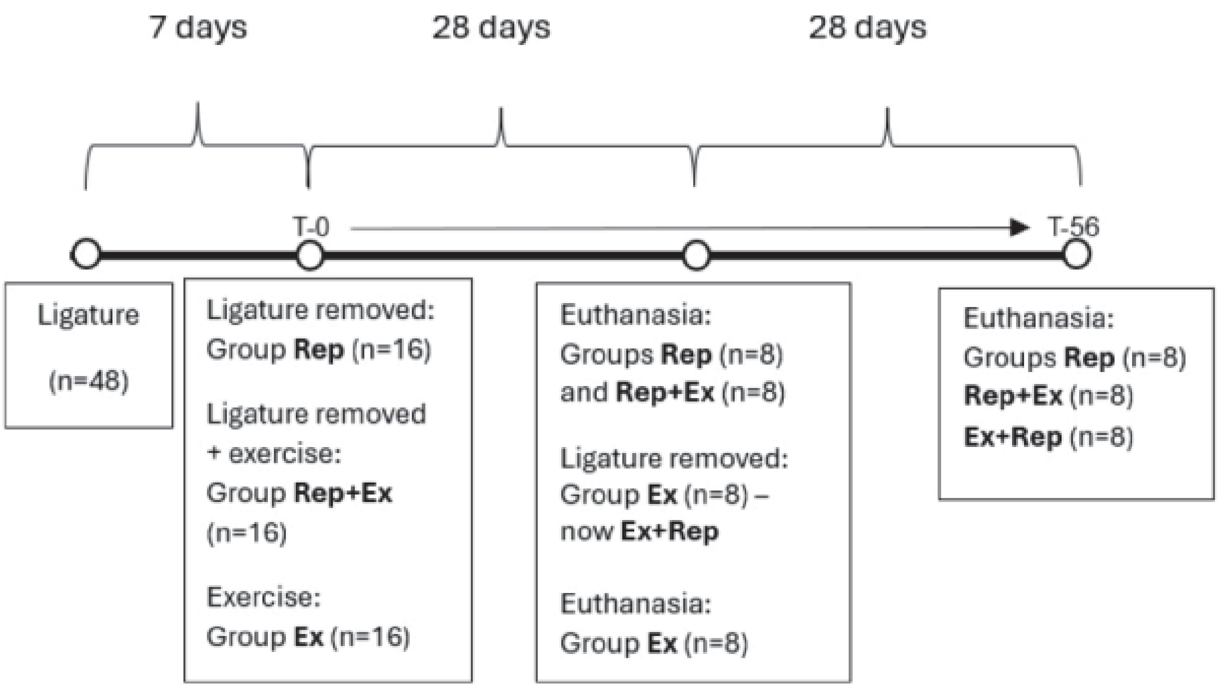
7 days
28 days
28 days
T-0
T-56
Ligature
(n=48)
Ligature removed:
Group Rep (n=16)
Ligature removed
+ exercise:
Group Rep+Ex
(n=16)
Exercise:
Group Ex (n=16)
Euthanasia:
Groups Rep (n=8)
and Rep+Ex (n=8)
Ligature removed:
Group Ex (n=8) –
now Ex+Rep
Euthanasia:
Group Ex (n=8)
Euthanasia:
Groups Rep (n=8)
Rep+Ex (n=8)
Ex+Rep (n=8)

