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ORIGINAL ARTICLE

Radiographic evaluation of the effects of a high-fat-diet on mandibular bone in animals

Avaliação radiográfica dos efeitos da dieta hiperlipídica no osso mandibular em animais

Karina M.M.C. Pinto[1], Vitor M. Encarnação[2], Denise G.P. Dantas[2], Moises J. Oliveira[2], Camila S. Sodré[2], Marco Orsini[3], Carlos A. S. Costa[4], Rachel M. M. Santos[5], Sabrina C. Brasil[6], Luciana Armada[1]

[1]Estácio de Sá University, Rio de Janeiro, RJ, Brasil

[2]Uninassau University, Rio de Janeiro, RJ, Brasil

[3]Iguaçu University (UNIG), Nova Iguaçu, Brasil

[4]Federal University of Rio de Janeiro (UFRJ), Macaé, RJ, Brasil

[5]Fluminense Federal University (UFF), Niterói, RJ, Brasil

[6]Grande Rio University (Unigranrio Afya), Duque de Caxias, RJ, Brasil

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Correspondence: Luciana Armada, luadias@hotmail.com

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Abstract

Introduction: Considering the pathophysiology of apical periodontitis (AP), an inflammatory disease characterized by bone resorption, it is likely that bone changes promoted by high-fat- diet (HFD) are also able to influence the mechanism of AP lesions development. *Objective:* To evaluate by periapical radiography the main effects of a high-fat- diet on mandibular bone in animal models. *Methods:* Isogenic Wistar rats (n=16), 8 weeks old, were used. Half of the animals were submitted to HFD and the other half to control diet (CD). AP lesions were induced 8 weeks later by creating pulp exposure of the mandibular left first molars. After 40 days, the animals were euthanized. Blood was collected for biochemical analysis (total cholesterol, HDL, VLDL and serum triglycerides) and mandible for radiographic analysis (periodontal ligament space, condyle diameter and mandibular thickness).

Results: The HFD promoted significant increase in serum triglyceride ($p<0.05$) and VLDL ($p<0.05$) concentrations in the HFD group. The HFD group animals showed a reduction in both condyle diameter ($p<0.05$) and mandibular thickness ($p<0.05$), and increased periodontal ligament space ($p<0.05$) when compared to the CD group. *Conclusion:* The HFD produced significant changes in the mandibular bone, modifying the periradicular response to endodontic infection.
Keywords: Bone; Diet high-fat; Mandible.

Resumo

Introdução: Considerando a fisiopatologia da periodontite apical (PA), uma doença inflamatória caracterizada pela reabsorção óssea, é provável que as alterações ósseas promovidas pela dieta hiperlipídica (DH) também sejam capazes de influenciar o mecanismo de desenvolvimento das lesões de PA. *Objetivo:* Avaliar através de radiografia periapical os principais efeitos da dieta hiperlipídica no osso mandibular em modelos animais. *Métodos:* Foram utilizados ratos Wistar (n=16), isogênicos, com 8 semanas de idade. Metade dos animais foi submetida à DH e a outra metade à dieta normal (C). Após 8 semanas, foi estimulado o desenvolvimento de lesão perirradicular (LP) nos primeiros molares inferiores esquerdos através da exposição pulpar. Após 40 dias, os animais foram eutanasiados. O sangue foi coletado para a realização da análise bioquímica (colesterol total, HDL, VLDL e triglicerídeos séricos) e mandíbula para análise radiográfica (espaço do ligamento periodontal, diâmetro do côndilo e espessura mandibular). *Resultados:* A DH promoveu aumento significativo nas concentrações séricas de triglicerídeos ($p<0.05$) e VLDL ($p<0.05$) no grupo DH. Os animais do grupo DH apresentaram redução tanto no diâmetro do côndilo ($p<0.05$) como na espessura mandibular ($p<0.05$), e aumento no espaço do ligamento periodontal ($p<0.05$) quando comparados ao grupo C. *Conclusão:* A DH provocou alterações significativas no osso mandibular, influenciando inclusive na resposta perirradicular à infecção endodôntica.
Palavras-chave: Osso; Dieta hiperlipídica; Mandíbula.

