Cross Talk between Apical Periodontitis and Metabolic Disorders: Experimental Evidence on the Role of Intestinal Adipokines and *Akkermansia muciniphila*

Cauana O. Tavares, PhD,[*†] *Fernanda L. Rost, DDS,*[†] *Rodrigo B.M. Silva, PhD,*[†‡] *Ana Paula Dagnino, MSc,*[†§] *Bruno Adami, MSc,*[§] *Helena Schirmer, PhD,*[‖] *José Antônio P. de Figueiredo, PhD,*[*] *André Arigony Souto, PhD,*[§] *Fábio Dal Moro Maito, PhD,*[¶] *and Maria M. Campos, PhD*[*†‡§¶]

Abstract

Introduction: Infection and dysbiosis present a close relationship with metabolic diseases although the influence of apical periodontitis (AP) in this context needs further investigation. This study evaluated the influence of AP in a rat model of metabolic syndrome induced by 10% fructose supplementation. **Methods:** Male Wistar rats were used. Animals that received a high-fructose diet (HFD, n = 30) or filtered water (control, n = 30) were subdivided into the following groups: (1) without induction of AP (no AP, n = 10 each), (2) with AP induction 2 weeks before euthanasia (AP 14 days, n = 10 each), and (3) with AP induction 4 weeks before euthanasia (AP 28 days, n = 10 each). **Results:** HFD triggered metabolic syndrome, as indicated by the induction of overweight and hyperglycemia, besides polydipsia, regardless of the AP induction. Serum or intestinal tumor necrosis factor, interleukin 1 beta, and interleukin 6 levels were undetectable, regardless of the experimental group. Serum leptin and adiponectin levels were significantly elevated in the HFD group without AP induction. The intestinal levels of leptin were significantly increased in the groups with 28 days of AP induction despite HFD. A significant elevation of liver glutathione levels was observed in animals submitted to HFD and AP for 14 days. AP induction (14 or 28 days) led to pulp and periapical tissue inflammation without any influence of HFD. Either HFD or AP induction led to dysbiosis, as indicated by a significant reduction of fecal *A. muciniphila* expression. **Conclusions:** We provide novel evidence that AP can have systemic impacts on metabolic disorders, likely by modulating intestinal metabolism and microbiota. *(J Endod 2019;45:174–180)*

Key Words

Adipokines, *Akkermansia muciniphila*, apical periodontitis, fructose, gut, metabolic syndrome

Significance

We provide novel evidence that AP can have systemic impacts on metabolic disorders, likely by modulating intestinal adipokines and microbiota.

Obesity is an alarming disease with epidemic proportions; the estimates indicate that more than 600 million individuals are obese, and 2 billion are overweight worldwide (1). Obesity and overweight are closely related to metabolic diseases, such as diabetes and hypertension, representing a serious public health problem with elevated morbidity and mortality (2). Studies have been gradually showing the strong correlation between obesity and altered gastrointestinal microbiota (3, 4). Fat intake dramatically modifies the gut microbiota, which greatly disturbs the host metabolism, triggering low-grade inflammation (5, 6). The microbiota undergoes dysbiosis, leading to an elevated susceptibility for metabolic diseases (7). Fat intake also enhances the permeability for bacterial toxins (mainly lipopolysaccharides from gram-negative bacteria) through the gut cell barrier into the bloodstream. Toxin invasion elicits endotoxemia, maintaining the inflammatory state and turning into a cyclic condition (4).

Among the diversity of gut microbiome, *Akkermansia muciniphila* has gained attention because of its contribution to host protection. This species presents an inverse correlation with inflammation, diabetes, and altered adipose tissue metabolism (8). Of note, animals treated with *A. muciniphila* showed a reduction of body weight and glycemia (9), and, therefore, these bacteria have been pointed out as candidates for the development of novel food or pharmaceutical supplements (10).

From the *Programa de Pós-Graduação em Odontologia, †Centro de Pesquisa em Toxicologia e Farmacologia, Escola de Ciências da Saúde, PUCRS, Porto Alegre, Rio Grande do Sul, Brazil; ‡Programa de Pós-Graduação em Medicina e Ciências da Saúde, Escola de Medicina, PUCRS, Porto Alegre, Rio Grande do Sul, Brazil; §Programa de Pós-Graduação em Biologia Celular e Molecular, Escola de Ciências, PUCRS, Porto Alegre, Rio Grande de Sol, Brazil; ‖Departamento de Ciências Básicas da Saúde, Universidade Federal de Ciências da Saúde, Porto Alegre, Rio Grande so Sul, Brazil; and ¶Laboratório de Patologia Bucal, Escola de Ciências da Saúde, Pontifícia Universidade Católica do Rio Grande do Sul Porto Alegre, Rio Grande do Sul, Brazil.

