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Impact of endodontic and periodontal diseases and treatments on the aorta and liver of obese and non-obese rats

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Abstract

Aim: To evaluate the impact of the presence and treatment of periodontal disease (PD) and apical periodontitis (AP) on the aorta and liver of obese and non-obese rats.

Methodology: One hundred and forty Wistar rats were divided into two groups, according to the diet administered: normal diet (-n), without obesity; and cafeteria diet (-c), with induced obesity. These groups were divided into seven subgroups according to the specific experimental protocols: naïve control (NC); AP; AP with treatment (APt); PD; PE with treatment (PDt); AP and PD (APPD); and AP and PD with treatment (APPDt). AP and PD lesions were induced for four weeks. Four weeks after treatments, the animals were euthanatized, and the aorta and liver were dissected for histological evaluation. For the comparison of the thickness of the aorta between groups, the Kruskal–Wallis test was used, followed by the Mann–Whitney test. For the analysis of other variables related to the aorta and liver outcomes, logistic regression was carried out.

Results: Both PD and AP were associated with the development of histological alterations in the aortic arch, with no significant difference between obese and non-obese animals ($p = .17$). The aorta thickness was increased significantly ($p < .05$) with the combination of PD and AP in obese rats (APPDt-c group) compared with the other groups (NC-n, APt-n, APt-c and AP-c). The logistic regression models revealed that the untreated (OR = 7.78; 95%CI = 2.4–25) and treated (OR = 2.9; 95%CI = 1.0–8.4) groups were significantly more likely to have endothelial alterations compared with the control groups ($p = .002$). Obesity (OR = 16.5; 95%CI = 3.4–81.3) was the only predictor variable of liver steatosis ($p < .001$).

Conclusion: Histological alterations in the aortic arch of obese and non-obese rats were observed in the presence of periodontal disease and apical periodontitis. The combination of PD and AP increased the aorta thickness in obese rats. A reduction of vascular endothelial lesions was observed with the treatments of PD and AP.

KEYWORDS
animal model, apical periodontitis, obesity, periodontitis, steatosis, vascular diseases

INTRODUCTION

Obesity is a contemporary public health problem, whose prevalence has increased sixfold in the last 40 years (World Health Organization, 2021). It is associated with several additional health problems, including increased risk of insulin resistance, type 2 diabetes, liver steatosis, degenerative disorders, dementia, airway disease, some cancers and atherosclerosis (Ozcan et al., 2004; Semenkovich, 2006). Epidemiological evidence has linked obesity to a broad spectrum of cardiovascular diseases (CVD) including coronary heart disease, heart failure, hypertension, stroke, atrial fibrillation and sudden cardiac death Koliak et al., 2019).

Atherosclerotic CVD remains the major cause of global deaths (World Health Organization, 2017). Atherosclerosis is a chronic inflammatory process which affects the medium and large size blood vessels in the entire cardiovascular system (Davis, 2005; Libby et al., 2002). Obesity has an important role in atherosclerosis and coronary artery disease. Both obesity and atherosclerosis are considered chronic inflammatory conditions, in which the activation of nonspecific and adaptive immune processes is present (Rocha & Libby, 2009).

Considering the inflammatory nature of obesity, it has also been associated with chronic oral inflammatory diseases (Arboleda et al., 2019; Prpić et al., 2012). Data indicate that increased body mass index, waist circumference, percentage of subcutaneous body fat and serum lipid levels are associated with increased risk to present periodontal disease (PD) (Dahiya et al., 2012). The biological plausibility of this association involves adipose tissue-derived cytokines, which contributes to the development of a low-grade systemic inflammation (Arboleda et al., 2019).

Chronic oral inflammatory diseases, such as PD and apical periodontitis (AP), have been suggested as potential risk factors for the occurrence of CVDs (Jakovljevic et al., 2020; Mattila et al., 1989). This association has been supported by a number of biologically plausible mechanisms, including direct infection and systemic inflammation (Carrizales-Sepúlveda et al., 2018; Cullinan & Seymour, 2013; Gomes et al., 2013; Hegde & Awan, 2019). The increase of systemic inflammatory mediators seems to be the key to explain the connection between all the above-mentioned pathologies.

Previous studies (Fischer et al., 2020; Piconi et al., 2009) reported that the treatment of PD might limit the inflammation, reduce endothelial dysfunction and normalize the intima-media thickness of the carotid arteries. However, it should be highlighted that one of those studies (Piconi et al., 2009) did not include a control group, which is an important methodological limitation. Moreover, the evidence regarding the systemic repercussions of AP is limited (Chauhan et al., 2019; Jakovljevic et al., 2020). One recent clinical study (Bergandi et al., 2019) suggested that AP may drive early vascular endothelial dysfunction and that root canal treatment ameliorated the vascular outcome.

NAFLD (Non-alcoholic fatty liver disease) is another condition that has been linked to PD (Alakhali et al., 2018). NAFLD represents a spectrum of liver diseases ranging from simple steatosis to non-alcoholic steatohepatitis (Neuschwander-Tetri & Caldwell, 2003). The known risk factors for NAFLD include obesity, diabetes, insulin resistance, oxidative stress and inflammation (Marchesini et al., 2003). The mechanism by which PD may affect the liver is not fully elucidated, and there is a lack of prospective clinical studies in the field (Grønkjær, 2015). Considering that PD and AP share the same chronic inflammatory nature (Kerekes & Olsen, 1990; Li et al., 2014; Rupf et al., 2000), the relationship between AP and NAFLD should be further studied.