Introduction

Lipids are organic molecules that have physiological, structural and nutritional functions. They act as hormone precursors, promote the transport of vitamins and serve as an energy reserve [1]. The association between lifestyle, diet, and genetic factors may be responsible for the development of a hyperlipidemic state, which is considered a risk factor for the development and progression of some diseases [2-5], grouped in the so-called metabolic syndromes: hypercholesterolemia; hyperglycemia; cardiovascular diseases; atherosclerosis and hepatic steatosis [6,7].

The hyperlipidemia is associated with a pro-inflammatory state, characterized by an expressive increase in cytokines, such as interleukins (IL) and tumor necrosis factor alpha (TNF-α) [6-8] . And the inflammatory process, associated with hyperlipidemia, is also negatively correlated with bone architecture, reducing bone quantity and quality due to inhibition of osteoblast differentiation by bioactive lipids and increased functional activity of osteoclasts [4,9,13].

Considering the pathophysiology of apical periodontitis (AP), an inflammatory disease characterized by bone resorption, it is likely that bone changes promoted by high-fat- diet (HFD) are also able to influence the mechanism of AP lesions development [10,12]. There are indications that bone resorption associated with AP occurs by the involvement and interaction between pro-inflammatory cytokines and the triad Nuclear factor kappa B activating receptor (RANK)/ Nuclear factor kappa B activating receptor ligand (RANK-L) / Osteoprotegerin (OPG) [14,15].

Therefore, the aim of this study was to perform a radiographic evaluation of the main effects of an HFD on mandibular bone in animal models. The research hypotheses were: (1) The HFD has an inflammatory potential that may interfere with bone microarchitecture, reducing mandibular bone quantity and quality; and (2) It is believed that animals in hyperlipidemic state will show greater bone changes when compared to control groups.

Methods

Animals were cared for in accordance with the "Guide for the Care and Use of Laboratory Animals", National Academy of Sciences. Washington DC and have the approval of an institutional committee (594).

Sample Selection and Diet

For this study 16 adult male Wistar rats were used, which, after weaning, at 8 weeks of age, were kept in individual cages, with controlled room temperature (25 to 27ºC), constant humidity, and a 12-hour light/dark cycle (6:00 am to 6:00 pm). After an acclimatization period of 1 week, the animals were randomly divided into 2 groups control diet (CD) group, with normal diet and HFD group, with high-fat diet). Water was provided *ad libitum* and the feed was weighed and quantified at each feeding for a period of 8 weeks.

The formulation of the high-fat diet followed the protocol established by AIN- 93G [16] (Table 1). The animals were kept in the animal house of the Experimental Nutrition Department of the Universidade Federal Fluminense (UFF).

Table 1 - *Nutritional Composition of high-fat diet (AIN 93-G).*

Ingredient	Control diet (g/kg)	High-fat diet (g/kg)
Casein	20	20
Maize starch	52.9	35.9
Sucrose	10	10
Soy oil	7	7
lard	0	17
Cellulose	5	5
AIN-93G Mineral Mix	3.5	3.5
AIN-93G Vitamin Mix	1	1
L-Cystine	0.3	0.3
Choline Bitartrate	0.25	0.25
BHT (mg)	14	14

Quantity of ingredients used in the preparation of 100g of feed.

Apical periodontitis induction

After the 8-week feeding period [11], all animals were anesthetized with Thiopenthal (0.1 mL/100 g body weight), and the enamel and dentin of the left mandibular first molars were worn with a ½ spherical carbide bur (KG Sorensen, São Paulo) with a low rotation motor (Dentec, CS 421, Brazil). The opening was performed in the mesial fossula of the occlusal surface until pulpexposure by a single operator previously calibrated. After 40 days, the animals were euthanized by exsanguination under anesthesia with Thiopenthal (0.2 mL/100 g body weight) and blood and mandible were collected.

