Received: 29 September 2021 | Revised: 3 January 2022 | Accepted: 14 February 2022

DOI: 10.1002/JPER.21-0575

JOURNAL OF Periodontology

ORIGINAL ARTICLE

Effects of periodontitis and periodontal treatment on systemic inflammatory markers and metabolic profile in obese and non-obese rats

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Abstract

Background: Little is known about a synergistic effect of periodontitis and obesity on systemic biomarkers and a possible effect periodontal treatment may exert. This study aimed to evaluate the impact of periodontitis and periodontal treatment on systemic inflammation and metabolic profile in obese and non-obese rats.

Methods: Sixty male Wistar rats were randomly divided in six groups differentiated by diet and periodontal status: no periodontitis (G1 and G4), untreated ligature-induced periodontitis (G2 and G5), and treated ligature-induced periodontitis (G3 and G6). Groups G4, G5, and G6 were exposed to cafeteria diet to induce obesity. Periodontitis was induced by silk ligatures over 4 weeks (G2, G3, G5, and G6). Rats in G3 and G6 received scaling and root planing and were followed for additional 4 weeks. After sacrifice, serum levels of C-reactive protein (CRP), interleukin (IL)-1β, IL-6, IL-10, IL-17a, tumor necrosis factor alfa (TNF-α), glucose, triglycerides, and total cholesterol (TC) were compared between groups.

Results: CRP was significantly higher in obese rats with than without periodontitis (G5 = 10.15 versus G4 = 4.47 μg/L, $P = 0.01$). No beneficial effects of periodontal treatment were observed for CRP levels, IL-6, IL-1β, IL-17a, and TNF-α, glucose and triglycerides. Treated periodontitis (G6) exhibited significantly lower TC than the periodontitis group (G5) in obese rats.

Conclusion: Periodontitis increased serum CRP in obese rats, indicating a synergistic role of periodontitis in the systemic inflammatory burden triggered by obesity. The treatment of induced periodontitis reduced TC levels in obese rats.

KEYWORDS
inflammation, obesity, periodontitis, rats, scaling and root planing

1 | INTRODUCTION

In the past decades, obesity has emerged as a condition strongly associated with periodontitis.[1] Obesity can be defined as a systemic disease characterized by extreme body fat accumulation related to lean body mass that can negatively impact health conditions.[2] Obesity is associated with a systemic inflammatory burden due to increased levels of serum pro-inflammatory markers, triggering the establishment and/or worsening of non-communicable chronic diseases.[3]

Inflammation plays an essential role in the link between obesity and periodontitis. It has been suggested that the low-grade chronic inflammatory state observed in periodontitis, as a consequence of the high levels of pro-inflammatory markers, can contribute to the maintenance of a systemic inflammatory phenotype and, in this way, correlate with several other diseases.[4] It has also been observed that obesity and periodontitis share common biological and behavioral risk factors, such as insulin resistance, stress, and smoking.[5,6]

Nonetheless, additional evidence concerning the biological plausibility underlying the association between periodontitis and obesity is still needed. Likewise, there is insufficient evidence to determine the effect of periodontal treatment on systemic levels of inflammatory markers and lipid profile when obesity is present. Several studies have assessed the efficacy of periodontal therapy in the management of periodontitis among obese and non-obese patients, with conflicting results.[7–13] These contradictory findings suggest a need to evaluate the association between obesity and periodontitis and the potential benefit of periodontal treatment in controlling the systemic inflammatory burden and metabolic status. In this regard, animal models have the advantage of isolating exposure and outcome, controlling for sociodemographic, cultural, economic, educational, and other factors that have influence on the observed associations in human studies.[14–17] This is the case for periodontitis and obesity, which may be induced in rats applying validated methodologies, resulting in a scenario where the sole effects of periodontitis and obesity may exert in systemic inflammation and metabolism can be analyzed.

Therefore, the present study aimed to evaluate the systemic effects of periodontitis and its treatment on serum levels of systemic inflammatory biomarkers and metabolic profile in obese and non-obese Wistar rats. The hypothesis is that periodontitis and its treatment are able to modify systemic biomarkers in obese and not in normal weight rats.