A position statement of the American Heart Association concluded that there is no evidence of a causal relationship between PD and CVD but acknowledged an association between these diseases (Lockhart et al., 2012). The observed associations may occur due to a large number of shared risk factors, as well as genetic predispositions. Causality is assessed especially by the effect of the treatment of the exposure on the reduction in the frequency of the outcome (Hill, 1965). In this sense, experimental studies in animal models allow a comparison between treated and untreated groups, whilst confounding factors are better controlled. Therefore, considering the inherent ethical and methodological barriers of interventional clinical trials to analyse the impact of oral diseases and their treatments on histological parameters of remote organs, the present study aimed to evaluate the impact of the presence and treatments of PD and AP on the aorta and liver of obese and non-obese Wistar rats.

METHODS

This study was approved by the Ethics Committee on the Use of Animals (CEUA) of the Pontifical Catholic University of Rio Grande do Sul (Nº. 7863). The manuscript of this animal study has been written according to the Preferred Reporting Items for Animal studies in Endodontology (PRIASE) 2021 guidelines (Nagendrababu et al., 2021). Figure 1 describes the key stages of the study. One hundred and forty male Wistar rats of three-month-old, weighing 250–300 g, were used. The animals were housed under standard and controlled conditions of

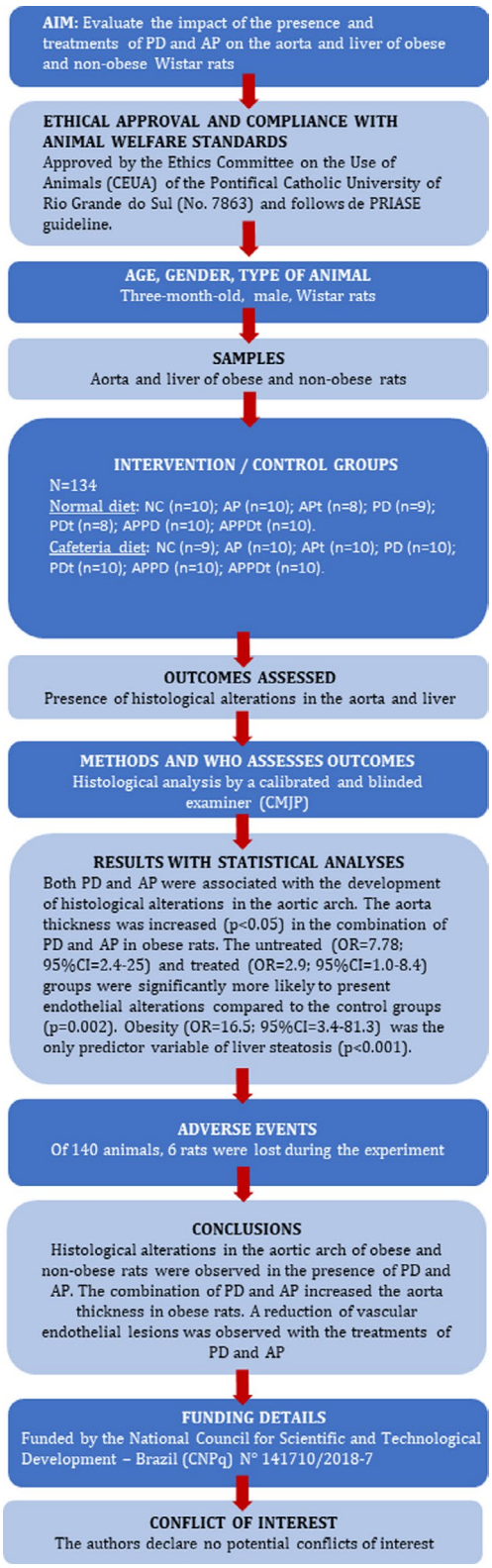

FIGURE 1 PRIASE 2021 Flowchart. NC: naïve control; AP: Apical periodontitis; PD: Periodontal disease; APPD: Apical periodontitis +Periodontal disease; t: treated group

temperature (22 ± 2ºC), light (12-h light-dark cycle) and humidity (50%–70%), in ventilated cages, with autoclaved wood chip bedding.

The sample size calculation was performed based on the results of a previous study (Cintra et al., 2014), considering the mean and standard deviations of serum IL-17 levels in Wistar rats with AP+PD (2.08 ± 0.58) and without oral lesions (1.00 ± 0.99), with $\alpha = 5\%$ and 90% power, resulting in 7 animals per group. Estimating a possible loss of 20%, a sample of 10 animals per group was used.

Induction of obesity–Normal diet and cafeteria diet

The 140 rats were randomly divided into two large groups, differentiated by the administered diet. In the standard diet group, it was provided a standard commercial chow and filtered water *ad libitum*. In the other group, obesity was induced by the administration of hyperlipidic and hypercaloric diet, adapted from a diet known as the Cafeteria Diet (Miesel et al., 2010; Sampey et al., 2011). Thus, the high-fat diet consisted of processed foods such as sausage, stuffed biscuits, crackers, condensed milk, chips and soda in addition to the standard commercial chow as previously reported (Cavagni et al., 2016). All feed and water offered to the animals were quantified prior to the daily consumption of each cage (Table S1).