Serum Analysis

Blood samples were collected by cardiac puncture and total cholesterol, VLDL- cholesterol, HDL-cholesterol, and triglycerides values were analyzed using BT Plus 3000 (Biotecnica Instruments, Rome, Italy) equipment and Wiener Lab Group (Rosario, Argentina) kits (Colestat enzimatico AA líquida, HDL Colesterol monofase AA plus, TG Color GPO/PAP AA líquida).

Radiographic evaluation

The left hemi-mandibles were washed and immersed in buffered formalin solution. After this process, the radiographs were taken in a digital radiographic device (Radioesfera model - Siemens - SP, Brazil), and the positioning of the pieces on the radiographic film was standardized in order to avoid image distortion.

Image J software (National Institute of Mental Health, Bethesda, USA) was used to measure: the periodontal ligament space near the mesial roots of the left mandibular first molars, the diameter of the condyle and the thickness of the mandible. Image analysis was performed separately by two calibrated evaluators who were blinded to the groups analyzed. After image analysis, the evaluators compared the results. The few cases of disagreement between them were resolved by joint discussion.

Statistical Analysis

The comparative analysis of the data obtained was performed through non- parametric *Kruskal-Wallis* and *Dunn's Multiple Comparison* tests using the *GraphPad Prism* 6 program (*GraphPad Software*, Inc, California, USA). The statistical significance considered was $p<0.05$.

Results

Biochemical evaluation

The numerical values of the mean serum levels of total cholesterol, HDL, VLDL and triglycerides per group can be seen in Table 2. The serum levels of total cholesterol and HDL were similar in all HFD and CD groups, showing no statistically significant difference ($p>0.05$). VLDL and triglyceride values were higher in the HFD group, with statistical significance ($p<0.05$).

Table 2 - *Biochemical evaluation per group*

Groups	Cholesterol total (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	Triglycerides (mg/dL)
CD	28.4 ± 6.1	14.5 ± 2.8	5.8 ± 2.1	29.0 ± 11.6
HFD	27.1 ± 3.0	11.0 ± 2.8	15.6 ± 6.2*	79.5 ± 32.8*

Results are expressed by mean ± DPM. * $p<0.05$

Radiographic evaluation

Periodontal ligament space

All animals in the HFD and CD groups showed increased periodontal ligament space (Table 3), since both developed AP lesions. However, it was possible to observe that the thickening was significantly greater in the HFD group ($p=0,0015$). Some of the images obtained through digital radiography can be seen in Figure 1.

Table 3 - *Radiographic evaluation per group*

Groups	Ligament space periodontal (pixel)	Head diameter of the condyle(pixel)	Thickness mandibular (pixel)
CD	303.8 ± 24.1	46.6 ± 10.2	1453 ± 55.49
HFD	323.7 ± 32.2*	32.3 ± 8.6*	1278 ± 106.6 *