Address requests for reprints to Maria M. Campos, Escola de Ciências da Saúde, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, Partenon, 90619-900 Porto Alegre, Brazil. E-mail address: maria.campos@pucrs.br

0099-2399/$ - see front matter

https://doi.org/10.1016/j.joen.2018.10.013

The close relationship among obesity, low-grade inflammation, and microbiota sparked our interest to evaluate the role of apical periodontitis (AP) in the outcome of systemic metabolic diseases. AP is associated with a complex microbiota; because this lesion has no epithelial barrier, oxidative stress products and inflammatory cytokines can easily reach the bloodstream (11). There is a lack of scientific studies on AP and its systemic effects. Thus, the present study investigated the cross talk between systemic inflammatory changes and intestinal dysbiosis, and their correlation with AP outcomes in a rat model of metabolic syndrome induced by a high-fructose diet.

Material and Methods

Animals

Male Wistar rats (8 weeks old, total $N = 60$ animals) weighing 240–280 g at the onset of the experiments were obtained from the Central Animal House of the Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil. The animals were housed under standard conditions of temperature (22° ± 2 °C), light (12-hour light-dark cycle), and humidity (50%–70%) in ventilated cages with autoclaved wood chip bedding. They received a standard rat chow diet (Nuvilab; Nuvital Nutrients, Colombo, PR, Brazil) with free access to filtered water or 10% fructose solution depending on the experimental group. The experimental protocols followed the current Brazilian guidelines for the care and use of animals for scientific and didactic procedures from the National Council for the Control of Animal Experimentation. The local animal ethics committee evaluated and approved all of the protocols (CEUA 14/00428). We followed the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines to report *in vivo* experiments (12). The number of animals and the intensity of noxious stimuli were the minimum necessary to show consistent effects.

Induction of Metabolic Syndrome and Experimental Groups

Metabolic syndrome was induced as described previously (13). The rats received 10% fructose in the drinking water for 8 weeks. The control animals received filtered water during the same period. Rats and chow were weighted (g) 3 times a week. The water was refilled, and the consumed volume (mL) was measured every day; the results were pooled weekly. Glucose levels were measured at the end of the experiments with a digital glucometer (Accu-Check III; Boehringer Mannheim, Mannheim, Germany). Animals submitted to a high-fructose diet (HFD, $n = 30$) or a normal diet (control, $n = 30$) were subdivided into additional experimental groups as follows: without induction of AP (no AP, $n = 10$ each), with AP induction 2 weeks before euthanasia (AP 14 days, $n = 10$ each), and with AP induction 4 weeks before euthanasia (AP 28 days, $n = 10$ each).

AP Rat Model

AP induction was accomplished as previously described (14). Animals were anesthetized by an intraperitoneal injection of xylazine (10 mg/kg) plus ketamine (100 mg/kg). The pulp of the mandibular first left molar was surgically exposed with a ¼-size round steel bur in high-speed rotation under constant irrigation. Pulps were left exposed to the oral environment for 14 or 28 days according to the experimental group, as described previously, to allow the establishment of AP (14).

Assessment of Cytokines and Adipokines

The animals were euthanized 8 weeks after the onset of HFD by deep anesthesia with sevoflurane. Serum and ≈3 cm large intestine were collected to evaluate the levels of tumor necrosis factor (TNF), interleukin (IL) 1 beta, IL-6, leptin, and adiponectin. The samples were stored at −80°C until use. The following cytokines and adipokines were analyzed by sandwich enzyme-linked immunosorbent assay using DuoSet kits according to the manufacturer's instructions (R&D Systems, Minneapolis, MN): rat TNF-α catalog number DY510 (no cross-reactivity with human or porcine TNF-α with 2.4% of cross-reactivity with mouse TNF-α), rat IL-6 catalog number DY506 (no cross-reactivity with human or porcine IL-6 with 0.7% of cross-reactivity with mouse IL-6), rat IL-1β/IL-2F2 catalog number DY501 (no cross-reactivity with human or porcine IL-1β/IL-2F2, with 0.79% of cross-reactivity with mouse IL-1β/IL-2F2), rat total adiponectin/Acrp30 catalog number RRP300 (no significant cross-reactivity was observed), and mouse/rat leptin catalog number MOB00 (no significant cross-reactivity was observed). The results are expressed in pg/mL or pg/mg tissue for serum and intestine, respectively.