Figure 1

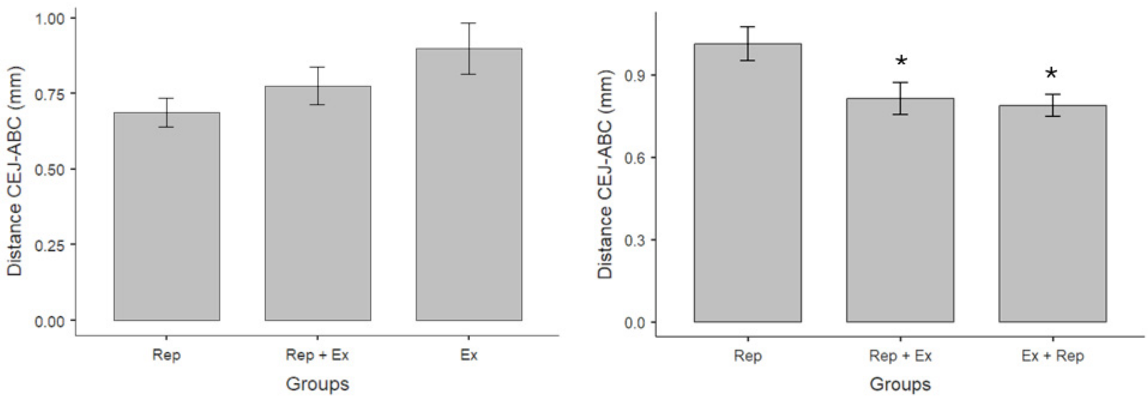
1.00
0.75
0.50
0.25
0.00
Distance CEJ-ABC (mm)
Rep
Rep + Ex
Ex
Groups
0.9
0.6
0.3
0.0
Distance CEJ-ABC (mm)
*
*
Rep
Rep + Ex
Ex + Rep
Groups

Figure 2

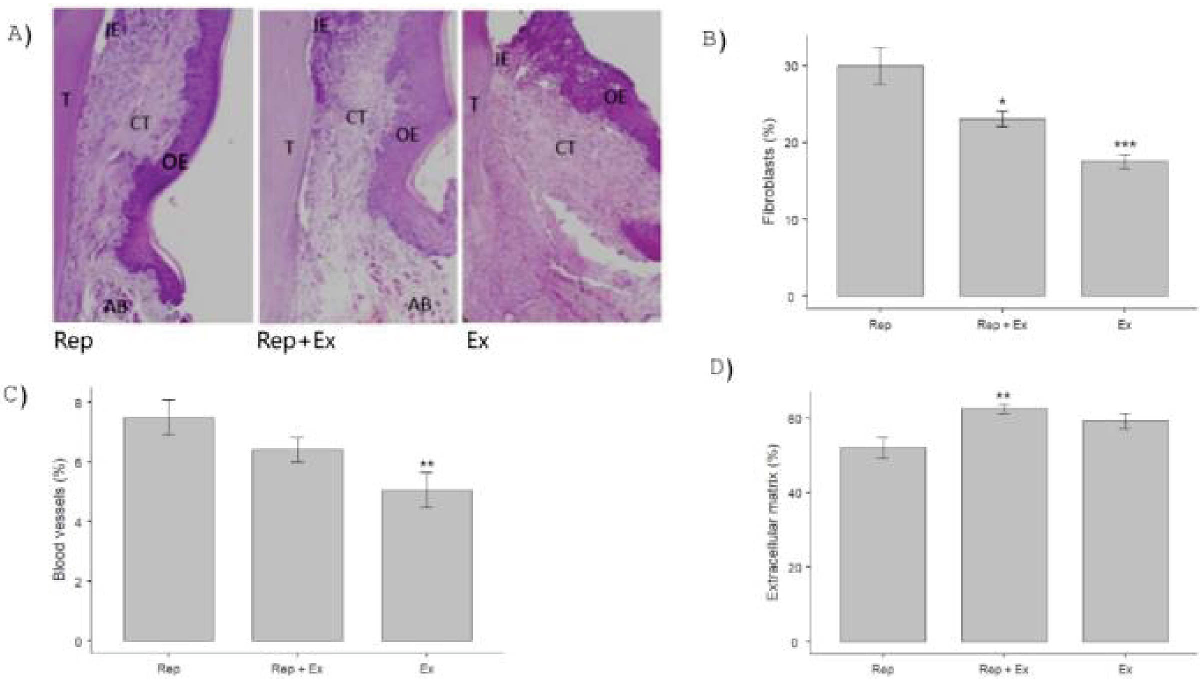
A)
IE
T
CT
OE
AB
Rep
Rep+Ex
Ex
B)
Fibroblasts (%)
30
20
10
0
*
***
Rep
Rep + Ex
Ex
C)
Blood vessels (%)
8
6
4
2
0
**
Rep
Rep + Ex
Ex
D)
Extracellular matrix (%)
60
40
20
0
**
Rep
Rep + Ex
Ex