The Cafeteria Diet groups were administered for a period of 12 weeks prior to the experimental procedures, according to pre-established protocols (Cavagni et al., 2016). Weighing was measured using an electronic scale (Prix 3 Light Benchtop Battery), and an increase of ≈18% in weight of the cafeteria diet group compared with the standard diet group was considered as a possible induction of obesity (Svensson et al., 1996).

Experimental groups

The animals of both the normal diet and the cafeteria diet groups were then divided into seven subgroups, with 10 animals in each experimental group (Table 1).

Induction of oral lesions–PD and AP

All experimental procedures were performed under anaesthesia by intraperitoneal injection of a combination of xylazine (10 mg/kg) and ketamine (90 mg/kg). PD was induced by ligature placement in the mandibular first molars of rats. A silk thread (4–0) was placed with the support of two needle clamps in the interproximal spaces and a knot was placed on the buccal surface, remaining for a period of four weeks, according to procedures previously

TABLE 1 Distribution of the experimental groups

Oral conditions								
		Naïve control	**AP**	**AP treated**	**PD**	**PD treated**	**AP and PD**	**AP and PD treated**
Systemic condition	Normal Diet	NC-n	AP-n	APt-n	PD-n	PDt-n	APPD-n	APPDt-n
		n = 10	n = 10	n = 10	n = 10	n = 10	n = 10	n = 10
	Cafeteria Diet	NC-c	AP-c	APt-c	PD-c	PDt-c	APPD-c	APPDt-c
		n = 10	n = 10	n = 10	n = 10	n = 10	n = 10	n = 10

Abbreviations: AP, Apical periodontitis; APPD, Apical periodontitis +Periodontal disease group; -c, cafeteria diet; -n, normal diet; NC, naïve control; PD, Periodontal disease; t, treated group.

described (Galvão et al., 2003; Vargas-Sanchez et al., 2020). The presence and correct position of the ligature were verified weekly by clinical examination until the end of the study.

AP was induced by pulp exposure in the mesial fossa of the maxillary first molars, according to procedures previously described (Jara et al., 2018). The teeth were left exposed to the oral environment for a period of four weeks (Kakehashi et al., 1965).

Treatment of oral lesions–PD and AP

The treatments were carried out in three groups of normal diet (APt-n, PDt-n, APPDt-n) and three groups of the cafeteria diet (APt-c, PDt-c, APPDt-c). The AP-n, PD-n, APPD-n, AP-c, PD-c and APPD-c groups remained untreated, acting as positive controls (Table 1).

Four weeks after PD induction, the ligatures were removed, and periodontal treatment was performed by mechanical instrumentation with scraping and root planing on all surfaces of the selected tooth. Adapted Gracey curette, model mini-five, from series 11–12 and 13–14 (Hu-Friedy, Chicago, IL, USA) were used, according to pre-established protocols (Fernandes et al., 2010).

Four weeks after AP induction, the extraction of injured teeth was performed. Using an exploratory probe (Golgran), the epithelial tissue was separated from the tooth, and later with the alveolotome (Golgran), the dislocation movement was performed until extraction of the piece, followed by gentle socket curettage. The haemostasis of the lesion was done with the help of saline-soaked gauze. The treatments groups had a follow-up period of 4 weeks; then, euthanasia was performed.

Sample processing and histological analysis

The euthanasia was performed by the intracardiac puncture technique (Hadie & Abdul, 2013), blood samples were collected for another study, and periapical and periodontal status were checked with control radiographs. In the AP groups, the radiographic limits of periapical lesions were observed. In PD groups, periodontal bone support was analysed as described by Pontes Andersen et al., (2006).

A midline incision was made in the thoracic region. The aorta and liver were dissected and fixed in 10% formalin saline for 48 hours. Tissues were processed routinely and included in paraffin. The aorta arch was dissected and three-sectional cuts of 5 μm thickness were prepared per slide and then coloured with HE. The stained sections were examined with a microscope and digital photomicrographs (100×–200× magnification). All the histological analyses were performed by a calibrated and blinded examiner (CMJP).

The thickness of the aortic wall was measured histologically using 100× magnification and Image J program (Version of image Tools 3.0; University of Texas Health Science Centre at San Antonio). The mean value of three portions of the histological image (one medium and two lateral measurements) was calculated. The intima and media tunica were included on the measurements.

The endothelial alterations were also evaluated. After going through the entire section of the slide (100× and 200×), a representative image was selected and evaluated considering the presence or absence of cell alterations on the tunica intima and media according to previously reported histological criteria (Zhang et al., 2016).

To assess the prevalence of liver steatosis, three portions of each sample were obtained, each portion corresponding to the – section six and seven – section one and four, and – section two and three of the liver. With a 200× magnification, two images were obtained per portion, with a total of six images per slide. Subsequently, the steatosis was evaluated according to the presence or absence of lipid vacuoles, according to previously reported histological parameters (Zhang et al., 2016). Some samples of liver were lost during processing, and five samples from each experimental and control group were evaluated in the liver analysis.

Statistical analysis

To determine the normality of the data, Shapiro–Wilk tests were performed. For the analysis of the thickness of the aorta, the Kruskal–Wallis test was used followed by the Mann–Whitney test. For dichotomous analysis of the aorta and liver condition, logistic regression was used. Pairwise comparison (Tukey's least significant difference) was conducted if a significant difference was found. The statistical package SPSS version 25 (SPSS Statistics; IBM) was used for all analyses, with a significance level of 5%.