2 | MATERIALS AND METHODS

This study comprised a randomized controlled prospective animal model experiment. The protocol was approved by the Institutional Review Board of the Pontifícia Universidade Católica do Rio Grande do Sul (registration #7863) and followed the ARRIVE guidelines.[18] It complied with the Universal Declaration of Animal Rights and the International Ethical Guidelines for Biomedical Research Involving Animals (Council for International Organizations of Medical Sciences).

Sixty male Wistar rats (CrlCeMBE: WI), 60 days old, weighing ≈ 250 g, were used in the study. An adaptation time of 7 days was given before the beginning of the experimental procedures. Animals were housed in groups of 2 to 3, under a light/dark cycle of 12 hours, room temperature (24 ± 2°C), maximum allowed noise of 85 dB and controlled humidity (50% ± 5%).

2.1 | Diet and induction of obesity

Animals were randomly allocated to receive two different diets. The standard diet comprised a regular rat chow* and filtered water. The cafeteria diet was delivered to induce obesity, as previously described in the literature,[19–21] comprising a palatable hyperlipidic and hypercaloric diet, consisting of carbohydrates, lipids, proteins and other elements such as sodium, calcium, vitamins, preservatives, and minerals. It comprised processed foods such as sausage, filled biscuits, wafers, condensed milk, chips, and soft drinks, in addition to the standard rat chow and filtered water.

Food and water were provided ad libitum for all animals. The diets were replaced daily to allow consumption of fresh food, as well as soft drinks, condensed milk, and water.

2.2 | Experimental periodontitis and periodontal treatment

Experimental periodontitis was induced after 12 weeks of diet exposure by placing silk ligatures† in the lower first molars bilaterally. The knot was positioned at the buccal side, and the ligature remained for 4-weeks. Presence and correct position of the ligatures were clinically checked weekly until the end of the study. This

* Nuvilab CR1, Quimtia S/A, Paraná, Brazil.
† 4-0 Ethicon, Johnson & Johnson, São Paulo, Brazil.

procedure followed the recommendations of previous studies.[22,23]

The diet allocated to each animal remained during the experimental periodontitis period, that is, once an animal was allocated to one of the two diets; it received the diet until sacrifice. In two randomly selected groups of rats, one receiving standard and another receiving cafeteria diet, the ligatures were removed, and periodontal treatment was performed. Non-surgical treatment consisted of scaling and root planing using mini-five Gracey curettes[‡] in all periodontitis-affected teeth.[24,25]

2.3 | Experimental groups

Figure 1 shows the flowchart of the study. Groups were formed by stratified randomization by tertiles of body weight. A random sequence of numbers was generated, dividing the animals in six experimental groups according to diet, induction of experimental periodontitis and periodontal treatment, as follows:

- G1: Standard diet during 12 weeks and no experimental periodontitis (naive controls).
- G2: Standard diet during 16 weeks. After the 12th week, experimental periodontitis was induced during 4 weeks.
- G3: Standard diet during 20 weeks. After the 12th week, experimental periodontitis was induced, at the 16th week scaling and root planing was performed, and animals were sacrificed 4 weeks after periodontal treatment at the 20th week.
- G4: Cafeteria diet during 12 weeks and no experimental periodontitis (naive controls).
- G5: Cafeteria diet during 16 weeks. After the 12th week, experimental periodontitis was induced during 4 weeks.
- G6: Cafeteria diet during 20 weeks. After the 12th week, experimental periodontitis was induced, at the 16th week scaling and root planing was performed, and animals were sacrificed 4 weeks after periodontal treatment at the 20th week.

2.4 | Body weight and Lee index

Each animal was weighted on a digital scale, and the naso-anal length was measured with a flexible measuring tape once a week throughout the experimental period by a trained and calibrated examiner. The Lee index[26] was calculated by the ratio between the cube root of body weight (g) by naso-anal length (cm), multiplied by 10. A weight 15% higher in animals exposed to cafeteria diet compared to control animals has been considered as the presence of obesity.[27]

2.5 | Sacrifice and blood samples

Animals were sacrificed according to the timeline established for each group (Figure 1). After general anesthesia, blood samples were collected through intracardiac puncture technique and centrifuged for 15 minutes at 2000 rpm. Supernatant serum was collected and stored at −80°C.