Determination of Oxidative Stress

After euthanasia, the livers and hearts were immediately collected for the determination of catalase and reduced glutathione activities, 2 classic indicators of tissue oxidative stress, as described previously (14).

Histopathologic Analysis

To confirm the induction of tooth pulp inflammation, the mandibles were collected and fixed in 10% neutral-buffered formalin solution. The samples were decalcified with 17% EDTA (pH = 7.0). The paraffin blocks containing the maxillae were serially cut (6-μm thickness) in the longitudinal plane. The sections were stained with hematoxylin-eosin and examined under light microscopy. A microscope (Axio Imager A1; Carl Zeiss, Oberkochen, Germany) coupled to an image capture system (Axio Vision Rel. 4.4 Software Multimedia, Carl Zeiss) was used (200× magnification). Periapical events were qualitatively analyzed as previously described (15). Histologic slides (3 fields in each) were evaluated for every tooth, which included the root dentin, the apical foramen, and the periapical tissues. The intensity of the inflammatory infiltrate was classified according to the following scores: 0, absent; 1, discrete; 2, moderate; or 3, severe. An experienced pathologist (F.D.M.M.) in addition to an experienced endodontist with advanced histology skills (J.A.P.F.) evaluated the slides in a blinded manner.

Evaluation of Fecal *A. muciniphila*

The day before the euthanasia, ≈4 g feces was collected from each animal. Cellular DNA was extracted using the Wizard Genomic Purification Kit (Promega, Madison, WI) according to the protocol suggested by the manufacturers. One milliliter of the extracted DNA was used in a total volume of 12.5 mL GoTaq qPCR Master Mix (Promega). The primer used to amplify *A. muciniphila* was F: 5′AGGCGGAGGAAATCCTAAAA -3′ and R: 5′GCGGTTGGCTTCAGATACTT -3′, which correspond to nucleotides 1231–1250 (sense) and 1395–1414 (antisense) (accession number NR_074436.1) in a concentration of 0.64 μmol per reaction. Quantitative polymerase chain reaction amplification was confirmed with the amplicon by melting curve analysis (melting temperature = 88.7). The specific parameters of the primers for bacterial detection were evaluated using the BLAST search program (Basic Local Alignment Search Tool; NCBI, NIH, Bethesda, MD). Negative (0.9% sodium chloride) and positive controls (the oligo sequence design according to the sequence of the primers) were used in each quantitative polymerase chain reaction. To evaluate the concentration of bacteria and the limit of detection, a serial dilution was made with the positive control, with

concentrations varying from 10^4–10^7. All samples were analyzed in duplicate. To confirm that the amplified product was from *A. muciniphila*, we performed specific control sequencing for this bacterium.

Statistical Analysis

The results are expressed as the mean ± the standard error of the mean. Our data were submitted to the Bartlett test for equal variances that indicated a Gaussian distribution for the results. Data were subjected to 2-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test (GraphPad Software Inc, San Diego, CA). *P* values <.05 were considered statistically significant. The variations in the experimental sample size are described in each figure legend.

Results

We used a model of metabolic syndrome induced by the supplementation with 10% fructose (HFD) to evaluate the cross talk between metabolic changes and AP in rats. Regarding the general metabolic alterations, the groups submitted to HFD for 8 weeks displayed an increase of body weight gain, irrespective of the AP induction. The increased body weight gain in HFD groups was significant from 40 days after the onset of experiments when compared with control animals with or without AP (Fig. 1*A*). HFD elicited hyperglycemia in all animals submitted to the HFD, confirming the induction of type 2 diabetes (Fig. 1*B*). This evidence was supported by data showing an increase of water intake in HFD groups, regardless of the AP intervention (Supplemental Fig. S1*A–D* is available online at www.jendodon.com).