Results are expressed by mean ± DPM. * $p<0.05$

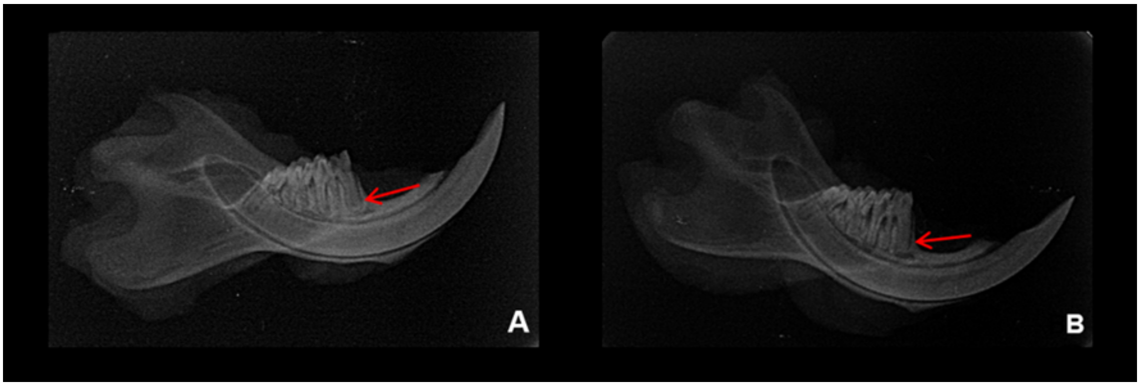

Fig. 1 - *Radiographs of rat mandibles. A: CD group, B: HFD group. Arrows indicate increased periodontal ligament space in the mesial roots of the left mandibular first molars*

Condylar head diameter

The condylar head diameter (Table 3) of the HFD group was significantly smaller ($p<0.05$) when compared to the CD group. Some of the images obtained by digital radiography can be seen in Figure 2.

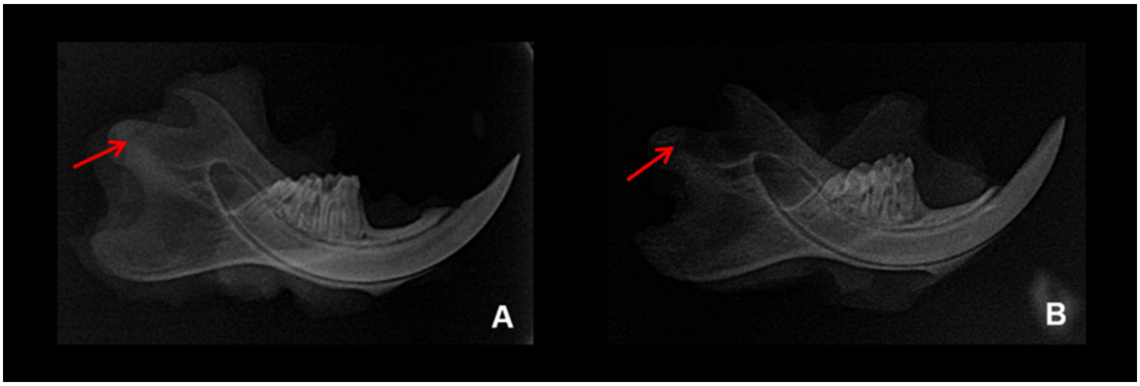

Fig. 2 - *Radiographs of rat mandibles. A: CD group, B: HFD group. Arrows indicate the left condyles.*

Thickness of the left hemi-mandibles

The mandibular thickness (Table 3) of the HFD group was significantly lower ($p<0.05$) when compared to the CD group. Some of the images obtained through digital radiography can be seen in Figure 3.

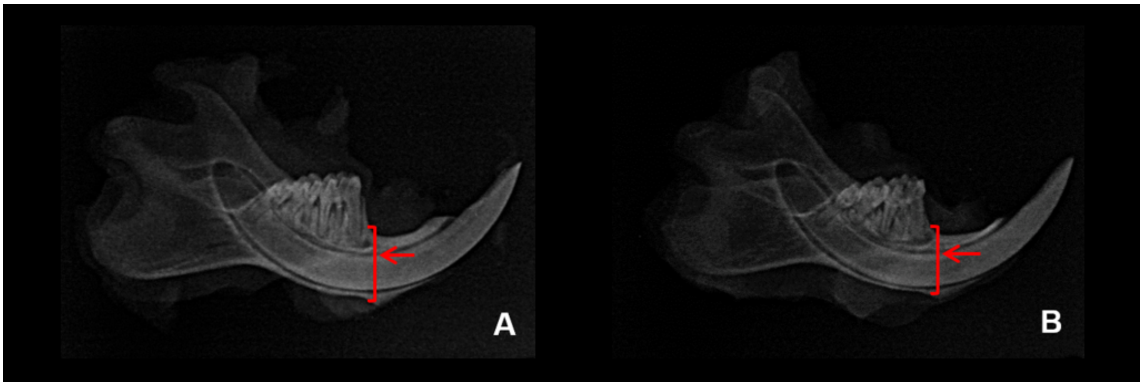

Fig. 3 - *Radiographs of rat mandibles. A: CD group B: HFD group. Arrows indicate mandibular thickness*