2.6 | Serum inflammatory markers

The primary outcome of this study was the serum concentration of C-reactive protein (CRP). Serum samples were diluted according to manufacturer's instructions and measured using an assay kit[§]. Multiple cytokines interleukin (IL)-1β, tumor necrosis factor alfa (TNF-α), IL-17A, IL-6, and IL-10 were simultaneously measured using another multiplex kit[**]. The levels of cytokines and CRP were determined by MagPix[††], and the results were analyzed through specific software[‡‡].

2.7 | Metabolic markers

Serum levels of glucose[§§], total cholesterol (TC), and triglycerides[***] were measured using specific assay kits. The dosage (mg/dL) of these markers was obtained according to the manufacturers' instructions using a spectrophotometer at 505 absorbance.

2.8 | Quality control

Reproducibility of body weight and the naso-anal length was performed weekly in 10% of the sample. The calculated intra-class correlation coefficients were always above 0.98 for weight and varied from 0.87 to 0.95 for naso-anal length. Cytokine and biochemical assays were performed by a trained and blinded examiner, respecting all the manufacturer recommendations.

[‡] Hu-Friedy, Chicago, IL.

[§] ProcartaPlex, Thermo Fisher Scientific, Waltham, MA.
[**] Procarta Plex, Thermo Fisher Scientific, Waltham, MA.
[††] MILLIPLEX, Millipore, Germany.
[‡‡] xPONENT 4.2, MILLIPLEX, Millipore, Germany.
[§§] Glucose Liquiform, Labtest Diagnóstica, Minas Gerais, Brazil.
[***] Colesterol Liquiform e Triglicérides Liquiform, Labtest Diagnóstica, Minas Gerais, Brazil.