Next, we examined the serum and intestinal levels of cytokines and adipokines. The proinflammatory cytokines, namely TNF, IL-1β, and IL-6, were undetectable in either the serum or intestine of any experimental groups (data not shown). However, there was a significant increase in the serum levels of the adipokine leptin in the HFD group, without AP induction (Fig. 2*A*). Moreover, the intestinal levels of leptin were significantly increased in the groups with 28 days of AP induction, regardless of the supplementation with 10% fructose (Fig. 2*B*). The levels of adiponectin were significantly increased in the serum of the HFD group without AP induction (Fig. 2*C*) although no variation in the intestinal levels of this adipokine was noticed in any other groups (Fig. 2*D*). Considering the relationship between metabolic syndrome and oxidative stress, we also assessed the activities of catalase and glutathione in the liver and heart. No significant alteration was noted when comparing the different experimental groups, except a significant elevation of glutathione levels in the liver of animals submitted to HFD and AP for 14 days (Supplemental Fig. S2*A–D* is available online at www.jendodon.com).

Considering the induction of AP, the histologic analysis indicated that control groups (without coronal opening) showed no alterations in the pulp or periapical tissues (Fig. 3*A* and *B*). It is noteworthy that 100% of these teeth presented healthy pulp and periapical tissues without any significant effect of HFD (Fig. 3*G*). Those teeth submitted to coronal opening (14- or 28-day opening) showed necrosis in the coronal region in 100% of the cases (Fig. 3*C* and *E*). Additionally, the groups with 28 days of AP induction showed root pulp necrosis in 100% of the cases (Fig. 3*E* and *F*), apart from the supplementation with 10% fructose. Data analysis revealed a time-related increase of periapical inflammation, with mild periapical inflammation at 14 days and intense periapical inflammation at 28 days (Fig. 3*G*).

The fecal expression of *A. muciniphila* was reduced in all of the experimental groups with HFD, with or without AP induction, in relation to the control animals. This decline was significant in animals that received 10% fructose supplementation and had been submitted to pulp exposure for 14 and 28 days. The groups with pulp exposure for 14 or 28 days without HFD also displayed a significant reduction of *A. muciniphila* expression in feces (Fig. 4).

Discussion

This study investigated the influence of AP in a model of metabolic disease induced by long-term ingestion of fructose in rats. For this purpose, we investigated the influence of AP on inflammation, oxidative

