Life Sciences 265 (2021) 118747

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Caloric restriction prevents alveolar bone loss in the experimental periodontitis in obese rats

Evelyn Lopez Soares [a,*], Fábio André dos Santos [a], Tayline Mroczek [b], Debora Cristina de Lima [c], Hemily Victória Bardini Josefino [c], Bruna Carolina Mehret Scorsin [a], Dionizia Xavier Scomparin [d], Gilson Cesar Nobre Franco [b]

[a] Department of Dentistry, Universidade Estadual de Ponta Grossa, PR, Brazil
[b] Department of Health Sciences, Universidade Estadual de Ponta Grossa, PR, Brazil
[c] Department of Pharmaceutical Sciences, Universidade Estadual de Ponta Grossa, PR, Brazil
[d] Department of Biology, Universidade Estadual de Ponta Grossa, PR, Brazil

ARTICLE INFO

Keywords:
Obesity
Caloric restriction
Periodontitis

ABSTRACT

Aims: It has been shown that periodontitis, can be modified by systemic changes, including behavioral factors, such as diet. Caloric restriction is one of the dietary therapeutic strategies indicated for obesity. It is associated with several benefits, among them, modulation of the inflammatory response. The aim of this study was to verify whether caloric restriction in obese rats changes the progression of experimental ligature-induced periodontitis.
Materials and methods: Forty-eight Wistar rats were used for 24 weeks and initially fed with cafeteria diet during 12 weeks. The animals were divided into four groups according to the caloric restriction and experimental periodontitis. The cotton thread was placed around the mandibular first molars, for 15 days, before the end of the experiment. Rats submitted to caloric restriction received, from the 13th week of the experimental protocol, 70% of the food intake compared to the *ad libitum* animals of other study of the our research group. Alveolar bone loss was assessed using macroscopic morphometric analysis. Analyzes of clinical periodontal measures, biometrics, serum biomarkers and biochemical parameters were performed.
Key findings: Caloric restriction decreased the alveolar bone loss in the periodontitis group when compared to the group that received a cafeteria diet with periodontitis. Moreover, the results demonstrate the improvement in the glycemic profile, without prejudice to bone tissue biomarkers.
Significance: Based on the results, caloric restriction reduces the progression of alveolar bone loss in rats with experimental periodontitis, in addition to presenting benefits in biometric data, decreasing both glycemic profile and clinical periodontal measures.

1. Introduction

The deleterial effects of obesity on the progression and severity of periodontitis have been reported in epidemiological and experimental studies [1–3]. Among the therapeutic methods indicated for reducing obesity, there are diets and exercise [4].

Caloric restriction (CR) is one of the most recommended strategies for reducing body weight, currently considered extremely efficient [5]. It is mainly related to the host responses to inflammatory processes, such as obesity and periodontitis. In addition, CR can act on the mechanisms for maintaining hormonal balance and systemic physiological functions, regulates gene expression, reduces damage caused by oxidative stress and reduces the expression of inflammatory cytokines, such as tumor necrosis-alpha (TNF-alpha) and interleukin 6 (IL-6). These mechanisms have been suggested as the basis of the relationship between CR and periodontitis [6].

Actually, caloric restriction has been suggested to attenuate periodontal parameters and inflammatory molecules in human studies and animals submitted to experimental periodontitis ligature-induced [6,7]. However, the effect on alveolar bone tissue and the condition of the diet used as therapy for obesity, still needs to be elucidated. Experimental animal models have been used to evaluate the inflammatory, immunological and clinical processes of progression and severity of periodontitis, for the reason that studies in humans of progression of periodontal

* Corresponding author at: Rua Carlos Cavalcanti, 4748 Bloco M, Uvaranas, Ponta Grossa, Paraná 84030-900, Brazil.
E-mail address: evelynlsoares.uepg@gmail.com (E.L. Soares).

https://doi.org/10.1016/j.lfs.2020.118747
Received 21 September 2020; Received in revised form 5 November 2020; Accepted 7 November 2020
Available online 10 November 2020

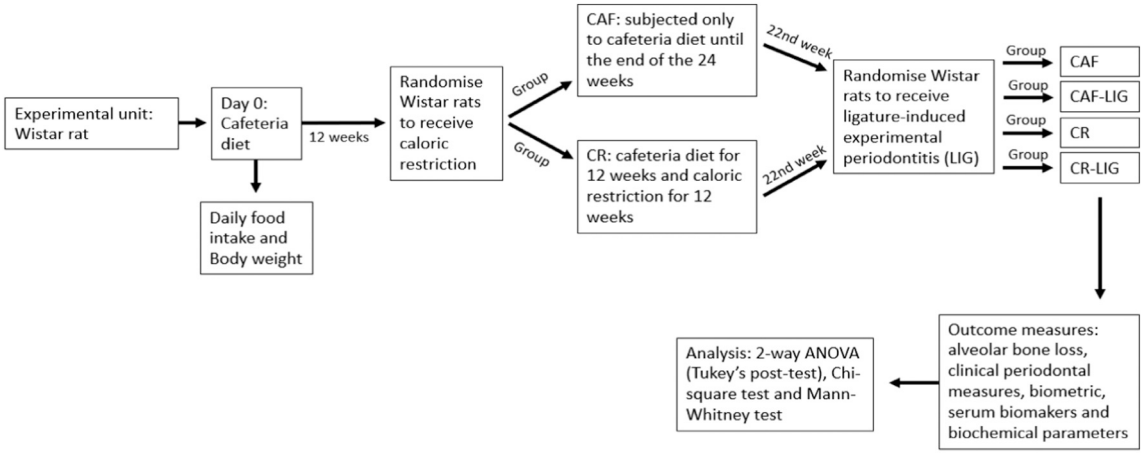

Fig. 1. Overview of the study design and workflow

disease are considered extremely difficult to be evaluated due to the unpredictability of progression [4].

Periodontitis is characterized by immune and inflammatory responses in defense against bacterial colonization and involves the protective and supporting tissues of the tooth [8,9]. The inflammatory process in periodontitis can result in degradation of connective fibers and resorption of alveolar bone in a susceptible individual. The susceptibility of each individual can be explained due to differences in the immune response in the context of imbalance in periodontal tissues. This imbalance is marked by the consequent clinical implication of alveolar bone loss, mainly through the release of inflammatory cytokines. Based on the increase in the circulating level of inflammatory molecules, the association of periodontitis with systemic and behavioral factors, such as CR, has been reported [7].

Therefore, the aim of this study was to evaluate whether the effect of caloric restriction can be beneficial in alveolar bone loss in experimental periodontitis after obesity induction with the cafeteria diet method in rats.

2. Material and methods

2.1. Experimental design and animals treatment

Male Wistar Rats (*Rattus norvegicus albinus*) were obtained from the State University of Ponta Grossa). The protocol was approved by the Ethics Committee on the Use of Animals (Protocol 12,203/2017) and was in accordance with the ARRIVE guidelines (Animal Research: Reporting of *In vivo* experiments) and following the National Institutes of (NIH) guide for the care and use of Laboratory animals [10,11]. The rats, with 30 days of life, were housed in polypropylene cages, maintained at a controlled temperature (± 23 °C) and on a 12/12 h light-dark cycle; fed a cafeteria diet without caloric restriction (CAF) or fed a cafeteria diet and 30% caloric restriction (CR), divided also according to the placement of periodontal ligature (CAF-LIG and CR-LIG). All animals had free access to water. For the sample calculation, alveolar bone loss was considered the primary outcome and the G* power 3.1 program (G * Power, Universität Düsseldorf, Düsseldorf, North Rhine-Westphalia, Germany) was used. It was found that for a power of 85%, the number used should be 10 animals per group. Considering possible losses during the experimental process and possible methodological error during the research, n of 12 animals per group were used, divided into four groups (total $n = 48$ animals, randomly distributed (Software Microsoft Excel®) (Fig. 1). No intentional exclusion of animals was necessary. However, due to animal deaths during the experimental period, statistical analyzes were performed with the following numbers: CAF ($n = 10$ animals), CR (n = 10 animals), CAF-LIG (n = 10 animals) and CR-LIG ($n = 12$ animals).