Discussion

In the present study, the formulation of the hyperlipidic diet used followed the protocol established by the American Institute for Nutrition for Animal Experimentation [16], using lard in the composition. The animals of the HFD group were subjected to this diet for a feeding period of 8 weeks, which according to a previous study [11] is considered the proper time for the establishment of changes in the lipidogram.

The hypercaloric diet was effective, as the biochemical evaluation performed showed higher serum levels of VLDL-cholesterol and triglycerides in the HFD group. However, serum concentrations of total and HDL-cholesterol were similar between the groups. A previous work [11] employed a hyperlipidic diet similar to the present study to assess the correlation of hyperlipidemia with the development of periodontal disease in mice, but with 12- week supply and found considerably higher levels of triglycerides, total cholesterol and HDL.

This increase in serum triglyceride values and decrease in HDL, called the atherosclerotic profile, is considered a potential risk factor for the development of some systemic disorders that have been well reported in previous studies, such as insulin resistance [17,18]; cardiovascular diseases [3, 5, 6, 8, 19-21] and hepatic steatosis [22].

The hyperlipidemic state also correlates with some alterations in bone homeostasis. The association with alterations in alveolar bone metabolism has been proven in several previous studies, both in humans [2, 9, 12, 17, 20] and in animal models [8, 11, 12, 21, 24] that have linked the hyperlipidic diet with periodontal disease. Such results suggested that this diet has an inflammatory potential that may accelerate bone loss, contributing to the development and progression of periodontitis, as well as, to the failure of periodontal therapy. Through a quantitative radiographic evaluation it was possible to observe similar results in the present study showed that animals in hyperlipidemic conditions showed a significant reduction in condylar head diameter and mandibular bone thickness compared to the control group. These results also corroborate the findings of previous studies, which stated that hyperlipidemia compromises alveolar bone quantity and quality by inhibiting osteoblastic differentiation and stimulating osteoclastic activity [10, 13]. Being AP, an inflammatory disease characterized by bone destruction, they proposed that this condition is also associated with its etiopathogenesis.

The present work is similar to several previous studies [19, 25-30] that used animal models with AP lesions induced by pulpal exposure to the oral environment to elucidate the main mechanisms involved in the pathogenesis of periradicular diseases. The rat was the experimental model of choice because it has some advantages over other animals, such as ease of obtaining and low cost.

But the most relevant aspect is related to the fact that the kinetics of development, as well as the aspects of the main peri-radicular alterations observed in this study model are very similar to those in humans, thus allowing greater credibility to the results [31].

When assessing the periodontal ligament space, we observed that there was an increase in this structure in all groups due to inflammatory edema formation in response to microbial aggression from root canal infection [32]. However, this thickening was significantly greater in the HFD group. Additionally, we found that all components of the HFD group developed lesions in the furcation region and on the mesial and distal root apices, and these radiolucent areas were significantly larger when compared to those present in the CD group. These results were consistent with those observed by previous studies [4, 33, 34], reinforcing the hypothesis that a hyperlipidic diet increases the speed of progression of periradicular tissue destruction. Under these conditions, we can assume that pro-inflammatory cytokines secreted by immunocompetent cells are a linking factor. Therefore, it would be of great scientific relevance that further studies be performed to evaluate and quantify the main inflammatory mediators that mediate this correlation between hyperlipidemia and peri-radicular disease.

The results revealed that the hyperlipidemic state caused significant changes in the mandibular bone, including the influence of the periradicular response to endodontic infection.