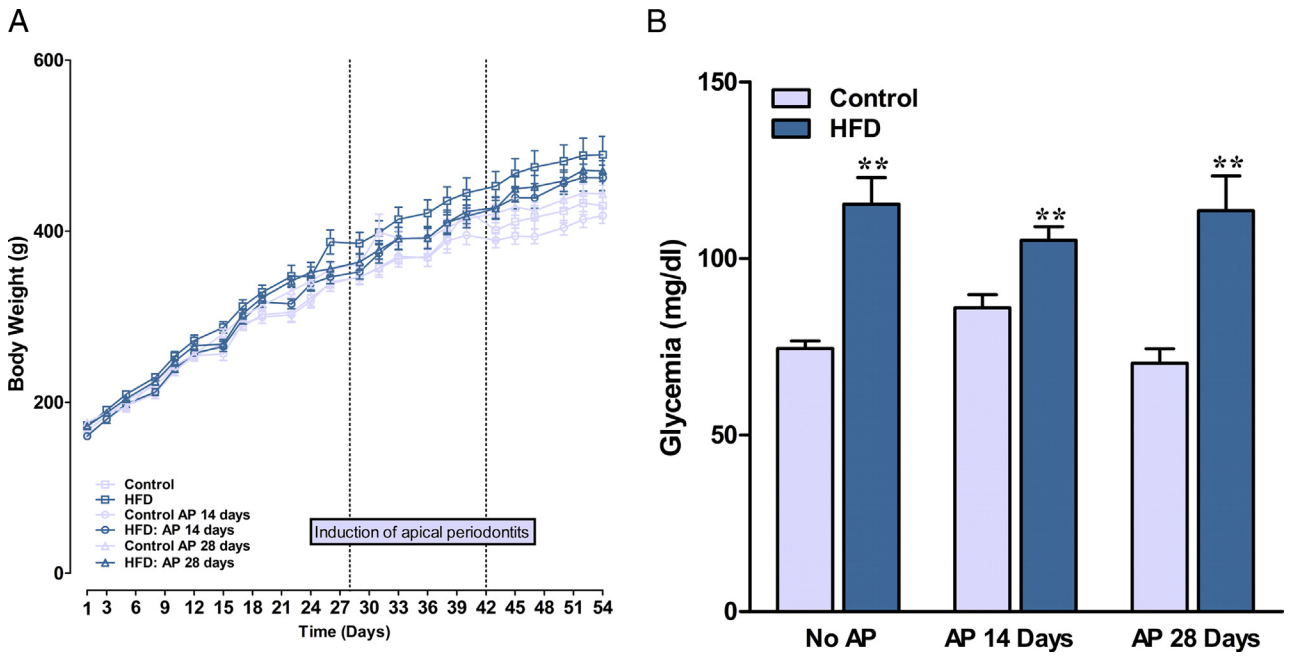

Figure 1. (*A*) The time-related body weight changes in the control or HFD groups. The *dotted lines* represent interventions to induce AP at 28 or 14 days. (*B*) Glycemic levels at the end of experiments. **A significant difference in relation to the control group ($P < .01$). The columns represent the mean of 10 animals, and the vertical lines indicate the standard error of the mean.