2.2. Diet protocol and experimental periodontitis ligature-induced

In order to evaluate the effect of caloric restriction as a treatment, obesity was induced in animals by the cafeteria diet method. All animals, at the beginning, received the cafeteria diet, available 3 times/week. The components of this diet consisted of 3 combinations with different menus containing high-caloric foods, distributed over the 3 days of the week [12–14]. After 12 weeks, half of the animals were randomized and submitted to caloric restriction (CR) and the other half remained on the cafeteria diet (CAF), until the 24th week. From the 13th week, in the groups submitted to CR, the animals were fed with 70% of the amount ingested by the group that received an *ad libitum* diet from another study that followed in parallel, in our research group, following the diet protocol established in the literature [4,15]. The animals remained fed the caloric restriction or cafeteria diet until the end of the 24 weeks. On the 22nd week, the rats in the CAF-LIG and CR-LIG groups were anesthetized with intramuscular injection of 10% ketamine (90 mg/kg) and 2% xylazine (10 mg/kg) and received the cotton thread placement in the mandibular first molars, to induce experimental periodontitis, according to the protocol recommended in the literature [16,17]. The animals that did not receive the experimental periodontitis, remained only submitted to diets until the 24th week. Euthanasia and blood collection, hemimandibles and analysis of clinical periodontal measures were performed at the end of the 24-week experiment. Biometric data and adipose tissue were collected for analysis and proof of the effectiveness of the diets.

2.3. Biometric data

Body weight was measured twice a week and the graph of evolution of body weight was made to verify the increase or decrease in weight according to the diet provided. The naso-anal length was determined to calculate the Lee Index, together with the measurement of abdominal circumference, according to Merigo et al. (2013) [3]. The adipose tissue from the epididymal, mesenteric, retroperitoneal regions were removed and weighed to determine the adiposity index, as proposed in previous studies [18,19].

2.4. Clinical periodontal measures

Previously to the euthanasia, clinical periodontal analysis of the molars that received the ligatures and also of the adjacent periodontal tissue was performed based on four parameters: Biofilm (0-absent; 1-present); Bleeding on probing (BOP) (0-absent; 1-present); Mobility: 0-

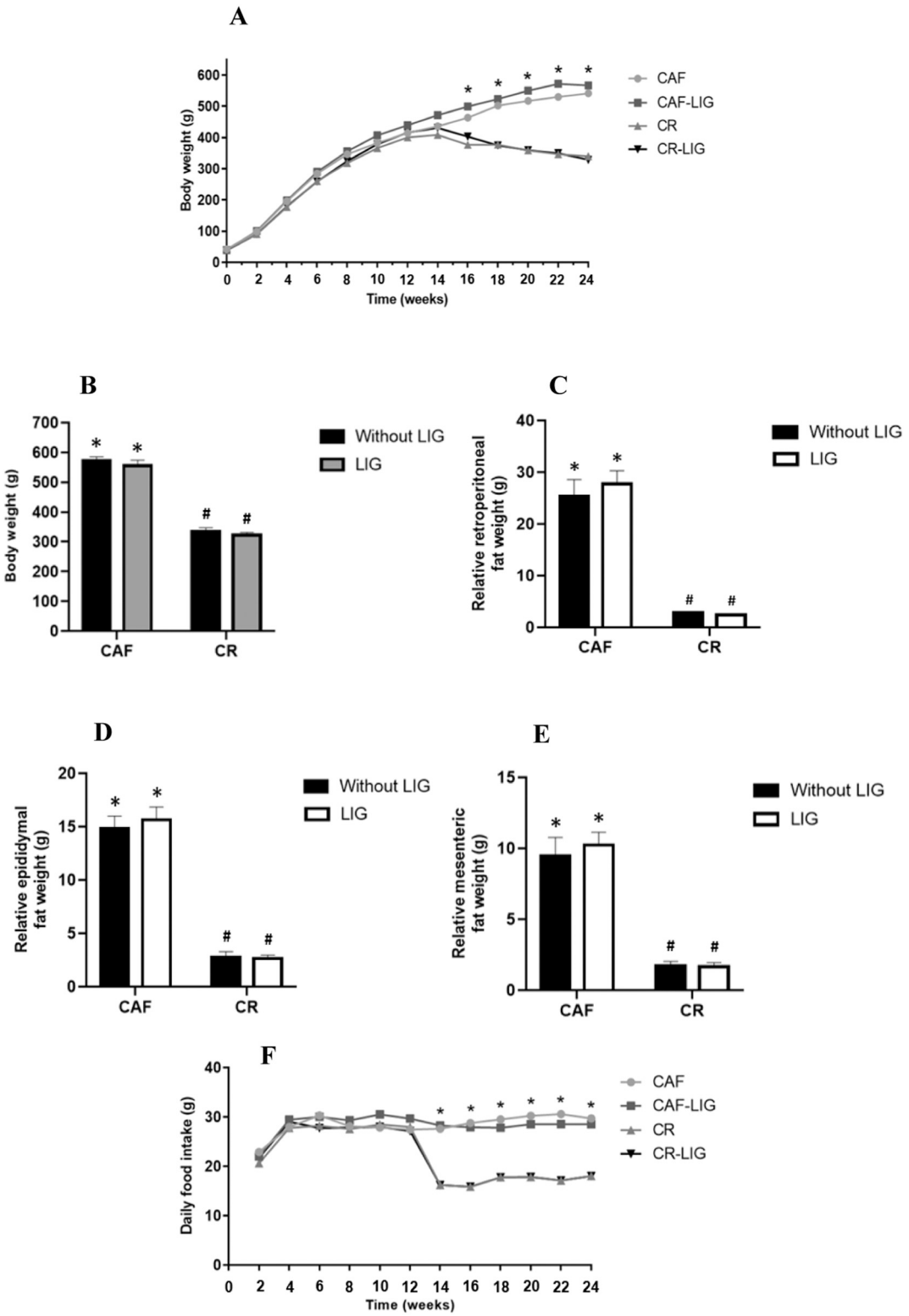

Fig. 2. Effect of caloric restriction on biometric parameters. (A) Body weight curve; (B) Final body weight; (C), (D) and (E) Relative retroperitoneal, mesenteric and epididymal fat weight, respectively; and (F) Daily food intake. CAF = cafeteria diet, CAF-LIG = cafeteria diet with experimental periodontitis ligature-induced, CR = caloric restriction, CR-LIG = caloric restriction and periodontitis ligature-induced. Different signs means a significant difference ($p < 0.05$). Two-way ANOVA followed by Tukey's post-test. Data expresses mean ± SEM. (A) and (F) *Significant difference ($p < 0.05$) between groups that received CR with CAF groups.

absent; 1-slight mobility (vestibular-palatal); 2-moderate mobility (vestibular-palatal and mesial-distal); 3-severe mobility (vertical, movement inside and outside the tooth socket); Gingival index (0-normal gingiva; 1-mild inflammation, mild edema, slight color change and no bleeding; 2-moderate inflammation, edema, redness and bleeding; 3-severe inflammation, extreme redness, presence of ulcer, edema and severe bleeding). To perform this analysis, the animals were initially weighed and sedated with intraperitoneal (IP) anesthesia with

Table 1
Biometric parameters in the experimentals groups submitted a caloric restriction and experimental periodontitis.