RESULTS

From the original sample of 140 animals, 6 rats were lost during the experiment. Thus, the final sample in the affected groups was as follows: APt-n (n = 8), PDt-n (n = 8), PD-n (n = 9) and NC-c (n = 9). All other groups consisted of 10 animals for the final analysis.

The mean final weight of rats with normal diet was 548.04 ± 47.48 g, whilst in the cafeteria diet, it was 673.71 ± 86.09 g. This difference was >18%, which confirmed the induction of obesity (Svensson et al., 1996). In the cafeteria diet group, after the induction of oral lesions, there was a small weight loss in some animals. Between weeks 12 and 13, the mean reduction was 9.2 ± 7.1 g. On the other hand, in the normal diet group, a mean reduction of 11 ± 5.4 g was reported. All weight loss was regained in the following weeks.

The aortic arch thickness

The aorta thickness was significantly increased ($p < .05$) in the APPDt-c group compared with other four groups: NC-n, APt-n, APt-c and AP-c (Figure 2).

Histological alteration of the aortic arch

The logistic regression model on the association between exposure variables (obesity; oral lesions PD, AP and combined APPD; treatment of oral lesions) and the outcome (histologic endothelial alterations) was significant ($p = .002$). Amongst the independent variables, obesity did not significantly influence the vascular outcome ($p = .174$). Moreover, the untreated (OR=7.78; 95%CI = 2.4–25) and treated (OR = 2.9; 95%CI = 1.0–8.4) groups were significantly more likely to have endothelial cells alterations compared with the control groups ($p = .002$).

In addition to the statistical analysis and for illustration purposes, the various types of histological alterations in the aortic arch were described in detail (Figure 3). Diverse initial pathologic conditions were observed in the aorta, and the most frequent were endothelial cell detachment (Figure 3C) and smoothing of the vascular wall (Figure 3B), compared with the normal endothelium (Figure 3A).

Some samples showed more obvious alterations, in which the tunica intima and media were affected. In some cases, the almost total detachment of the endothelium could be observed with the consequent infiltration of

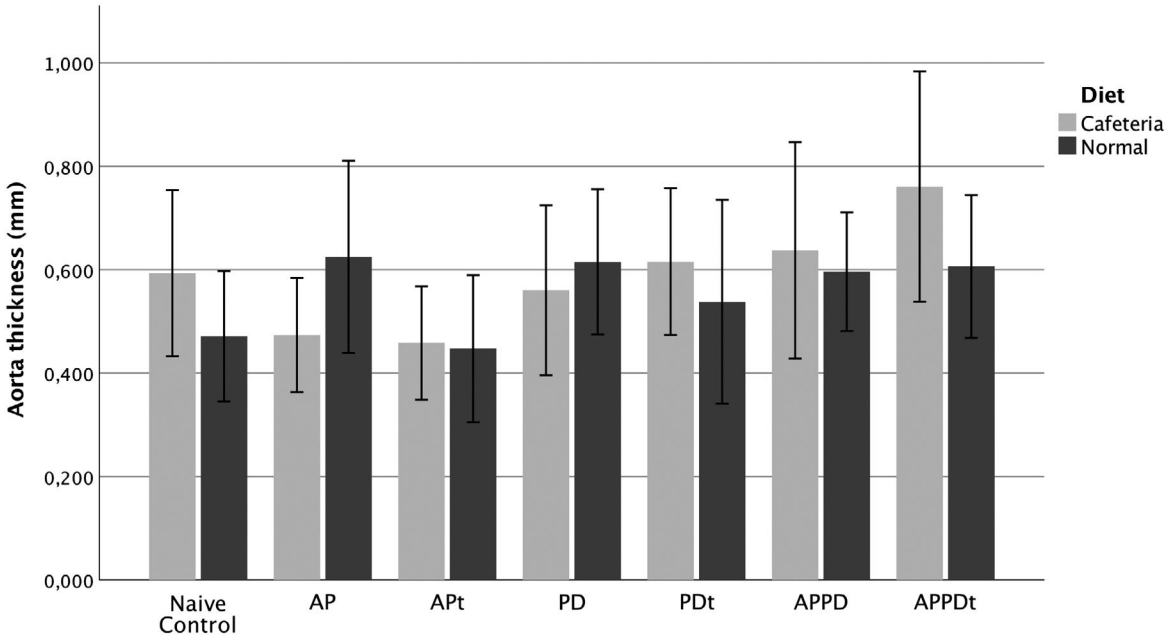

FIGURE 2 Aorta Thickness measured in millimetres (±SD). NC: naïve control; AP: Apical periodontitis; PD: Periodontal disease; APPD: Apical periodontitis +Periodontal disease; t: treated group