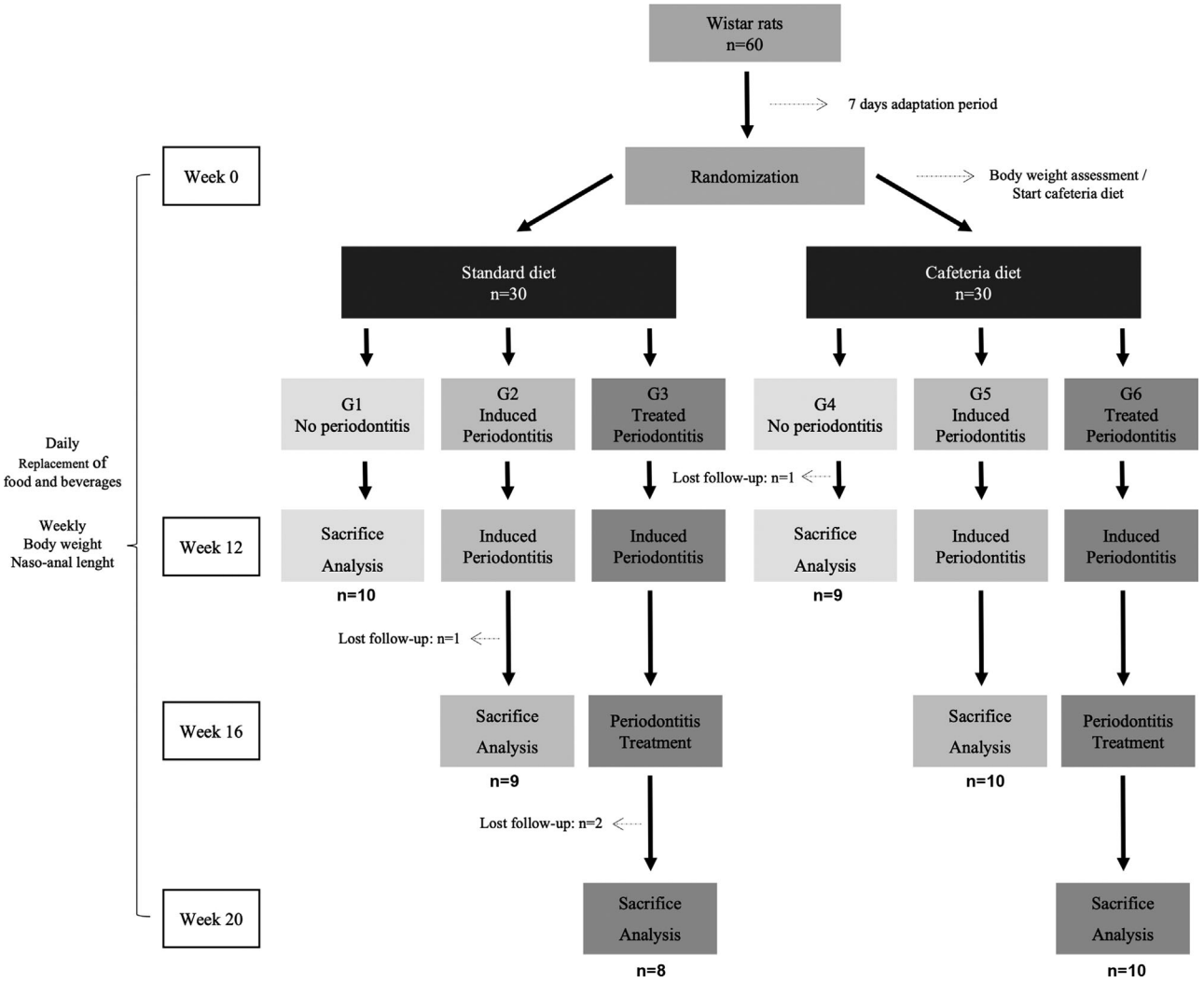

FIGURE 1 Flowchart of experimental groups

2.9 | Sample size

The sample size of this study was estimated, considering CRP as the primary outcome. Noteworthy, there is no established cut-off point to define a standard concentration of CRP in healthy rats. Also, when this study was planned, there were no previous studies evaluating periodontal treatment effects on CRP in obese Wistar rats to provide accurate estimates for sample size definition. Therefore, the calculation was based on an approximation of the estimates of mean and standard deviations found in studies that used similar designs and outcomes.[28–30] The concentration of CRP in the control group (regular diet without periodontitis) was expected to be 5.5 ± 2.0 μg/L, whereas it was estimated that it would increase to 10.0 ± 3.0 μg/L in the group of animals in which periodontitis was induced. Using alpha and beta errors of 5% and 20%, respectively, and the Mann–Whitney test, it was estimated that seven rats in each group would be necessary. Considering an attrition rate of up to 25%, it was defined that nine animals per group would be required.

2.10 | Statistical analyses

Given asymmetric data distribution, the results were expressed by median followed by minimum and maximum values. Comparisons between groups were performed using the Kruskal–Wallis test, followed by the Dunn's post-hoc test. All analyses were performed using a statistical package[†††]. Due to the great number of comparisons, only P-values < 0.05 were reported in the main text. P-values for all comparisons obtained by the Dunn's test are reported in Supporting Information Table S1, which is available online.

3 | RESULTS

Over the experimental period, one rat was lost in the standard diet periodontitis group (G2), one in the cafeteria diet without periodontitis group (G4), and two rats were lost in

[†††] Stata 14 for Macintosh, College Station, TX.

TABLE 1 Descriptive statistics for weight (grams) at the beginning of the study and after 12 weeks, percentage increase in weight until sacrifice, and Lee index at sacrifice (mean and standard deviation) according to the experimental groups

	Initial	12 weeks	% increase until sacrifice	P^a	Lee index at sacrifice	P^b
Standard diet						
G1. No periodontitis (n = 10)	291.9 (22.1)	531.4 (50.4)	*12 weeks* 81.9 (8.7)	A	3.1 (0.1)	a
G2. Periodontitis (n = 9)	252.2 (14.3)	550.6 (38.9)	*16 weeks* 133.1 (30.1)	B	3.1 (0.1)	a
G3. Treated periodontitis (n = 8)	257.0 (21.5)	544.1 (43.7)	*20 weeks* 131.7 (22.9)	B	3.1 (0.1)	a
Cafeteria diet						
G4. No periodontitis (n = 9)	283.0 (42.4)	613.5 (85.6)	*12 weeks* 117.3 (20.3)	B	3.3 (0.1)	b
G5. Periodontitis (n = 10)	238.9 (13.5)	621.7 (55.6)	*16 weeks* 190.5 (36.2)	C	3.3 (0.1)	b
G6. Treated periodontitis (n = 10)	250.5 (26.9)	637.2 (49.9)	*20 weeks* 87.0 (19.2)	C	3.3 (0.1)	b

[a]Between-groups comparison for the percentage increase (different letters indicate significant difference between groups).
[b]Between-groups comparison for the Lee index (different letters indicate significant difference between groups).