	CAF	CAF-LIG	CR	CR-LIG
Initial body weight (g)	36 ± 1.93	36 ± 1.20	34 ± 1.37	34 ± 2.15
Final body weight (g)	571 ± 14.14*	555 ± 18.81*	334 ± 11.69#	321 ± 9.77#
Body weight gain (g)	535 ± 13.93*	519 ± 18.27*	300 ± 11.19#	287 ± 9.59#
Adiposity index (%)	8.5 ± 0.73*	9.0 ± 0.63*	2.1 ± 0.24#	2.0 ± 0.20#
Naso-anal lenght (cm)	27 ± 0.40	26 ± 0.34	25 ± 0.39	25 ± 0.26
Lee index (g/cm)	313 ± 3.75*	315 ± 4.14*	276 ± 5.07#	277 ± 2.99#
Abdominal circumference (cm)	23 ± 0.47*	23 ± 0.45*	17 ± 0.20#	17 ± 0.33#

Data represented as mean ± SEM. ANOVA-two way, Tukey's post-test. Different signs indicated $p < 0.05$.

10% ketamine solution (90 mg/kg) and 2% xylazine muscle relaxant (10 mg/kg). Subsequently, the animals were positioned on the operating table and with the help of a spatula and probe, the clinical analysis of periodontal measures was performed by blinded investigator [20–22].

2.5. Biochemical parameters

After experiment, the blood was collected, centrifuged and stored at −80 °C. Glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride levels were determined using commercial kits following the manufacturer's instructions, using the Wiener CT300i analyzer equipment.

2.6. Macroscopic morphometric analysis

To assess the extent of alveolar bone loss, measurements were made on the left hemimandibles. After euthanasia, the mandible of each animal was removed, boiled during 30 min, immersed in hydrogen peroxide solution (3%) for 12 h and stained with methylene blue (1%) to differentiate between the tooth and bone tissue, fixed on a support and photographed. The images were made for the macroscopic analysis of the mandibles with a Zeiss stereoscopic loupe with a 20× magnification. Previously the digital images were randomized and then analyzed for alveolar bone loss with the Image J Software. The measurements were made on the lingual surface by a blinded investigator. In this procedure, the area (mm^2) corresponding to the exposed root of the molars was calculated. All acquired images were compared to an already known

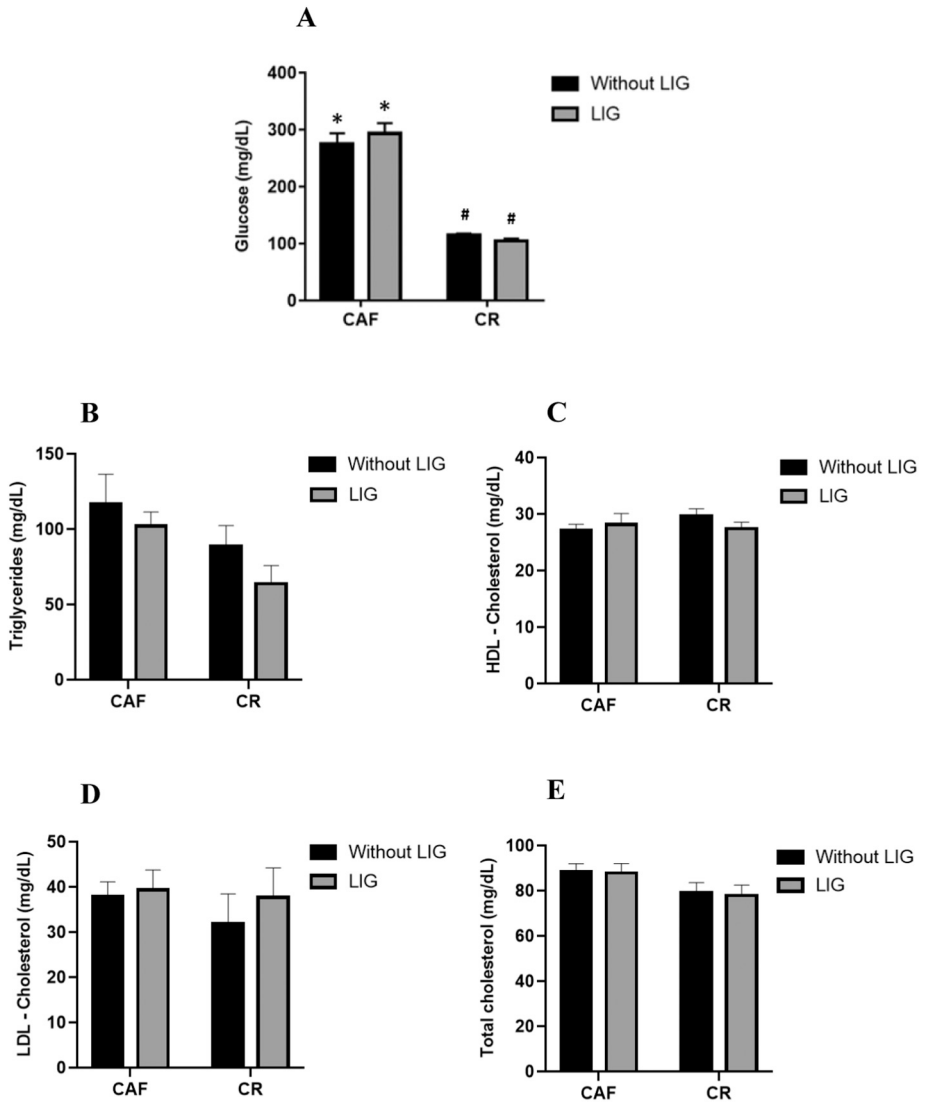

Fig. 3. Blood concentration of (A) Glucose (B) Triglycerides (C) HDL-cholesterol (D) LDL-cholesterol (E) Total cholesterol. Different signs means a significant difference ($p < 0.05$). Two-way ANOVA followed by Tukey's post-test. Data expresses mean ± SEM.

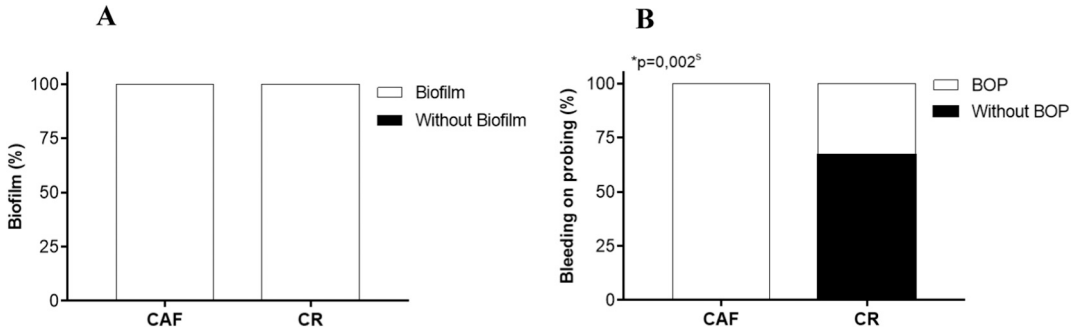

Fig. 4. Comparison of clinical periodontal measures between groups that received ligature-induced experimental periodontitis, expressed as a percentage. Chi-square test. (A) Biofilm (B) Bleeding on probing ($p = 0.002$).

area ($1.0 \times 1.0mm^2$) [23].