Conflict of interest

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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Author contribution statement

Conceptualization: Pinto KMMC, Brasil SC, Armada L; Data Curation: Pinto KMMC, Encarnação VM, Dantas DGP, Oliveira MJ; Research: Pinto KMMC, Encarnação VM, Dantas DGP, Oliveira MJ; Resources: Costa CAS, Santos RMM; Project Administration: Pinto KMMC, Encarnação VM, Dantas DGP, Oliveira MJ, Costa CAS, Santos RMM, Brasil SC, Armada L; Visualization: Pinto KMMC, Encarnação VM, Dantas DGP, Oliveira MJ, Gonçalves CSS, Orsini M, Costa CAS, Santos RMM, Brasil SC, Armada L, Methodology: Gonçalves CSS, Orsini M, Costa CAS, Santos RMM, Brasil SC, Armada L; Validation: Gonçalves CSS, Orsini M, Costa CAS, Santos RMM, Brasil SC, Armada L, Supervision: Brasil SC, Armada L; Formal Analysis: Gonçalves CSS, Orsini M; Acquisition of Funding: Armada L; Writing original draft: Pinto KMMC, Encarnação VM, Dantas DGP, Oliveira MJ; Writing analysis and editing: Pinto KMMC, Encarnação VM, Dantas DGP, Oliveira MJ, Gonçalves CSS, Orsini M, Costa CAS, Santos RMM, Brasil SC, Armada L.

References

1. Barrios V, Escobar C, Cicero AF, Burke D, Fasching P, Banach M, Bruckert E. A nutraceutial approach (Armolipid Plus) to reduce total and LDL cholesterol in individuals with mild to moderate dyslipidemia: review of the clinical evidence. Atheroscler Suppl. 2017; 24: 1-15. https://doi.org/10.1016/j.atherosclerosissup.2016.10.003
2. Hagh LG, Zakavi F, Hajizadeh F, Saleki M. The association between hyperlipidemia and periodontal Infection. Iran Red Crescent Med J. 2014; 16: 65-77. https://doi.org/10.5812/ircmj.6577
3. Stewart J, McCallin T, Martinez J, Chacko S, Yusuf S. Hyperlipidemia. Pediatr Rev. 2020 Aug;41(8):393-402. https://doi.org/10.1542/pir.2019-0053
4. Tiburcio-Machado CS, Lang PM, Campos MM, Wolle CFB, Barcelos RCS, Zago NB, Leite CE, Etges A, Bier CAS. High-fat diet effect on periapical lesions and hepatic enzymatic antioxidant in rats. Life Sci 2020; 23:118637. https://doi.org/10.1016/j.lfs.2020.118637
5. Yao YS, Li TD, Zeng ZH. Mechanisms underlying direct actions of hyperlipidemia on myocardium: an updated review. Lipids Health Dis. 2020 Feb 8;19(1):23. https://doi.org/10.1186/s12944-019-1171-8
6. Paniagua JA. Nutricion, insulin resistance and dysfunctional adipose tissue determine the different components of metabolic syndrome. World J Diabetes. 2016; 7: 483-514. https://doi.org/10.4239/wjd.v7.i19.483