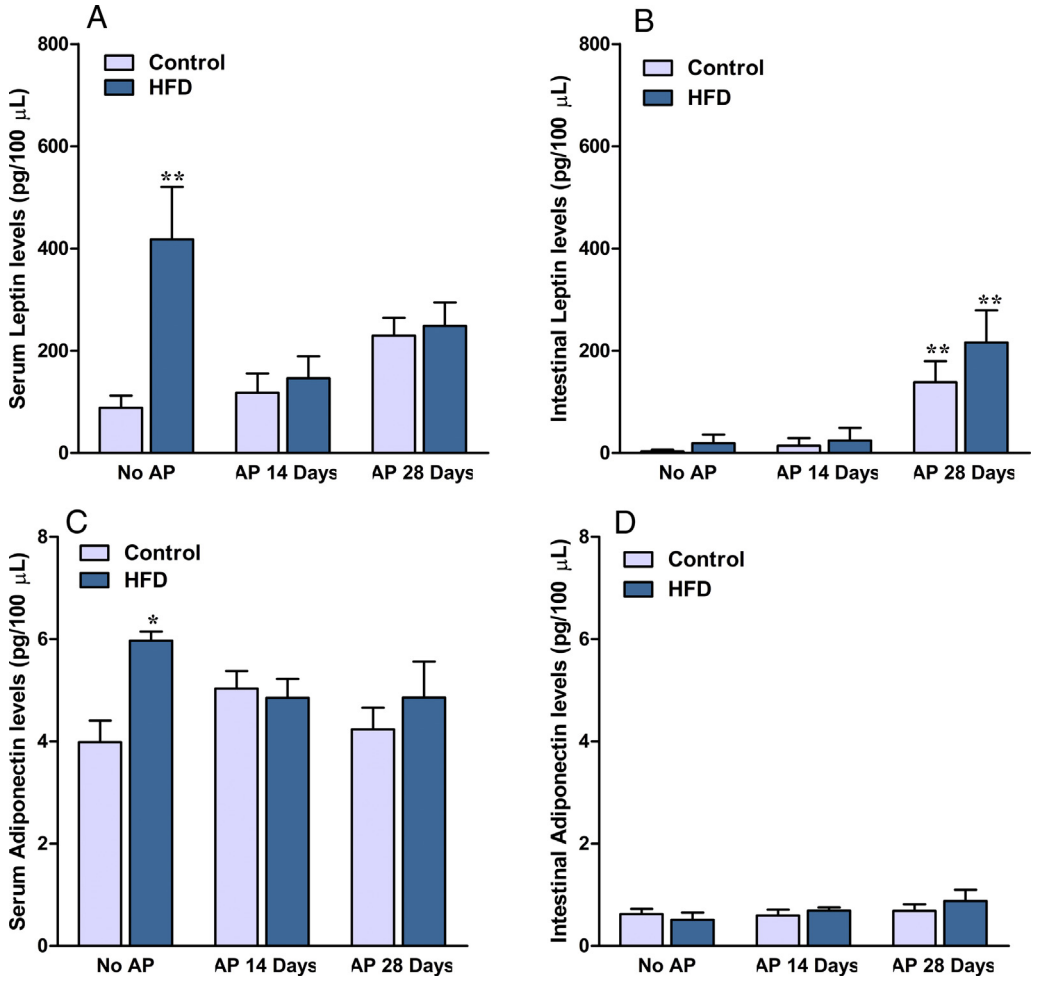

Figure 2. (*A*) The serum leptin levels in the HFD group are significantly increased in relation to the control (**$P < .01$). (*B*) The intestinal leptin levels in control AP 28-day and HFD AP 28-day groups are significantly increased in relation to the control (*$P < .05$) and between them (**$P < .01$). (*C*) Serum adiponectin levels in the HFD group are significantly increased in relation to the control (**$P < .01$). (*D*) The intestinal adiponectin levels showing no statistical differences among the groups. The columns represent the mean of 6–7 animals, and the vertical lines indicate the standard error of mean ($P < .05$).

stress and dysbiosis related to metabolic syndrome, as well as the influence of fructose-induced metabolic syndrome on AP development. The present findings carry significance on the potential systemic repercussions of AP.

Our data show that 10% fructose supplementation for 8 weeks was able to induce metabolic syndrome in rats. This model is well accepted, and it has been widely used for investigating the complications related to metabolic syndrome in rodents (16, 17). Data show that fructose supplementation induced marked body weight gain, which was more evident within ≈6 weeks of experiments, even with a decrease in the average food intake in the HFD groups, since the 3rd week. Moreover, the glycemic levels were increased, and the water consumption was higher in all HFD groups when compared with controls from the beginning of the experiments, confirming diabetes-related polydipsia (13). From the present results, it is possible to conclude that AP induction did not alter fructose-induced type 2 diabetes features, such as overweight, hyperglycemia, or polydipsia. Conversely, it was shown that periodontitis induction impaired glucose metabolism and insulin resistance in Zucker prediabetes fatty rats (18, 19). Nevertheless, AP and periodontitis display different pathogenesis and progression, which might partly justify the present discrepancy. Furthermore, genetic models of diabetes greatly differ from diet models, such as the one used by us.

As a next step, we evaluated the effects of HFD and AP induction on serum and intestinal levels of cytokines and adipokines. TNF, IL-1β, and IL-6 are well-known proinflammatory cytokines that exert a relevant role in local AP development, besides metabolic syndrome (20, 21). In this study, the levels of these cytokines were undetectable in either the serum or the intestine in any experimental group, confirming previous studies (21–23).

The adipose tissue itself can synthesize proinflammatory cytokines, named adipokines, such as leptin and adiponectin (21). Leptin is increased in obese individuals and is present under proinflammatory states (24). Adiponectin has an opposite role; it has anti-inflammatory effects and regulates insulin sensitivity (25). We found an increase in serum leptin and adiponectin levels in the HFD group compared with control animals. Additionally, there was an increase of intestinal leptin levels in groups with AP induction for 28 days, an effect that tended to be higher in animals submitted to HFD. An elevation of serum leptin levels in animals submitted to HFD was quite expected, whereas the adiponectin raise might represent a compensatory response to overcome leptinemia (26). However, to our knowledge, this is the first experimental evidence showing that AP induction alters the intestinal leptin levels. Partly upholding our data, periodontitis induction in a rat model of spontaneous type 2 diabetes led to an elevation of serum leptin levels when compared with diabetic rats without periodontal disease (27).