2.7. Bone biomakers

The measurement of biomakers bone tissue, calcium and alkaline phosphatase, was performed using commercial kits, following the manufacturer's instructions, and analyzed using the CT300i Wiener equipment.

2.8. Statistical analysis

Data regarding the biofilm and bleeding on probing, clinical periodontal measures, were analyzed using the Chi-square test and the mobility and gingival index data were analyzed using the Mann-Whitney test, evaluated by the Statistical Package for the Social Sciences - SPSS Program, version for Windows.The remaining data were analyzed using analysis of variance (two-way ANOVA) with Tukey's post-test, used for statistical comparisons (GraphPad Prisma software version 8.2.1, San Diego, CA, USA). The data were expressed as mean ± SEM. The level of significance used was 5% ($p < 0.05$).

3. Results

3.1. Biometric parameters

The effectiveness of caloric restriction was evidenced by the significant reduction in body weight of the CR groups compared to the obese animals ($p < 0.05$). The rats submitted to CR had a 42% decrease in body weight compared to the animals that received a cafeteria diet. The values of Lee index and abdominal circumference were also significantly reduced compared to groups with a high calorie diet ($p < 0.05$). Caloric restriction reduced the adiposity index 77% in CR animals compared to obese groups. (Fig. 2; Table 1).

3.2. Biochemical parameters

Caloric restriction reduced glucose concentrations, with a significant decrease compared to obese rats. The other biochemical parameters of triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol showed similar values, with no significant differences between groups ($p > 0.05$) (Fig. 3).

3.3. Clinical periodontal measures

Bleeding on probing was shown to be significantly increased in groups without caloric restriction, in which 100% of the rats were detected with bleeding, while approximately 70% of the animals with CR showed no BOP. However, for the biofilm parameter, no significant difference was found, and all animals in both groups were detected with the presence of biofilm. Beneficially, CR had a significant effect on the parameters of gingival index ($p < 0.001$) and mobility ($p = 0.001$), when comparing groups with and without induction of CR (Fig. 4 and Table 2).

Table 2
Distribution of frequency of the periodontal clinical parameters based in scores.

Parameter	Gingival index* ($p < 0.001$)				Mobility* ($p = 0.001$)			
Score	0	1	2	3	0	1	2	3
CAF-LIG	0	2	8	2	0	5	4	3
CR-LIG	1	10	1	0	7	5	0	0

* Significant diferences between the groups. Mann-Whitney Test. CAF-LIG: cafeteria diet experimental group without caloric restriction and with ligature-induced. CR-LIG: cafeteria diet experimental group with 30% caloric restriction and with ligature-induced.

3.4. Macroscopic morphometric – alveolar bone loss

The effect of CR was significant in reducing the values of alveolar bone loss, when comparing the groups that received ligature to induce experimental periodontitis ($p < 0.01$). Rats that were not induced to experimental periodontitis had no significant changes in alveolar bone loss after induction of CR ($p > 0.05$). Both in the analysis of the isolated factors ($p < 0.001$) and in the interaction between the factors ($p < 0.008$), the effect of the three conditions evaluated was significant (Fig. 5; Fig. 6).

3.5. Bone biomakers

The blood collected showed no significant changes in calcium and alcaline phosphatase levels when comparing the experimental groups (Fig. 7).

4. Discussion

Caloric restriction is worldwide used as a therapy for obesity and it is established in the literature that the reduction of caloric intake in the percentage between 20 and 40%, without malnutrition, results in several benefits, widely researched, such as the reduction of body weight and improvement in the health condition [24–29]. However, no study has tested the role of caloric restriction on alveolar bone loss in experimental periodontitis. In this study, Wistar rats were submitted to a cafeteria diet and subsequently to a caloric restriction of 30%, to analyze alveolar bone loss in the context of experimental periodontitis. Our results reveal a significant reduction in alveolar bone loss after caloric restriction for obesity therapy in rats with experimental periodontitis. These data demonstrate a pioneering and important finding in the impact and influence of caloric restriction on bone tissue, under the condition of ligature-induced periodontitis.

Periodontitis is an inflammatory condition that affects the protective and supporting tissues of the tooth, including the alveolar bone and that,