Figure 3

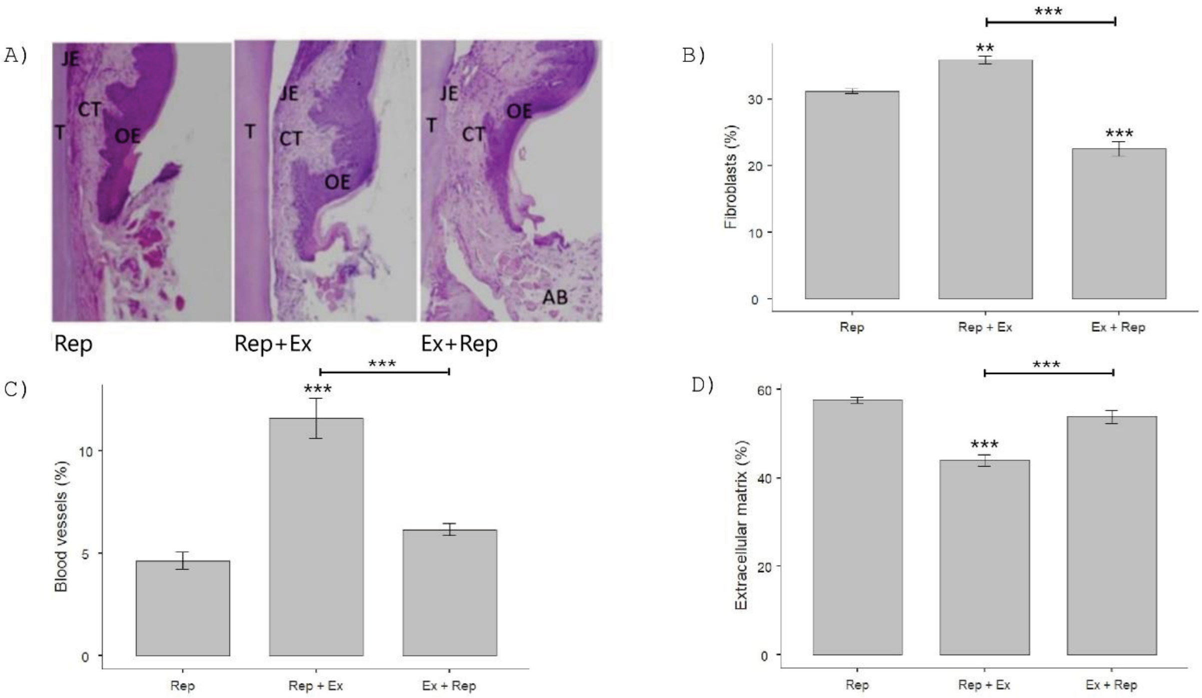
A)
JE
CT
T
OE
Rep
Rep+Ex
Ex+Rep
AB
B)
***
**
Fibroblasts (%)
30
20
10
0
Rep
Rep + Ex
Ex + Rep
C)
Blood vessels (%)
10
5
D)
Extracellular matrix (%)
60
40

Figure 4

CONSIDERAÇÕES FINAIS

Os achados do presente estudo clínico demonstraram que a prescrição de exercício físico, independentemente do momento de sua introdução (antes ou após a instrumentação subgengival), promoveu melhora significativa nos parâmetros periodontais após 90 dias de acompanhamento. De forma particularmente relevante, a prática de exercício físico anterior à instrumentação subgengival resultou em redução mais acentuada da profundidade de sondagem em bolsas periodontais moderadas e profundas, indicando que a introdução precoce dessa intervenção pode potencializar os desfechos clínicos do tratamento periodontal não cirúrgico.