TABLE 2 Concentrations of CRP (μg/L) according to the experimental groups

Diet	Group	Evaluation period (weeks)	Median (minimum–maximum)
Standard	G1. No periodontitis	12	7.04 (2.45–31.88)
	G2. Periodontitis	16	5.32 (3.23–7.68)[§]
	G3. Treated periodontitis	20	6.51 (4.58–10.27)
Cafeteria	G4. No periodontitis	12	4.47 (2.39–8.40)[*]
	G5. Periodontitis	16	10.15 (1.69–41.87)[*§]
	G6. Treated periodontitis	20	8.12 (1.36–35.75)

[§]Comparison between G2 and G5 ($P = 0.02$); *Comparison between G4 and G5 ($P = 0.01$).

the standard diet periodontitis group (G5) (Figure 1). The weight of animals receiving standard and cafeteria diets at the 12th week was equal to 541.5 and 624.1 g, representing a difference of 15.3% between the two types of diet. At the beginning of the study, there were no significant differences in the weight of animals (Table 1). A significant increase in weight was observed for all experimental groups. However, there was a significantly higher increase in the weight of rats exposed to cafeteria as compared to those exposed to standard diet. The Lee index was significantly higher ($P < 0.0001$) in groups exposed to cafeteria diet (3.3 ± 0.1) compared to those on standard diet (3.1 ± 0.1).

Table 2 shows the concentrations of CRP for all experimental groups. The induction and treatment of periodontitis did not result in significant changes in serum CRP concentration in animals receiving standard diet (G1, G2, and G3). In animals exposed to cafeteria diet, the periodontitis group (G5) had significantly higher serum CRP concentration than the no periodontitis group (G4). In addition, the periodontitis group exposed to cafeteria diet (G5) demonstrated significantly higher serum CRP concentration than periodontitis group receiving standard diet (G2). The other comparisons did not reveal statistically significant differences.

No significant differences were observed between groups for serum levels of IL-6, IL-1β, and IL-10 (Table 3). Among the groups on standard diet, periodontitis and treated periodontitis groups showed significantly higher serum levels of IL-17a than no periodontitis group. In regards to TNF-α, the treated periodontitis group exposed to cafeteria diet exhibited significantly higher serum levels than periodontitis and no periodontitis groups with the same diet and to no periodontitis group receiving standard diet.

Table 4 shows the concentrations of glucose, TC, and triglycerides according to experimental groups. There were no significant differences between any periodontitis, periodontitis and treated periodontitis groups in both diets for glucose concentrations. However, periodontitis and treated periodontitis groups (G5 and G6) on cafeteria diet exhibited significantly higher glucose levels compared to correspondent groups in standard diet (G2 and G3). Also, G4 had higher glucose levels than G3. Regarding cholesterol levels, treated periodontitis group (G6) exhibited significantly lower levels than periodontitis group (G5) exposed to cafeteria diet and standard diet (G2). There were no significant differences between any periodontitis, periodontitis and treated periodontitis groups in both diets for triglycerides concentrations; however, all groups in the

TABLE 3 Median (minimum and maximum) of concentrations of cytokines (pg/mL) according to the experimental groups