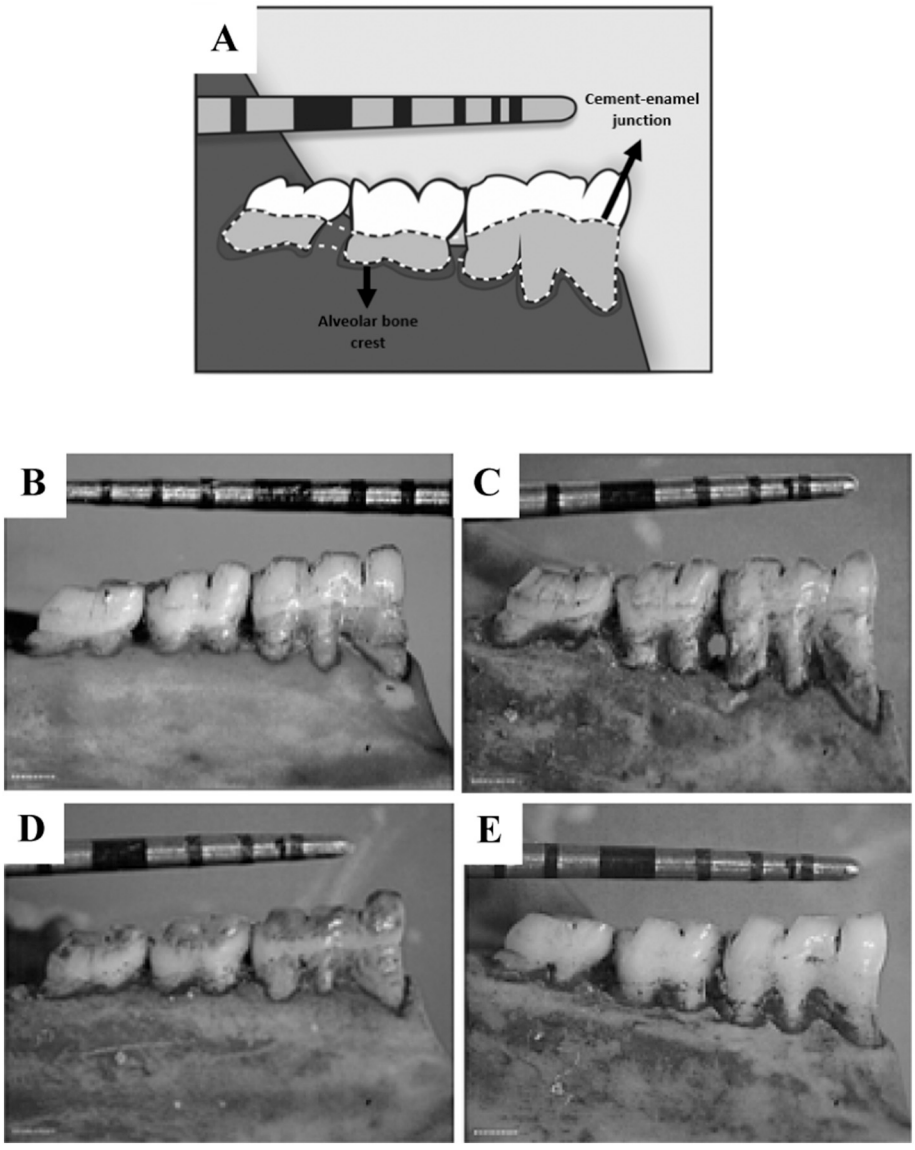

Fig. 5. Diagram of macroscopic morphometric analisys and images of alveolar bone loss representing the experimental groups. (A) Parameters used for measurement of the total area of alveolar bone loss. (B) CAF, cafeteria diet. (C) CAF-LIG, without caloric restriction and with experimental periodontitis. (D) CR, with CR induction and without ligature. (E) CR-LIG, with CR and with experimental periodontitis ligature-induced.

if untreated, may lead to tooth loss [30]. The method of ligature-induced periodontitis is currently well understood and widely used [16,22,31]. The fact that the presence of experimental periodontitis has been significantly influenced by caloric restriction, by decreasing the values of alveolar bone loss, interestingly reinforces the key role of caloric restriction on the body's inflammatory and immune response. The literature shows that obese patients, submitted to caloric restriction, showed a significant decrease in gingival fluid biomarkers, such as interleukin IL-1β and matrix metalloproteinases [7]. The cafeteria diet method for obesity induction is considered an experimental diet model that highly reflects the prevalent consumption of unhealthy foods in actual society and is a method proposed and established in the literature, considered efficient to result in obese rats [32–35]. Obesity has been associated with a low degree of chronic inflammation, which leads to the release of inflammatory mediators called adipokines [36–38]. Consequently, the increase in inflammation is accompanied by oxidative imbalance, with a greater possibility of damage to cellular structures, genetic material, lipids and proteins [33,39–41]. This imbalance in cellular processes and increased synthesis of pro-inflammatory cytokines trigger the overexpression of molecules related to the NF-κB signaling pathway, such as

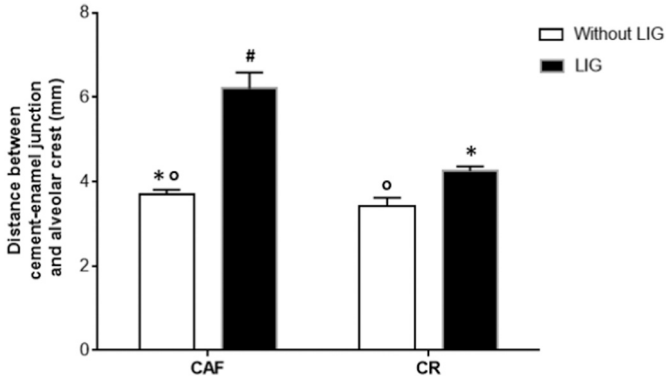

Fig. 6. Mean (EPM) of alveolar bone loss values (mm) of rats submitted to caloric restriction (CR) with and without experimental periodontitis ligature-induced (LIG) in the mandibular first molar. Caloric restriction factor, $p < 0.0001$ (significant difference); Ligature factor, $p < 0.0001$ (significant difference); Interaction of factors (caloric restriction with ligature), $p = 0.0008$ (significant difference). Different signs means significant differences ($p < 0.01$) between groups (two-way ANOVA with Tukey's post-test)

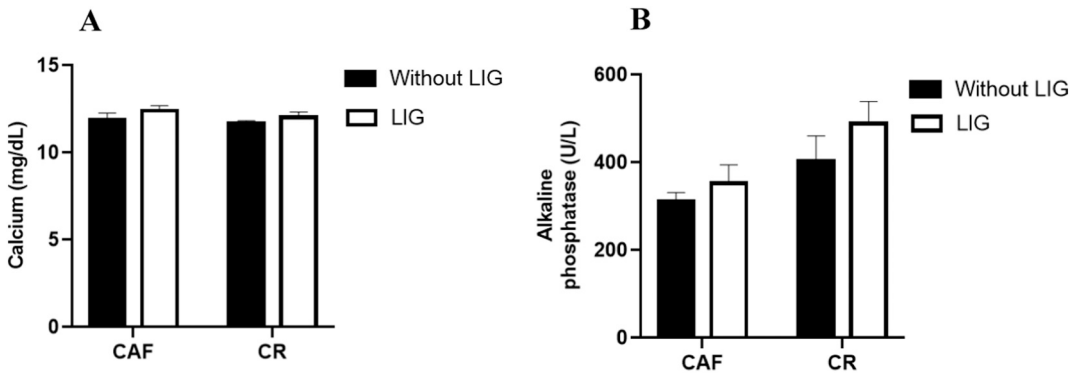

Fig. 7. Effect of caloric restriction on bone tissue biomarkers (A) Calcium (B) Alkaline phosphatase. Two-way ANOVA, followed by Tukey's post-test. Data represents mean ± SEM. No significant difference between the experimental groups.

the nucleus kappa beta activator receptor ligand (RANKL), by osteoblasts that act in differentiation and activation of the osteoclasts, resulting in alveolar bone resorption. The RANK/RANKL/OPG system is decisive in alveolar bone resorption [42]. In periodontitis, the imbalance and accumulation of RANKL, with less OPG production and secretion, makes the disease progression mainly resulting in alveolar bone loss [43].

In contrast, caloric restriction alters intracellular signaling pathways, such as NF-κB and thereby decreases the release of pro-inflammatory cytokines (RANKL), triggering the overexpression of regulatory molecules, such as SIRT-1 (sirtuin-1) [44–46]. SIRT-1 belongs to enzymes called deacetylases, of the sirtuin family and among the main beneficial functions: modulation of genetic stability, regulation of cell survival, endocrine signaling and gene transcription [45,47]. Sirtuins are involved in the processes of caloric restriction, more widely described and associated with health benefits [48–50]. With the regulation of the oxidative and inflammatory mechanism in cells, are greater the chances of preventing and decreasing inflammatory diseases, such as obesity and periodontitis [37]. In periodontitis, this mechanism suggests a reduction of cellular damage, improvement in the oxidative and inflammatory profile, and consequent decrease in the release of inflammatory mediators, characteristic of periodontitis [51]. In this sense, through the lower activation of bone resorption mechanism, it is suggested a decrease in the severity of periodontitis, wich explain the result found in our study by the significantly reduced values of alveolar bone loss after caloric restriction.

Regarding periodontal clinical parameters, mobility, bleeding and gingival index showed significant differences between obese groups and those who received caloric restriction. The findings of attenuated periodontal clinical parameters after caloric restriction suggest that the mechanism associated with the process of alveolar bone loss may be associated with the clinical manifestations of periodontitis. In addition, all animals tested presented biofilm, which indicates that the ligature-induced periodontitis method resulted in the expected microbiological accumulation. There are still no studies in the literature that evaluated periodontal clinical parameters in obese animals, submitted to both caloric restriction and experimental periodontitis. Therefore, our data suggest an important scientific basis for future research on this topic.