No modelo experimental em ratos, observou-se que a prática de exercício físico, realizada durante as fases iniciais ou intermediárias do tratamento periodontal, também favoreceu o reparo tecidual, quando comparada ao tratamento convencional isolado. A intervenção foi capaz de promover a preservação óssea, reduzir a inflamação gengival e modular negativamente a expressão de citocinas pró-inflamatórias, evidenciando um papel biológico ativo no controle do processo inflamatório e na regeneração periodontal.

Esses resultados reforçam a hipótese de que o exercício físico pode atuar como uma intervenção adjuvante eficaz no manejo da periodontite, tanto em modelos humanos quanto experimentais. A inclusão de programas de exercício físico no plano terapêutico periodontal representa uma abordagem inovadora, com potencial para ampliar os efeitos do tratamento convencional e contribuir para a manutenção da saúde periodontal a longo prazo.

Do ponto de vista da saúde pública, a adoção sistemática dessa estratégia pode trazer benefícios adicionais, como a redução da demanda por atendimentos especializados nos Centros de Especialidades Odontológicas, a racionalização do uso de recursos no sistema público de saúde e a melhoria da qualidade de vida dos pacientes. Dessa forma, o exercício físico configura-se não apenas como um adjuvante terapêutico, mas também como uma ferramenta viável e custo-efetiva para a promoção da saúde bucal em escala populacional.

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ANEXO A

Parecer: 7.1/2021

COMITÊ DE ÉTICA EM PESQUISA NO USO DE ANIMAIS DA UNIVERSIDADE POSITIVO – CEUA/UP

Curitiba – PR, 20 de maio de 2021

CERTIFICADO

Certificamos que o projeto **"Impacto do exercício físico sobre o reparo periodontal – Estudo *in vivo* sobre o momento da atividade no tratamento periodontal."**, sob a responsabilidade de **JOÃO CÉSAR ZIELAK,** que envolve a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi **APROVADO** pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA UNIVERSIDADE POSITIVO – CEUA/UP.

- **Finalidade:( ) Ensino (** X **) Pesquisa Científica ( ) Pós-Graduação**
- **Espécie/linhagem/raça:** *Rattus norvegicus, albinus, Wistar*
- **Nº de animais:** 80
- **Origem**: Biotério da Universidade Positivo

Thais Andrade Costa Casagrande
Coordenadora - CEUA/UP

Comissão de Ética no Uso de Animais da Universidade Positivo - CEUA/UP

Rua Prof. Pedro Viriato Parigot de Souza, 5300, Curitiba-PR,Tel: (41)3317-3260 www.up.edu.br / ceua@up.edu.br

ANEXO B

UFPR - SETOR DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DO PARANÁ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação da relação entre a prática de atividades físicas e a condição periodontal antes e após terapia periodontal não cirúrgica

Pesquisador: Joao Paulo Steffens

Versão: 2

CAAE: 02621018.4.0000.0102

Instituição Proponente: Departamento de Estomatologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.129.897

Apresentação do Projeto:

O projeto tem como título: " Avaliação da relação entre a prática de atividades físicas e a condição periodontal antes e após terapia periodontal não cirúrgica" que tem como coordenador o Prof. Dr. Joao Paulo Steffens com vínculo ao Dep. de Estomatologia da UFPR.

"Tendo em vista que a prevalência da periodontite é de 10% na população mundial e que população vem mantendo cada vez mais dentes e por mais tempo, a identificação de possíveis fatores modificadores do quadro se faz necessária. Além dos benefícios gerais à saúde tais como proteção ao desenvolvimento de doenças cardíacas, Diabetes Mellitus, osteoporose, hipertensão arterial, obesidade etc., a prática regular de atividades físicas parecem estar fortemente associados à melhores condições periodontais. Por isso, o objetivo dessa pesquisa será avaliar os possíveis efeitos que a condição periodontal (saúde e doença) podem gerar na capacidade de exercer atividade física dos participantes, bem como os possíveis efeitos da prática de atividade física na condição periodontal destes".

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Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CURITIBA, 04 de Fevereiro de 2019

Assinado por:
IDA CRISTINA GUBERT
(Coordenador(a))