Diet	Group	IL-6	IL-1β	IL-10	IL-17a	TNF-α
Standard	G1. No periodontitis	1.7 (0.7–4.1)	14.1 (3.4–23.9)	11.8 (4.7–21.0)	0.8 (0.3–1.5)[#¶]	0.1 (0.02–0.5)[‡]
	G2. Periodontitis	1.7 (0.5–6.5)	13.2 (2.9–59.2)	12.2 (4.5–54.7)	1.1 (0.6–4.8)[¶]	0.2 (0.03–1.2)
	G3. Treated periodontitis	1.4 (0.9–5.1)	13.2 (7.3–49.8)	13.3 (7.9–43.1)	1.3 (0.7–3.6)[#]	0.2 (0.03–1.2)
Cafeteria	G4. No periodontitis	1.9 (0.7–3.1)	9.7 (2.7–23.9)	11.9 (6.5–16.4)	0.8 (0.4–1.3)	0.2 (0.03–0.2)[§]
	G5. Periodontitis	1.9 (1.2–2.7)	16.1 (4.3–20.2)	14.2 (5.5–25.7)	1.0 (0.4–1.5)	0.2 (0.05–0.3)[Ψ]
	G6. Treated periodontitis	2.1 (1.3–5.8)	22.5 (4.3–39.3)	20.3 (7.4–238.4)	0.9 (0.5–3.1)	0.5 (0.03–0.8)[‡§Ψ]

The following comparisons between groups, with regard to each cytokine, were found to be statistically significant:
IL-17a: G1 versus G2 $^{¶}P = 0.01$; G1 versus G3 $^{\#}P = 0.01$.
TNF-α: G1 versus G6 $^{‡}P = 0.02$; G4 versus G6 $^{§}P = 0.02$; G5 versus G6 $^{Ψ}P = 0.03$.
All other *P*-values are presented in the Supporting Information Materials in the online *Journal of Periodontology*.

TABLE 4 Median (minimum and maximum) of concentrations of metabolic markers (mg/mL) according to experimental groups

Diet	Group	Glucose	TC	Triglycerides
Standard	G1. No periodontitis	322.9 (245.9–390.9)	94.6 (52.0–133.6)	105.5 (66.7–173.1)[#€¶]
	G2. Periodontitis	269.2 (211.2–433.2)[*!]	99.6 (85.4–137.3)[#]	135.1 (71.8–266.7)[†‡δ]
	G3. Treated periodontitis	251.7 (204.5–397.3)[¶€†]	91.9 (43.5–141.8)	144.6 (57.7–296.2)[§Ψ¥]
Cafeteria	G4. No periodontitis	326.1 (148.8–455.1)[€]	69.4 (53.9–127.7)	263.3 (193.2–362.9)[#‡Ψ]
	G5. Periodontitis	354.7 (228.3–443.7)[*¶]	87.8 (67.2–132.1)[*]	229.8 (121.2–507.3)[€†¥]
	G6. Treated periodontitis	340.7 (279.3–474.5)[†!]	56.3 (30.2–96.0)[#*]	263.6 (118.7–370.9)[¶§δ]

The following comparisons between groups, in regards to each marker, were found to be statistically significant:
Glucose: G2 versus G5 $^{*}P = 0.01$; G2 versus G6 $^{!}P = 0.01$; G3 versus G4 $^{€}P = 0.01$; G3 versus G5 $^{¶}P = 0.002$; G3 versus G6 $^{†}P = 0.01$.
TC: G2 versus G6 $^{\#}P = 0.002$; G5 versus G6 $^{*}P = 0.02$.
Triglycerides: G1 versus G4 $^{\#}P < 0.001$; G1 versus G5 $^{€}P = 0.001$; G1 versus G6 $^{¶}P = 0.001$; G2 versus G4 $^{‡}P = 0.001$; G2 versus G5 $^{†}P = 0.001$; G2 versus G6 $^{δ}P = 0.002$; G3 versus G4 $^{Ψ}P = 0.02$; G3 versus G5 $^{¥}P = 0.01$; G3 versus G6 $^{§}P = 0.001$.
All other *P*-values are presented in the Supporting Information Materials in the online *Journal of Periodontology*.

cafeteria diet differed significantly from all groups in the standard diet.

4 | DISCUSSION

The present study aimed to understand the possible role of periodontitis and its treatment on systemic inflammatory markers and metabolic profile of obese rats. CRP was considered the primary outcome due to its biological role in the pathogenesis of numerous conditions such as cardiovascular diseases[31,32] and metabolic disorders.[33,34] Even though there is evidence that periodontitis is associated with increased levels of CPR in humans, the majority of the studies are cross-sectional.[35] With regard to clinical trials evaluating periodontal treatment and obesity, no randomization may be performed due to ethical and methodological reasons. Therefore, animal studies are warranted to understand the possible causality of the associations between periodontitis and obesity, lowering bias by the control of several confounding factors.