The caloric restriction markedly reduced the values of body weight and other biometric parameters, such as adiposity index, abdominal circumference, Lee index and body weight gain, in comparison with the groups that were not submitted to caloric restriction. Our data converge with the literature, in which both obese patients and animals showed a reduction in body weight after caloric restriction [7,47]. Interestingly, patients from a randomized clinical study also showed a decrease in body weight and glucose level, after caloric restriction in obesity. Converging with our findings, the clinical study also found no significant difference in the parameters of triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol [26]. Additionally, the literature is established regarding the reduction of body weight and blood glucose levels, after caloric restriction [52]. The potential of CR improves periodontal condition as well as systemic condition, such as hyperglicemia and obesity, can be explained by the fact that obesity and periodontitis might share the same pathophysiologic mechanism, suggesting a risk factor for periodontitis. Besides that, obesity has been directly related with diabetes because affects the glycemic control and diet control in CR could regulate cytokine responses in diet-induced obese, suggesting an improvement also in hyperglicemia [7]. CR regulates several biological processes *via* deacetylation of the main metabolic molecules, including NF-κB. The deacetylation of these molecules results in an increase in the concentration of adiponectin, decreasing inflammation and increased sensitivity to insulin [53]. In contrast to our finding, Ding et al. (2017) observed a significant difference in the parameters of triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol after caloric restriction [54].

Other data from our study, such as calcium and alkaline phosphatase levels, did not show significant difference after induction of caloric restriction. This fact suggests that the 30% caloric restriction did not cause malnutrition. Alkaline phosphatase has been postulated as a prognostic predictor and adjunct in routine methods to determine periodontitis activity. Alkaline phosphatase is a calcium and phosphate binding glycoprotein, present in various organs and tissues, mainly in bone tissue. It is produced by several cells, such as polymorphonuclear leukocytes, macrophages, osteoblasts, present in the periodontium [55,56]. Therefore, it is an important indicator of osteoblastic activity and therefore, it was also the target of investigation in our study.

5. Conclusion

The caloric restriction effectively reduced the alveolar bone loss and attenuated the clinical periodontal measures in the CR-LIG group, after cafeteria diet. Moreover, this therapy also improved the biometric parameters and glycemic profile. Considering our findings, we demonstrate the important role of caloric restriction in inflammatory conditions, mainly the periodontitis and potential regulation of alveolar bone metabolism.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgment

The authors greatly acknowledge the Coordination of Improvement of Higher Education Personnel – CAPES – Brasil by the scholarship during a period of the research.

References

[1] M. Martinez-Herrera, J. Silvestre-Rangil, F.J. Silvestre, Association between obesity and periodontal disease. A systematic review of epidemiological studies and controlled clinical trials, Med. Oral Patol. Oral Cir. Bucal. 22 (2017) e708–e715, https://doi.org/10.4317/medoral.21786.

[2] A. Solini, J. Suvan, E. Santini, S. Gennai, M. Seghieri, S. Masi, M. Petrini, F. D'Aiuto, F. Graziani, Periodontitis affects glucoregulatory hormones in severely obese individuals, Int. J. Obes. 43 (2019) 1125–1129, https://doi.org/10.1038/s41366-018-0253-4.

[3] C.M. do Nascimento, T. Cassol, F.S. da Silva, M.L. Bonfleur, C.A. Nassar, P. O. Nassar, Radiographic evaluation of the effect of obesity on alveolar bone in rats with ligature-induced periodontal disease, Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 6 (2013) 365–370, https://doi.org/10.2147/DMSO.S50105.

[4] G.L. Branch-Mays, D.R. Dawson, J.C. Gunsolley, M.A. Reynolds, J.L. Ebersole, K. F. Novak, J.A. Mattison, D.K. Ingram, M.J. Novak, The effects of a calorie-reduced diet on periodontal inflammation and disease in a non-human primate model, J. Periodontol. 79 (2008) 1184–1191, https://doi.org/10.1902/jop.2008.070629.

[5] A. Picca, V. Pesce, A.M.S. Lezza, Does eating less make you live longer and better? An update on calorie restriction, Clin. Interv. Aging 12 (2017) 1887–1902, https://doi.org/10.2147/CIA.S126458.

[6] M.A. Reynolds, D.R. Dawson, K.F. Novak, J.L. Ebersole, J.C. Gunsolley, G. L. Branch-Mays, S.C. Holt, J.A. Mattison, D.K. Ingram, M.J. Novak, Effects of caloric restriction on inflammatory periodontal disease, Nutrition 25 (2009) 88–97, https://doi.org/10.1016/j.nut.2008.07.003.

[7] H.S. Park, H.S. Nam, H.S. Seo, S.J. Hwang, Change of periodontal inflammatory indicators through a 4-week weight control intervention including caloric restriction and exercise training in young Koreans: a pilot study, BMC Oral Health 15 (2015), https://doi.org/10.1186/s12903-015-0094-7.

[8] R.A.G. Khammissa, R. Ballyram, Y. Jadwat, J. Fourie, J. Lemmer, L. Feller, Vitamin D deficiency as it relates to oral immunity and chronic periodontitis, International Journal of Dentistry 2018 (2018), https://doi.org/10.1155/2018/7315797.

[9] A.A. de Araújo, H.B. de Morais, C.A.C.X. de Medeiros, G.A. de Castro Brito, P.M. M. Guedes, S. Hiyari, F.Q. Pirih, R.F. de Araújo Júnior, Gliclazide reduced oxidative stress, inflammation, and bone loss in an experimental periodontal disease model, J. Appl. Oral Sci. 27 (2019), https://doi.org/10.1590/1678-7757-2018-0211.

[10] C. Kilkenny, W.J. Browne, I.C. Cuthill, M. Emerson, D.G. Altman, Improving bioscience research reporting: the arrive guidelines for reporting animal research, PLoS Biol. 8 (2010), https://doi.org/10.1371/journal.pbio.1000412.

[11] National Research Council (U.S.). Committee for the Update of the Guide for the Care and Use of Laboratory Animals., Institute for Laboratory Animal Research (U. S.), Guide for the Care and Use of Laboratory Animals, National Academies Press, 2011.

[12] C. de Moraes, A.P.C. Davel, L.V. Rossoni, E. Antunes, A. Zanesco, Exercise training improves relaxation response and SOD-1 expression in aortic and mesenteric rings from high caloric diet-fed rats, BMC Physiol. 8 (2008), https://doi.org/10.1186/1472-6793-8-12.

[13] A.F. Nascimento, M.M. Sugizaki, A.S. Leopoldo, A.P. Lima-Leopoldo, R.A. M. Luvizotto, C.R. Nogueira, A.C. Cicogna, A hypercaloric pellet-diet cycle induces obesity and co-morbidities in Wistar rats, Arquivos Brasileiros de Endocrinologia e Metabologia 52 (2008).

[14] D. Didek, M.M. Cordeiro, J.L. de P. Xavier, P.R. Ribeiro, T. Rentz, G.C.N. Franco, C. C. Kanunfre, H.R. de O. Emilio, D.X. Scomparin, Association between exercise and treatment with liraglutide in obese rats by cafeteria diet, Braz. Arch. Biol. Technol. 62 (2019), https://doi.org/10.1590/1678-4324-2019180563.

[15] S. Ding, J. Jiang, G. Zhang, Y. Bu, G. Zhang, X. Zhao, Resveratrol and caloric restriction prevent hepatic steatosis by regulating SIRT1-autophagy pathway and alleviating endoplasmic reticulum stress in high-fat diet-fed rats, PLoS One 12 (2017), https://doi.org/10.1371/journal.pone.0183541.