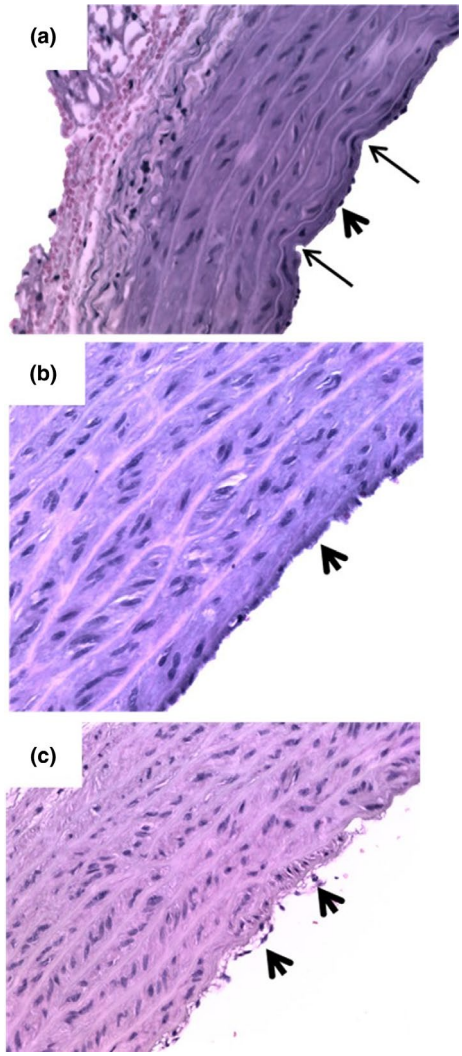

FIGURE 3 Photomicrography of aortic arch sections. (A) – Normal aortic arch showing tunica intima faces the lumen and endothelial cells present (arrowhead), with the wavy corrugated endothelium (long arrow). (B) – Endothelium with incipient lesion showing the almost total absence of endothelial cells and the loss of endothelial wavy (arrowhead). (C) – Endothelium with incipient lesion showing detachment of cells (arrowheads) (HE ×200)

monocytes and macrophages (foam cells). In other samples, cellular hypertrophy and hyperplasia with muscular disorganization could be noticed. Finally, in a few isolated cases, the total disorganization of the tunica intima and media could be observed (Figure 4). A representative histological image of each of the fourteen groups is shown in Figure 5.

Liver steatosis

The Figure 6 illustrates the histological findings in the liver. The logistic regression model on the association between exposure variables (obesity; oral lesions PD, AP and combined APPD; treatment of oral lesions) and the outcome (histological liver alterations) was statistically significant ($p < .001$). Amongst the predictor variables, only obesity was statistically significant (OR = 16.5; 95%CI = 3.4–81.3). Noteworthy, despite no significant differences were found, the only two groups with normal diet that presented steatosis were the APPD groups with and without treatment (Figure 7).

DISCUSSION

This novel study evaluated the impact of the presence and treatments of PD and AP on histological parameters of the aorta and liver of obese and non-obese rats. The main findings revealed that the presence of oral lesions negatively influenced the histological condition of the aortic arch, resulting in an early vascular endothelial dysfunction. Of note, the treatment of the oral lesions resulted in decreased risk to the endothelial alterations.

The level of evidence for an association between PD and atherosclerosis is greater and more extensive (Kapellas et al., 2014; López et al., 2012; Zeng et al., 2016), compared with the evidence on the association between AP and atherosclerosis. Even though many findings report an association between endodontic variables and systemic outcomes, the moderate–low quality of the publications and the lack of prospective experimental studies challenge the establishment of a causal relationship (Berlin-Broner et al., 2017). Moreover, the existence of an association does not imply causation. Two variables can be statistically related to each other without either variable directly affecting the values of the other (Segura-Egea et al., 2019). Following the causality criteria reported by Hill (1965), experimental and prospective studies are necessary in order to evaluate the effect of the treatment on the outcome. In view of the complexity in performing a randomized clinical interventional study, different research using animal models have been developed to understand the potential mechanism for the linkage between oral diseases and atherosclerosis (Conti et al., 2020; Zhang et al., 2016). Therefore, one of the strengths of this study is its experimental nature that allows inferences to be made about the repercussions of 'treating or not treating' oral diseases. The use of an animal model also allows a more detailed and invasive analysis of the tissues and organs involved, and in this case allowed the evaluation of the aorta and the liver by histological methods.

One methodological aspect that should be highlighted is the model used for atherosclerosis induction in the present study. The atherosclerosis model in rats has limitations. Generally, rats respond poorly to a cholesterol

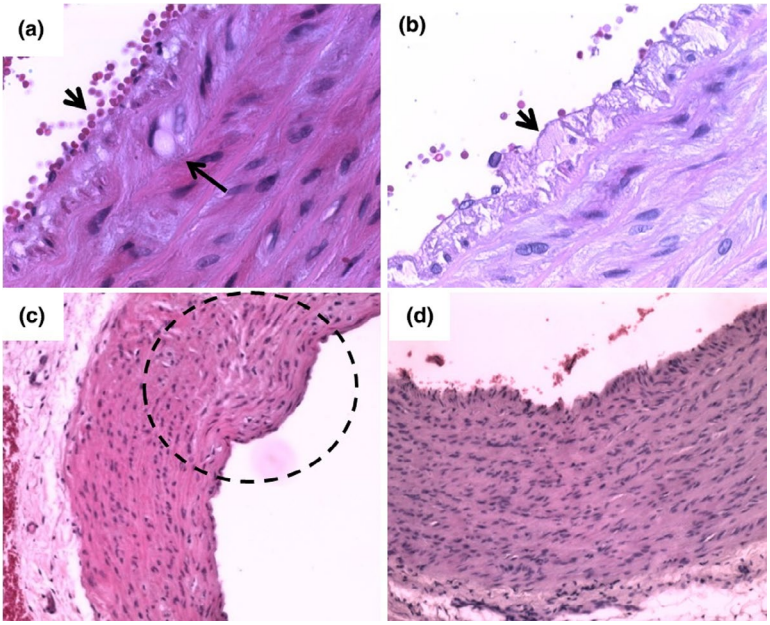

FIGURE 4 Photomicrography of the aortic arch showing alterations in the tunica intima and media. (A) – Endothelial detachment and adhesions of red blood cells to the surface of the intima (arrowhead). Vacuolated cells in the sub-endothelial layer (foam cell) (long arrow). (B)– Endothelial detachment (arrowhead). (C) – Increase of vascular tone and muscular disorganization (in circle). (D) – Disorganization of the elastic fibres and smooth muscle of the tunica intima and media (HE ×200)