In this scenario, the present animal study demonstrated that periodontitis and obesity had a synergistic negative effect on CRP concentrations. The results revealed that CRP levels were two times higher in obese rats exposed to experimental periodontitis than in obese animals non-exposed to the oral disease. Interestingly, the presence of periodontitis did not modify the CRP levels in non-obese rats receiving the standard diet. To the best of the authors' knowledge, this is the first study in rats to demonstrate this finding. A previous cross-sectional observational study in humans has observed higher CRP concentrations in obese

individuals with periodontitis than normal weight individuals without periodontitis.[36] In the present study, this finding was corroborated; however, with temporality and under controlled research conditions, strengthening the evidence that periodontitis may be a modifier of systemic inflammation in obese individuals, highlighting the importance of preventing the oral disease in this specific population.

It has been demonstrated that periodontal treatment reduces systemic inflammation, including CRP, in healthy non-obese humans.[37] In obese individuals, there is scarce and controversial evidence of the effect of periodontal treatment on CRP levels.[12,38] In this study, two groups of animals were periodontally treated. Although periodontal treatment aiming to disrupt subgingival biofilm with the aid of mini-curettes is an established model in the literature,[24,39] there is no consensus on how to evaluate if periodontal treatment in rats is effective, which may be considered a limitation of the method. With the experimental model applied herein, the treatment of periodontitis did not result in CRP changes. One previous study showed that obese rats with treated periodontitis had significantly lower CRP levels when compared to the group of obese animals with untreated periodontitis.[25] However, important methodological differences between the two studies should be noted, mainly the use of ligatures soaked in *P. gingivalis*, the 2 weeks after periodontal treatment to the end of the experiment and the induction of obesity by a high fat diet in the study by Ni and coworkers. Regarding human interventional studies, the number of studies is also scarce, and controversial findings have also been reported.[12]

Systemic inflammatory status is also represented by levels of different cytokines. Unlike what would be expected, it was observed that IL-17a and TNF-α were significantly higher in rats exposed to periodontal therapy as compared to those without periodontitis. This pattern of cytokine expression observed in this study represents a challenge to be interpreted and explained. It could be speculated that periodontal therapy, with the timeline of the present study, was not able to level off cytokine expression. However, the lack of previous similar studies in the literature makes difficult to infer consistent comparisons with the present findings.

In this study, animals exposed to the cafeteria diet that were periodontally treated had significantly lower serum TC values than rats with untreated periodontitis exposed to the same diet. In the group exposed to the standard diet, this difference was not observed, suggesting a direct benefit of periodontal therapy only in the group of obese animals. One study in humans also achieved similar findings, in which a significant reduction in TC levels was observed after periodontal therapy in the group of patients with obesity but not in normal weight individuals.[7]

The evaluation of other metabolic parameters in this study showed significant differences only between groups exposed to cafeteria diet (obese animals) compared to their respective reference groups exposed to the standard diet (normal weight animals). The increase observed in serum levels of triglycerides and glucose only indicated the effect of obesity-inducing diet in the groups exposed to it, not indicating the influence of periodontitis or its treatment. Clinical studies in obese humans also found no significant differences in serum triglyceride levels between patients with and without periodontitis[40] or after periodontal therapy.[10] However, Cavagni et al.,[41] using an experimental model similar to that used in this study, showed that the group of obese rats with periodontitis had significantly higher triglycerides and TC values than the only obese and only periodontitis groups. It is noteworthy that the analysis of these lipid markers in that study[41] was made from a tissue sample of the liver, suggesting that the assessment site can be decisive for obtaining more accurate and significant data that represent this outcome in rats.