[16] P.K. Vargas-Sanchez, M.G. Moro, F.A. dos Santos, A.L. Anbinder, E. Kreich, R. M. Moraes, L. Padilha, C. Kusiak, D.X. Scomparin, G.C.N. Franco, Agreement, correlation, and kinetics of the alveolar bone-loss measurement methodologies in a ligature-induced periodontitis animal model, J. Appl. Oral Sci. 25 (2017) 490–497, https://doi.org/10.1590/1678-7757-2016-0517.

[17] P.H.F. Silva, Luiz, F.F. Oliveira, R.S. Cardoso, Milla, S.T. Ricoldi, Luciene, C. Figueiredo, S.L. Salvador, D.B. Palioto, Flávia, A.C. Furlaneto, M.R. Messora, A. Café, R. Preto, The Impact of Predatory Bacteria on Experimental Periodontitis, 2019, https://doi.org/10.1902/jper.10318.

[18] A.S. Maeda Júnior, J. Constantin, K.S. Utsunomiya, E.H. Gilglioni, F.R.S. Gasparin, F.O. Carreño, S.M.F. de Moraes, M. Rocha, M.R.M. Natali, C.V. de C. Ghizoni, A. Bracht, E.L. Ishii-Iwamoto, R.P. Constantin, Cafeteria diet feeding in young rats leads to hepatic steatosis and increased gluconeogenesis under fatty acids and glucagon influence, Nutrients 10 (2018), https://doi.org/10.3390/nu10111571.

[19] C.J. Teixeira, L.M. Ribeiro, K. Veras, L.C. da Cunha Araujo, R. Curi, C.R. de Oliveira Carvalho, Dehydroepiandrosterone supplementation is not beneficial in the late postmenopausal period in diet-induced obese rats, Life Sci. 202 (2018) 110–116, https://doi.org/10.1016/j.lfs.2018.03.052.

[20] C.O. Rodini, A.C. Batista, T.J. Dionísio, C.F. Santos, F.Q. Cunha, V.S. Lara, Morphologic evaluation and expression of matrix metalloproteinases-2 and 9 and nitric oxide during experimental periodontal disease in rat, J. Mol. Histol. 39 (2008) 275–282, https://doi.org/10.1007/s10735-008-9163-4.

[21] R.S.B. de Andrade, L.F. de C. França, L.D.S. Pessoa, B. de A.A. Landim, A. A. Rodrigues, E.H.P. Alves, D. di Lenardo, H.M.S. Nascimento, F.B. de M. Sousa, A. L.D.R. Barbosa, J.V.R. Medeiros, A.C.C.G. Vasconcelos, D.F.P. Vasconcelos, High-fat diet aggravates the liver disease caused by periodontitis in rats, J. Periodontol. 90 (2019) 1023–1031, https://doi.org/10.1002/JPER.18-0564.

[22] Y. Xu, W. Wei, A comparative study of systemic subantimicrobial and topical treatment of minocycline in experimental periodontitis of rats, Arch. Oral Biol. 51 (2006) 794–803, https://doi.org/10.1016/j.archoralbio.2006.03.018.

[23] C.S. Martins, R.F.C. Leitão, D.V.S. Costa, I.M. Melo, G.S. Santos, V. Lima, V. Baldim, D.V.T. Wong, L.E. Bonfim, C.B. Melo, M. G. de Oliveira, G.A.C. Brito, Topical HPMC/S-nitrosoglutathione solution decreases inflammation and bone resorption in experimental periodontal disease in rats, PLoS One 11 (2016), https://doi.org/10.1371/journal.pone.0153716.

[24] M.D. Jensen, D.H. Ryan, C.M. Apovian, J.D. Ard, A.G. Comuzzie, K.A. Donato, F. B. Hu, V.S. Hubbard, J.M. Jakicic, R.F. Kushner, C.M. Loria, B.E. Millen, C. A. Nonas, F.X. Pi-Sunyer, J. Stevens, V.J. Stevens, T.A. Wadden, B.M. Wolfe, S. Z. Yanovski, 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association task force on practice guidelines and the obesity society, Circulation. 129 (2014), https://doi.org/10.1161/01.cir.0000437739.71477.ee.

[25] M.F. Dutra, I.J. Bristot, C. Batassini, N.B. Cunha, A.F.K. Vizuete, D.F. de Souza, J.C. F. Moreira, C.A. Gonçalves, Effects of chronic caloric restriction on kidney and heart redox status and antioxidant enzyme activities in Wistar rats, BMB Rep. 45 (2012) 671–676, https://doi.org/10.5483/BMBRep.2012.45.11.094.

[26] P. Ruggenenti, M. Abbate, B. Ruggiero, S. Rota, M. Trillini, C. Aparicio, A. Parvanova, I.P. Iliev, G. Pisanu, A. Perna, A. Russo, O. Diadei, D. Martinetti, A. Cannata, F. Carrara, S. Ferrari, N. Stucchi, G. Remuzzi, L. Fontana, Renal and systemic effects of calorie restriction in patients with type 2 diabetes with abdominal obesity: a randomized controlled trial, Diabetes 66 (2017) 75–86, https://doi.org/10.2337/db16-0607.

[27] B. Ott, T. Skurk, L. Hastreiter, I. Lagkouvardos, S. Fischer, J. Büttner, T. Kellerer, T. Clavel, M. Rychlik, D. Haller, H. Hauner, Effect of caloric restriction on gut permeability, inflammation markers, and fecal microbiota in obese women, Sci. Rep. 7 (2017), https://doi.org/10.1038/s41598-017-12109-9.

[28] M.E. Francois, N.M. Gilbertson, N.Z.M. Eichner, E.M. Heiston, C. Fabris, M. Breton, J.H. Mehaffey, T. Hassinger, P.T. Hallowell, S.K. Malin, Combining short-term interval training with caloric restriction improves β-cell function in obese adults, Nutrients 10 (2018), https://doi.org/10.3390/nu10060717.

[29] S. Wang, M. Huang, X. You, J. Zhao, L. Chen, L. Wang, Y. Luo, Y. Chen, Gut microbiota mediates the anti-obesity effect of calorie restriction in mice, Sci. Rep. 8 (2018), https://doi.org/10.1038/s41598-018-31353-1.

[30] K.E. Izuora, E.E. Ezeanolue, M.F. Neubauer, C.L. Gewelber, G.L. Allenback, G. Shan, G.E. Umpierrez, Changes in inflammatory and bone turnover markers after periodontal disease treatment in patients with diabetes, Am J Med Sci 351 (2016) 589–594, https://doi.org/10.1016/j.amjms.2016.02.004.

[31] H. Toker, H.B. Yuce, A. Yildirim, M.B. Tekin, F. Gevrek, The effect of colchicine on alveolar bone loss in ligature-induced periodontitis, Brazilian Oral Research 33 (2019), https://doi.org/10.1590/1807-3107bor-2019.vol33.0001.

[32] B.P. Sampey, A.M. Vanhoose, H.M. Winfield, A.J. Freemerman, M.J. Muehlbauer, P.T. Fueger, C.B. Newgard, L. Makowski, Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet, Obesity 19 (2011) 1109–1117, https://doi.org/10.1038/oby.2011.18.