diet, so atherogenesis can only be induced in small quantity with diets rich in cholesterol and fats (Navarro et al., 2005). Rats are classified as an HDL mammal, because, unlike humans, these lipoproteins predominate in its plasma (Chapman, 1986). In addition, the cholesterol ester transfer protein, which plays an important role in human lipoprotein profiles, is absent in the rat (Santos-Gallego et al., 2013). These main differences are the reason why, despite a hyperlipidic and hypercaloric diet were provided, the rats developed only abnormalities of an apparently initial stage or prior to atherosclerosis. For example, in Figure 4-C, the hypertrophy and hyperplasia of the tunica media protruding towards the lumen were observed, a fact that in the human aorta could already correspond to the formation of the atheromatous plaque.

There is no literature about the influence of age on the development of atherosclerosis in rats. However, following the parameters that predispose humans to CVD (Criqui et al., 2010), it is suggested that for future studies using Wistar rats, these animals should be more than 6 months old, considering that they have an average lifespan of 2 years.

Another methodological point that must be debated is the control and standardization of the exposure time (4 weeks) to periodontal and endodontic infections for the comparison between untreated and treated groups. The untreated groups were euthanized four weeks after induction of the oral lesions, and the treated groups were euthanized four weeks after the AP and PD treatments, which necessarily resulted in a difference of four weeks in the age of the untreated and treated animals. This age difference is likely to be a less important factor compared with the standardization of the exposure time to the periodontal and endodontic infections. If the untreated groups had lived exactly the same time as the treated groups, the time of exposure to the oral lesions would be overestimated, biasing the comparison with the groups in which the injuries had already been treated/eliminated. According to the classic experimental model of AP in rats (Kakehashi et al., 1965; Yu & Stashenko, 1987), the lesion expands rapidly between day 7 and day 15–20, with a slower expansion thereafter. Also, as occurs in humans, anaerobic bacteria gradually increase in parallel with the chronicity of the lesion, as well as the expressions of different inflammatory mediators (Stashenko et al., 1994). Considering the clear difference between the two periods of AP progression, it was decided to standardize the exposure time to the oral lesions, allowing similar microbial and inflammatory conditions for the comparison between untreated and treated groups. The present methodological approach allows a fair estimate of the effect of the oral treatments, comparing it with groups that reflect the pre-treatment AP and PD conditions.

The surgical protocol for the treatment of AP was applied in view of the infeasibility of root canal treatment (RCT) of all roots in rats. A pilot study was previously carried out, and it was observed that due to the anatomical complexity of the first molars of rats, RCT could be performed correctly only in the mesial root, leaving the other roots untreated. Therefore, it was decided to carry out the extraction of the tooth, to eliminate the infection focus related to AP. Considering that tooth extraction is an invasive surgical procedure, that lead to an acute exacerbated inflammation (Graziani et al., 2017), it may have had a more aggressive impact on the results, compared with a conventional RCT. It is possible to infer that non-surgical

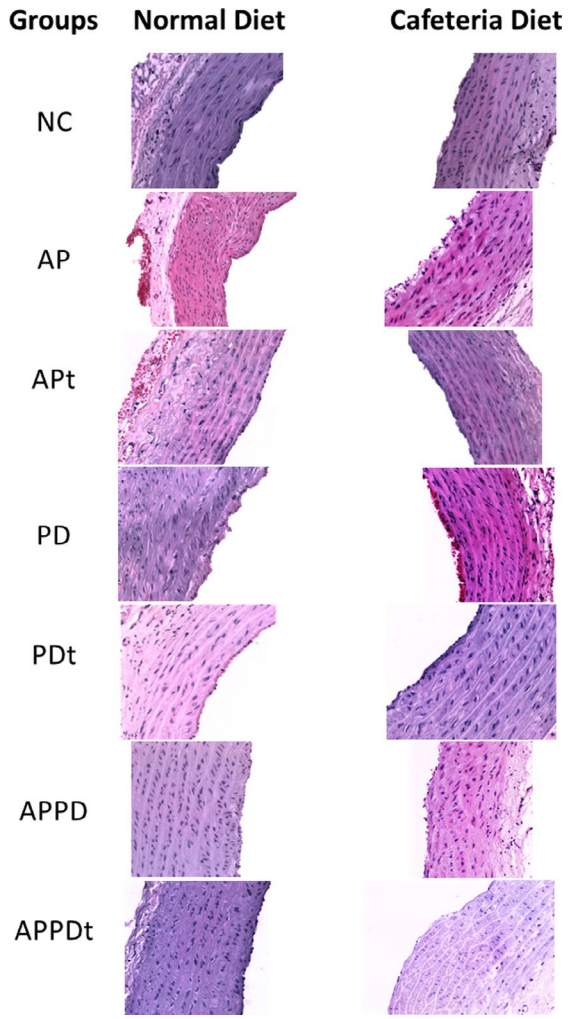

FIGURE 5 The histological representative images of each experimental group (HE ×200). Note that the most evident alterations were found in groups where oral lesion was induced and combined. NC: naïve control; AP: Apical periodontitis; PD: Periodontal disease; APPD: Apical periodontitis +Periodontal disease; t: treated group

RCT could have improved even more the outcome in relation to tooth extraction.