7. Drummer CIV, Saaoud F, Sun Y, Atar D, Xu K, Lu Y, Shao Y, Johnson C, Liu L, Shen H, Jhala NC, Jiang X, Wang H, Yang X. Hyperlipidemia May Synergize with Hypomethylation in Establishing Trained Immunity and Promoting Inflammation in NASH and NAFLD. J Immunol Res. 2021 Nov 23; 2021:3928323. https://doi.org/10.1155/2021/3928323
8. Antona ME, Ramos C, Stranges A, Monteiro AF, Chaves MMG, Mandalunis P, Zago V, Friedman SM, Macri EV. Fish oil diet effects on alveolar bone loss, in hypercholesterolemic rats. *Arch Oral Biol*. 2020; 109:104553. https://doi.org/10.1016/j.archoralbio.2019.104553
9. Clemente-Postigo MMI, Queipo-Ortuño M, Murri M, Boto-Ordoñez, Perez- Martinez P, Andres-Lacueva C, Cardona F, Tinahones J. Endotoxin increase after fat overload is related to post prandial hyper-triglyceridemia in morbidly obese patients. J Lipid Res. 2012; 53: 973-978. https://doi.org/10.1194/jlr.P020909
10. Soares EA, Nakagaki WR, Garcia JAD, Camilli JA. Effect of hyperlipidemia on femoral biomechanics and morphology in low-density lipoprotein receptor gene knockout mice. J Bone Miner Metab. 2012; 30: 419-425. https://doi.org/10.1007/s00774-011-0345-x
11. Fujita Y, Maki K. High-fat diet-induced obesity triggers alveolar bone loss and spontaneous periodontal disease in growing mice. Obesity. 2016; 3: 4-9. https://doi.org/10.1186/s40608-016-0082-8
12. Muluke M, Gold T, Kiefhaber K, Al-Sahli A, Celenti R, Jiang H, Cremers S, Van Dyke T, Schulze-Spate U. Diet- induced obesity and its differential impact on periodontal bone loss. J Dent Res. 2016; 95: 223- 229. https://doi.org/10.1177/0022034515609882
13. Montalvany-Antonucci CC, Zicker MC, Ferreira AVM, Macari S, Ramos- Junior ES, Gomez RS, Pereira TSF, Madeira MFM, Fukada SY, Andrade I, Silva TA. High-fat diet disrupts bone remodeling by inducing local and systemic alterations, J. Nutr. Biochem. 2018; 59: 93-103. https://doi.org/10.1016/j.jnutbio.2018.06.006
14. Steeve KT, Marc P, Sandrine T, Dominique H, Yannick F. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth F R. 2004; 15: 49-60. https://doi.org/10.1016/j.cytogfr.2003.10.005
15. Nikolic N, Jakovljevic A, Carkic J, Beljic-Ivanovic K, Miletic M, Soldatovic I, Andric M, Ivanovic V, Milasin J. Notch signaling pathway in apical periodontitis: correlation with bone resorption regulators and proinflammatory cytokines. J Endod. 2018; 45:123-128. https://doi.org/10.1016/j.joen.2018.10.015
16. Reeves, PG. Components of the AIN-93 diets as Improvements in the AIN- 76 diet. J Nutr. 1997; 5: 838-841. https://doi.org/10.1093/jn/127.5.838S
17. Kwon Y, Park J, Lim H, Lee Y, Lee H, Shim J. Triglyceride to high density lipoprotein cholesterol ratio and its association with periodontal disease in Korean adults: findings based on the 2012-2014 Korean national health and nutrition examination survey. Clin Oral Investig. 2018; 22(1):515-522. https://doi.org/10.1007/s00784-017-2140-0
18. Zhou M, Zhu L, Cui X, Feng L, Zhao X, He S, Ping F, Li W, Li Y. The triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio as a predictor of insulin resistance but not of β cell function in a Chinese population with different glucose tolerance status. Lipids Health Dis. 2016; 15:1-9. https://doi.org/10.1186/s12944-016-0270-z