[33] L. Oliva, T. Aranda, G. Caviola, A. Fernández-Bernal, M. Alemany, J.A. Fernández-López, X. Remesar, In rats fed high-energy diets, taste, rather than fat content, is the key factor increasing food intake: a comparison of a cafeteria and a lipid-supplemented standard diet, PeerJ. 2017 (2017), https://doi.org/10.7717/peerj.3697.

[34] R.C. Bortolin, A.R. Vargas, J. Gasparotto, P.R. Chaves, C.E. Schnorr, K. B. Martinello, A.K. Silveira, T.K. Rabelo, D.P. Gelain, J.C.F. Moreira, A new animal diet based on human Western diet is a robust diet-induced obesity model: comparison to high-fat and cafeteria diets in term of metabolic and gut microbiota disruption, Int. J. Obes. 42 (2018) 525–534, https://doi.org/10.1038/ijo.2017.225.

[35] Y. Buyukdere, A. Gulec, A. Akyol, Cafeteria diet increased adiposity in comparison to high fat diet in young male rats, PeerJ. 2019 (2019), https://doi.org/10.7717/peerj.6656.

[36] M.D. DeBoer, Obesity, systemic inflammation, and increased risk for cardiovascular disease and diabetes among adolescents: a need for screening tools to target interventions, Nutrition 29 (2013) 379–386, https://doi.org/10.1016/j.nut.2012.07.003.

[37] D. Luo, L. Liu, F.X. Liang, Z.M. Yu, R. Chen, Electroacupuncture: a feasible sirt1 promoter which modulates metainflammation in diet-induced obesity rats, Evid. Based Complement. Alternat. Med. 2018 (2018), https://doi.org/10.1155/2018/5302049.

[38] M.M. Perez, J.S. Pessoa, A.L. Ciamponi, M.B. Diniz, M.T.B.R. Santos, H.H.D. O. Alves, R. Gorjão, R.O. Guaré, Correlation of salivary immunoglobulin A with body mass index and fat percentage in overweight/obese children, J. Appl. Oral Sci. 27 (2019), https://doi.org/10.1590/1678-7757-2018-0088.

[39] P. Galassetti, Inflammation and oxidative stress in obesity, metabolic syndrome, and diabetes, Exp. Diabetes Res. 2012 (2012), https://doi.org/10.1155/2012/943706.

[40] B. Ponugoti, G. Dong, D.T. Graves, Role of forkhead transcription factors in diabetes-induced oxidative stress, Exp. Diabetes Res. 2012 (2012), https://doi.org/10.1155/2012/939751.

[41] B.S. Karam, A. Chavez-Moreno, W. Koh, J.G. Akar, F.G. Akar, Oxidative stress and inflammation as central mediators of atrial fibrillation in obesity and diabetes, Cardiovasc. Diabetol. 16 (2017), https://doi.org/10.1186/s12933-017-0604-9.

[42] S.A. Hienz, S. Paliwal, S. Ivanovski, Mechanisms of bone resorption in periodontitis, J Immunol Res 2015 (2015), https://doi.org/10.1155/2015/615486.
[43] E. Papathanasiou, A. Kantarci, A. Konstantinidis, H. Gao, T.E. van Dyke, SOCS-3 regulates alveolar bone loss in experimental periodontitis, J. Dent. Res. 95 (2016) 1018–1025, https://doi.org/10.1177/0022034516645332.
[44] S. Rahman, R. Islam, Mammalian Sirt1: insights on its biological functions, Cell Communication and Signaling 9 (2011), https://doi.org/10.1186/1478-811X-9-11.
[45] L. Guarente, Calorie restriction and sirtuins revisited, Genes Dev. 27 (2013) 2072–2085, https://doi.org/10.1101/gad.227439.113.
[46] N. Makino, J. Oyama, T. Maeda, M. Koyanagi, Y. Higuchi, I. Shimokawa, N. Mori, T. Furuyama, FoxO1 signaling plays a pivotal role in the cardiac telomere biology responses to calorie restriction, Mol. Cell. Biochem. 412 (2016) 119–130, https://doi.org/10.1007/s11010-015-2615-8.
[47] M. Waldman, K. Cohen, D. Yadin, V. Nudelman, D. Gorfil, M. Laniado-Schwartzman, R. Kornwoski, D. Aravot, N.G. Abraham, M. Arad, E. Hochhauser, Regulation of diabetic cardiomyopathy by caloric restriction is mediated by intracellular signaling pathways involving "SIRT1 and PGC-1α", Cardiovasc. Diabetol. 17 (2018) https://doi.org/10.1186/s12933-018-0754-4.
[48] X. Qiu, K. Brown, M.D. Hirschey, E. Verdin, D. Chen, Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation, Cell Metab. 12 (2010) 662–667, https://doi.org/10.1016/j.cmet.2010.11.015.
[49] A. Ansari, M.S. Rahman, S.K. Saha, F.K. Saikot, A. Deep, K.H. Kim, Function of the SIRT3 mitochondrial deacetylase in cellular physiology, cancer, and neurodegenerative disease, Aging Cell 16 (2017) 4–16, https://doi.org/10.1111/acel.12538.
[50] W. Yu, J. Qin, C. Chen, Y. Fu, W. Wang, Moderate calorie restriction attenuates age-associated alterations and improves cardiac function by increasing SIRT1 and SIRT3 expression, Mol. Med. Rep. 18 (2018) 4087–4094, https://doi.org/10.3892/mmr.2018.9390.
[51] S. Hiyari, R.L. Wong, A. Yaghsezian, A. Naghibi, S. Tetradis, P.M. Camargo, F. Q. Pirih, Ligature-induced peri-implantitis and periodontitis in mice, J. Clin. Periodontol. 45 (2018) 89–99, https://doi.org/10.1111/jcpe.12817.
[52] Y.-B. Yang, X.-L. Wu, B. Ke, Y.-J. Huang, S.-Q. Chen, Y.-Q. Su, J. Qin, Effects of caloric restriction on peroxisome proliferator-activated receptors and positive transcription elongation factor b expression in obese rats, Eur. Rev. Med. Pharmacol. Sci. 21 (2017) 4369–4378.
[53] M.G. Heo, S.Y. Choung, Anti-obesity effects of Spirulina maxima in high fat diet induced obese rats via the activation of AMPK pathway and SIRT1, Food Funct. 9 (2018) 4906–4915, https://doi.org/10.1039/c8fo00986d.
[54] S. Ding, J. Jiang, G. Zhang, Y. Bu, G. Zhang, X. Zhao, Resveratrol and caloric restriction prevent hepatic steatosis by regulating SIRT1-autophagy pathway and alleviating endoplasmic reticulum stress in high-fat diet-fed rats, PLoS One 12 (2017), https://doi.org/10.1371/journal.pone.0183541.
[55] A. Cutando, A. López-Valverde, R. Gómez-de-Diego, S. Arias-Santiago, J. de Vicente-Jiménez, Effect of gingival application of melatonin on alkaline and acid phosphatase, osteopontin and osteocalcin in patients with diabetes and periodontal disease, Med. Oral Patol. Oral Cir. Bucal. 18 (2013), https://doi.org/10.4317/medoral.18832.
[56] S. Sridharan, P. Sravani, A. Satyanarayan, Kiran, V. Shetty, Salivary alkaline phosphatase as a noninvasive marker for periodontal disease in children with uncontrolled type 1 diabetes mellitus 41 (2017) 70–74, https://doi.org/10.17796/1053-4628-41.1.70.