Despite the methodological limitations reported above, the results obtained in this study were revealing with respect to the existing relationship between oral diseases and obesity-related outcomes, and the benefit provided by the treatment of AP and PD, which is in agreement with evidence raised by other studies (Bergandi et al., 2019; Chauhan et al., 2019; Piconi et al., 2009). Noteworthy, the present study seems to be the first to evaluate the impact of the treatment of oral lesions on the aorta and liver through histological analysis. In this sense, one study (Zhang et al., 2016) evaluated histologically the association of these organs with respect to oral lesions; however, treatments were not performed. The authors of that study reported that AP elevated inflammatory markers levels, causing reversible changes in the aortic arch, myocardium and spleen as well as irreversible changes in the liver. An important aspect to highlight is that, compared with the endothelial alterations (inflammatory infiltrate) found by Zhang et al., (2016), a more noticeable alteration was observed in the samples obtained in the present research; a complete endothelial detachment, presence of foam cells, red blood cell adhesion to the endothelium and disorganization of muscle cells are some of the observed results. Another study (Conti et al., 2020) evaluated the relationship between AP and atherosclerosis. The authors concluded that AP influenced triglyceride levels, increasing it even in the absence of atherosclerosis, and influenced the increase in the thickness of the carotid artery intima tunic in the presence of atherosclerosis. Regarding the thickness of the aorta, in the present study it could be noted that neither the diet nor the presence of oral pathology had a significant impact on the results. However, the differences observed were found only with respect to one group, the APPD-treated group from the cafeteria diet. It could be deduced that the overlap of inflammatory processes, produced by obesity, the previous presence of a combined lesion (AP + PD), and with a treatment carried out in a probable healing period, led to an increase in the thickness of the tunica intima and media of the aorta. For future studies, a longer post-operative period time for the evaluation is suggested, of at least 8-weeks, instead of the 4 weeks used in the present study.

With respect to the liver, one of the major problems presented was the difficulty for the histological processing of the sample. Only five samples from each group were viable for evaluation, differently from the aorta where no samples were lost. Therefore, the sample size could be affected, having an impact on the statistically non-significant results.

The cafeteria diet effectively induced obesity and steatosis in rats, as previously reported (de Melo et al., 2018; Maeda Júnior et al., 2018). On the other hand, the present study differs from others reporting an association of PD and steatosis (Komazaki et al., 2017; Nakahara et al., 2018). However, the presence of steatosis observed in all groups of the cafeteria diet and in two groups of the normal diet (APPD treated and untreated) suggests that the cafeteria diet was not imperative to observe manifestations in the liver. The possible mechanisms that link oral infections to the liver steatosis should be further investigated and may be related to the fact that oral infections alter the gut microbiota, causing abnormalities in the downregulation of fatty acid degradation (Komazaki et al., 2017). Tavares et al., (2019) provided evidence that AP can have systemic impacts on metabolic disorders, likely by modulating intestinal metabolism and microbiota. The authors

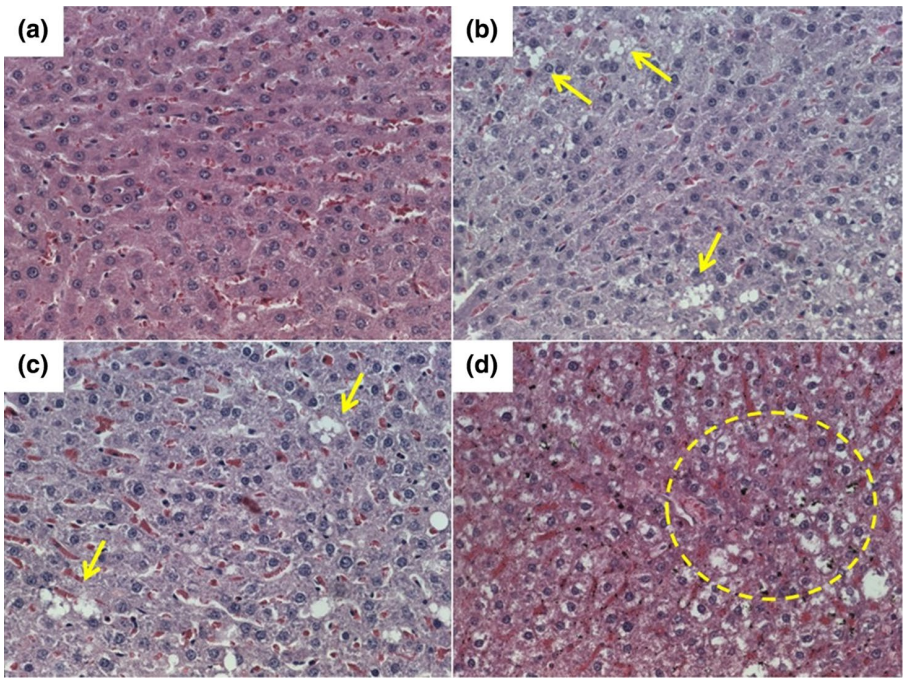

FIGURE 6 Histopathological changes in the liver. (A) – Normal liver. (B/C) – Presence of hepatocyte steatosis (arrows). (d) – Presence of steatosis with disorganization of hepatocyte trabeculae (circle)