19. Conti LC, Segura-Egea JJ, Cardoso CBM, Benetti F, Azuma MM, Oliveira PHC, Bomfim SRM, Cintra LTA. Relationship between apical periodontitis and atherosclerosis: Lipid profile and histological study. Int Endod J. 2020; 53:1387-1397. https://doi.org/10.1111/iej.13350
20. Lee S, Im A, Burm E, Ha M. Association between periodontitis and blood lipid levels in a Korean population. J Periodontol. 2018; 89(1):28-35. https://doi.org/10.1902/jop.2017.170111
21. Suh JS, Kim SYJ, Lee SH, Kim RH, N Park N. Hyperlipidemia is necessary for the initiation and progression of atherosclerosis by severe periodontitis in mice. Mol Med Rep. 2022;26(2):273. https://doi.org/10.3892/mmr.2022.12789
22. Tessari P, Coracina A, Cosma A, Tiengo A. Hepatic lipid metabolism and non-alcoholic fatty liver disease. Nutr Metab Cardiovac Dis. 2009; 19: 291- 302. https://doi.org/10.1016/j.numecd.2008.12.015
23. Amar S, Zhou Q, Shaik-Dasthgirisaheb Y, Leeman S. Diet-induced obesity in mice causes changes in immune responses and bone loss manifested by bacterial challenge. PNAS. 2007; 104: 20.466-20.471. https://doi.org/10.1073/pnas.0710335105
24. Silva NLC, Motta NAV, Soares MA, Araujo OMO, Espíndola LCP, Colombo APV, Lopes RT, Brito FCF, Miranda ALP, Tributino JLM. Periodontal status, vascular reactivity, and platelet aggregation changes in rats submitted to hypercholesterolemic diet and periodontitis. J Periodontal Res. 2020; 55(3):453-463. https://doi.org/10.1111/jre.12730
25. Akamine A, Anan H, Hamachi T, Maeda K. A histochemical study of behavior of macrophages during experimental apical periodontitis in rats. J Endod. 1994; 20: 474-478. https://doi.org/10.1016/S0099-2399(06)80042-7
26. Tani-Ishii N, Wang CY, Stashenko P. Immunolocalization of bone- resorptive cytokines in rat pulp and periapical lesions following surgical pulp exposure. Oral Microbiol Immunol. 1995; 10: 213-219. https://doi.org/10.1111/j.1399-302X.1995.tb00145.x
27. Kawashima N, Okiji T, Kosaka T, Suda H. Kinetics of macrophages and lymphoid cells during the development of experimentally induced periapical lesions in rat molars: a quantitative immunohistochemical study. J Endod. 1996; 22: 311-316. https://doi.org/10.1016/S0099-2399(96)80266-4
28. Lin S, Hong C, Chang H, Chiang C, Chen C, Jeng J, Kuo MY. Immunolocalization of macrophages and transforming growth factor β1 in induced rat periapical lesions. J Endod. 2000; 26: 335-340. https://doi.org/10.1097/00004770-200006000-00007
29. Minhoto GB, Khoury RD, Orozco EIF, Prado RF, Valera MC. Effect of chronic unpredictable stress on the progression of experimental apical periodontitis in rats. Int Endod J. 2021; 54:1342-1352. https://doi.org/10.1111/iej.13515
30. Pinto KP, Ferreira CMA, Guimarães AFC, Lima CO, Pires FR, Sassone LM, Silva EJNL. Effects of alcohol and nicotine consumption on the development of apical periodontitis in rats: a correlative micro-computed tomographic, histologic and immunohistochemical study. Int Endod J. 2020; 53:1238-1252. https://doi.org/10.1111/iej.13344
31. Eurasquim J, Muruzabal MA. A method for root canal treatment in the molar of rat. Oral Surg. 1967; 24: 540-546. https://doi.org/10.1016/0030-4220(67)90435-5

32. Abbott PV. Classification, diagnosis and clinical manifestations of apical periodontitis. Endod Topics. 2004; 8: 36-54. https://doi.org/10.1111/j.1601-1546.2004.00098.x

33. Kimak A, Strycharz-Dudziak M, Bachanek T, Kimak E. Lipids and lipoptroteins and inflammatory markers in patients with chronic apical periodontitis. Lipids Health Dis. 2015; 14:162-168. https://doi.org/10.1186/s12944-015-0156-5

34. Brasil SC, Santos RMM, Fernandes A, Lima RS, Costa CAS, Pinto KMMC, Pires FR, Santos MD, Siqueira Jr JF, Armada L. Influence of a high-fat diet in the progression of apical periodontitis. J Endod. 2021; 47: 600-605. https://doi.org/10.1016/j.joen.2020.12.015