Some methodological aspects related to the model of obesity of this study deserve discussion. Rats, like humans, are known to have different susceptibility to the development of diet-induced obesity.[42] In the present study, the cafeteria diet was used to induce obesity. This diet is mainly composed of foods with high lipids and carbohydrates and tries to reproduce the modern pattern of human food consumption known as *fast food*. This diet model was previously validated, being able to increase body weight, as well as lipid and glucose levels.[21] Nevertheless, some criticism has been pointed to the cafeteria diet due to lack of standardization of administered food and beverages to rats, leading to different lipidic profiles.[43] Independent of that, the group of animals exposed to the cafeteria diet in this study had ~15% more weight than the standard diet group, a magnitude pointed out as a real induction of obesity.[27] Similar results were observed in the analysis of the Lee index, which revealed a statistically significant difference between the groups, confirming the development of obesity. In addition, cafeteria diet includes sugar-containing components, that could both increase glucose levels and plaque formation; however, studies failed to demonstrate these suppositions.[44,45] Noteworthy, it would be expected that the cafeteria diet would lead to higher CRP levels than the standard diet, but this was not the case of this study. Previous studies also did not find increased levels of CRP after cafeteria diet.[46] This may be due to the high variability of this biomarker in animal studies or a limitation of the diet model to increase CRP in a standardized manner.

Another aspect to be addressed is related to the experimental time of 4 weeks applied to periodontitis induction in this study. Based on morphological and histological analysis, a recent study comparing different times of periodontitis induction in rats (0 to 60 days) observed that the period of 3 to 7 days was sufficient to obtain significant alveolar bone loss and, after 15 days, it remained stabilized.[47] On the other hand, maintaining the ligature in place makes the exposure to the microbial challenge for longer periods, which mimics chronic diseases. However, considering an analysis of inflammatory markers in the gingival tissues of rats with induced periodontitis, a significant increase in gene expression of IL-1β, IL-6, and TNF-α in the first week of that study was demonstrated, whereas in the second and third week of evaluation (chronic phase) no significant changes were observed in the gene expression of IL-6, IL-1β, and TNF-α.[48] Therefore, we chose to standardize the time of ligature exposure for all groups into 4 weeks. Nevertheless, the time of diet exposure differed between groups, which may have some influence on the observed outcomes of systemic inflammatory biomarkers. Also, the age of the animals differed between groups. The magnitude of the influences of different age and time to exposure to diets is difficult to be determined and this may be considered as a possible limitation of our findings. Importantly, we had to choose to standardize time to ligature exposure or diet since it was not possible to design a study with the same timepoints for the two exposures. By choosing to standardize the time of ligature exposure, we allowed similar microbial and inflammatory conditions for the comparison between untreated and treated groups. This is possibly more crucial to the outcomes of the study than a minor age difference (4 weeks) between the groups.

The present study has other strengths and limitations that should be highlighted. The controlled environment, age and sex standardization, random choice for group allocation (allowing obesity to occur in any of the study animals), and both groups receiving diets containing rat chow minimizing the impact of diet consistency, are among the main strengths of this study. The limitations relate to the possibility of comparing with other studies, since inflammatory markers were analyzed in the serum and not locally. This is a choice that could better relate to systemic inflammation; however, with less chances of demonstrating differences, since inflammatory markers are measured "at distance."

The findings of the present study contribute to the body of evidence of the inter-relationship between obesity and the occurrence of periodontal disease, as well as the impact of periodontal therapy in obese and non-obese animals. The present contribution focused on trying the separate the possible impacts of different components of this complex oral-systemic inter-relationship, which is clearly mediated by immune-inflammatory mechanisms.

5 | CONCLUSION

Periodontitis increased serum CRP levels in obese rats, indicating a synergistic role of periodontitis in the systemic inflammatory burden triggered by obesity. On the other hand, the treatment of induced periodontitis resulted in minimal impact on systemic inflammation. The treatment of periodontitis in rats was associated with low levels of TC, suggesting that periodontal treatment may be beneficial to metabolic control.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to this study.

AUTHOR CONTRIBUTIONS

Karina K. Y. Pereira, Cynthia M. Jara, and Géssica L. Antunes: conception and design, experimental procedures involving animals, analyses, and interpretation of the data. Writing, review, and approval of the manuscript. Maximiliano S. Gomes, Cassiano K. Rösing, Juliano Cavagni, and Alex N. Haas: conception and design, funding, analyses, and interpretation of the data. Writing, review, and approval of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Pereira KKY, Jara CM, Antunes GL, et al. Effects of periodontitis and periodontal treatment on systemic inflammatory markers and metabolic profile in obese and non-obese rats. *J Periodontol*. 2022;93:1411–1420. https://doi.org/10.1002/JPER.21-0575