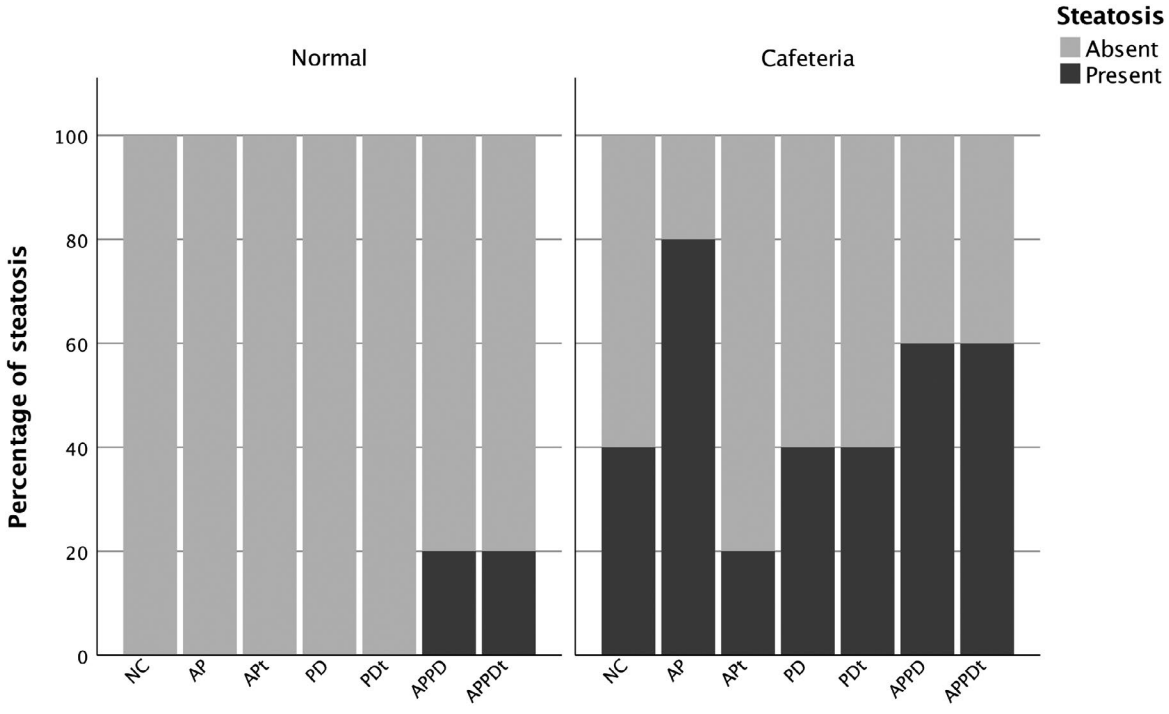

FIGURE 7 Prevalence of steatosis in the different experimental groups of obese and non-obese rats. NC: naïve control; AP: Apical periodontitis; PD: Periodontal disease; APPD: Apical periodontitis +Periodontal disease; t: treated group

report that AP induction led to dysbiosis, indicated by a significant reduction of faecal *A. muciniphila* expression, bacteria present in the intestinal microbiota, and is inversely correlated with the presence of obesity, diabetes, cardiometabolic diseases and chronic inflammation.

Findings from the present study emphasize the association between oral diseases and CVD and reinforce the importance of integrating oral health into primary care by providing patient education and developing referral networks to support collaborative practice between general practitioners and dentists. The prevention and treatment of oral diseases must be done from both local and systemic perspectives. Research efforts need to be increased to expand knowledge about systemic molecular activities

involving metabolic syndromes and the correlation between oral health and the systemic repercussions through well-delineated experimental animal and human studies.

CONCLUSION

Histological alterations in the aortic arch of obese and non-obese rats were observed in the presence of periodontal disease and apical periodontitis. Obesity was associated with liver steatosis. The combination of PD and AP increased the aorta thickness in obese rats. A reduction of vascular endothelial lesions was observed with the treatments of PD and AP.

ETHICAL STATEMENT

Authors affirm that this is an original work, which has not been previously published elsewhere. Furthermore, the paper reflects the authors' research and analysis wholly and truthfully. All sources used are appropriately disclosed and cited. We also affirm that authors have been personally and actively involved in substantial word leading to the paper and will take public responsibility for its content.

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CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

AUTHOR CONTRIBUTION

All authors contributed to the development of this original research. In addition, all authors read, revised, and approved the manuscript. The authors Cynthia Mireya Jara, Karina Kimiko Yamashina Pereira and Maximiliano Schünke Gomes also contributed to the original study design and performed the animal experiments. The authors Cynthia Mireya Jara, Karina Kimiko Yamashina Pereira and Fábio Luiz Dal Moro Maito contributed to the histological processing and histological analysis. The authors Carlos Gabriel Adorno and Maximiliano Schünke Gomes contributed to the statistical analysis. All authors contributed to the interpretation of data and preparation of text and manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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