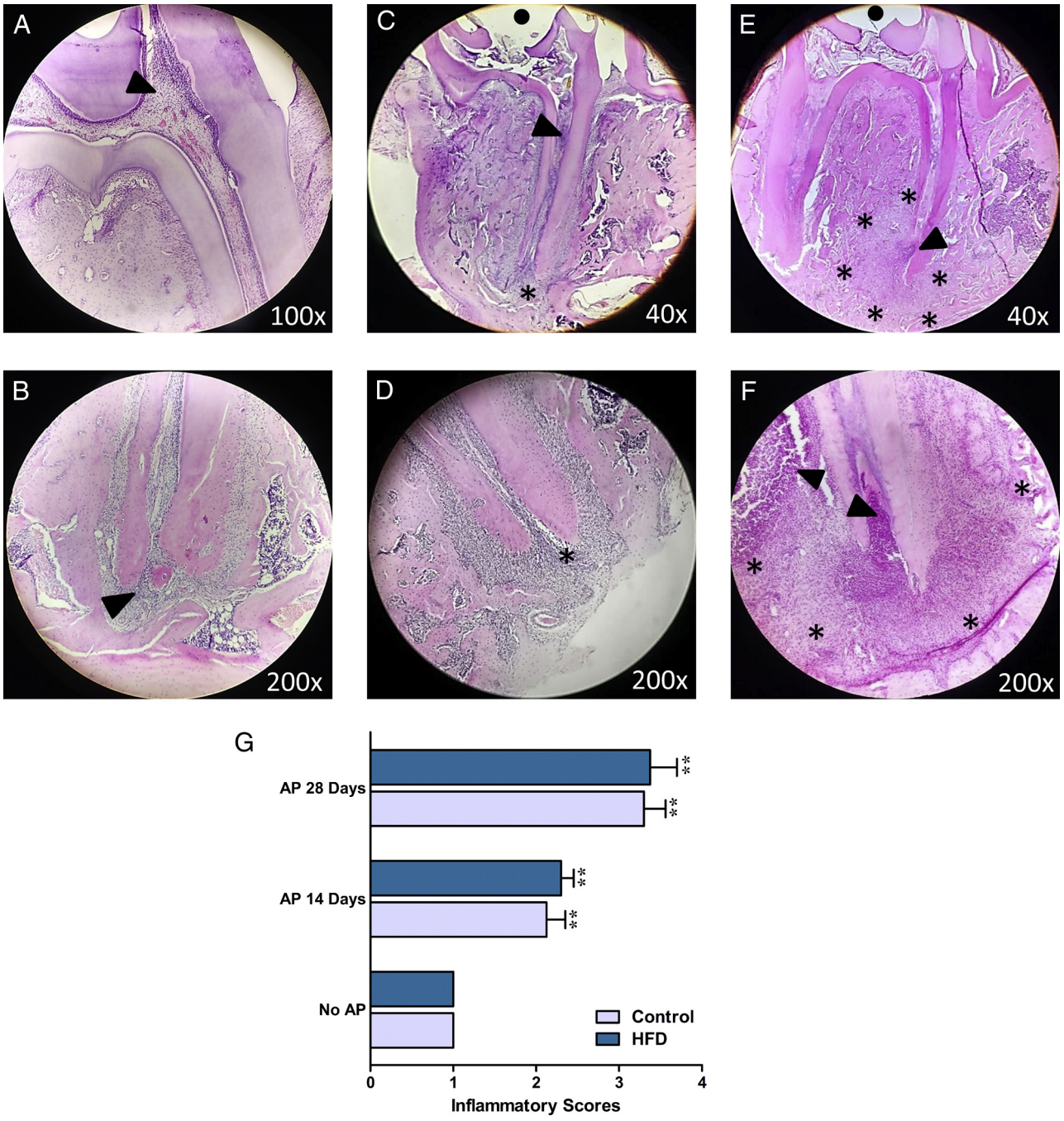

Figure 3. Representative images of histologic analysis (hematoxylin-eosin staining). (*A*) The control sample; the *arrowhead* shows the normal pulp tissue (100×). (*B*) The HFD sample; the *arrowhead* shows normal periapical tissue with a preserved periodontal ligament. No bone resorption is observed (200×). (*C*) The control AP 14-day sample showing coronal opening access (●), abscess inside the root (*arrowhead*), and mild inflammation at the periapical area (*) (40×). (*D*) HFD AP 14-day sample showing mild inflammation (*) at the periapical area without bone resorption (200×). (*E*) Control AP 28-day sample showing coronal opening access (●), totally necrotic pulp tissue, abscess at the periapical area (*arrowhead*), and extended inflammatory process with bone resorption (*) (40×). (*F*) HFD 28-day sample showing severe and extended inflammation (*) with abscess formation (*arrows*) (200×). (*G*) Data showing the time-related inflammatory scores at the periapical area in groups AP 14 days and AP 28 days. Significantly different in relation to control (**$P < .01$). The columns represent the mean of 7–10 experiments, and the vertical lines indicate the standard error of mean.

A previous study from our group showed a significant alteration of glutathione activity in the livers of animals submitted to chronic intake of 20% glucose solution and AP induction for 21 days (14). Here, the induction of liver oxidative stress was observed only in animals submitted to HFD and AP for 14 days, as indicated by an increase in glutathione levels. The other experimental groups with HFD only or HFD plus AP for 28 days did not display any significant change of this parameter. Accordingly, a previous study also showed unaltered glutathione or catalase activity in the liver of fructose-supplemented rats (28). This might suggest that liver

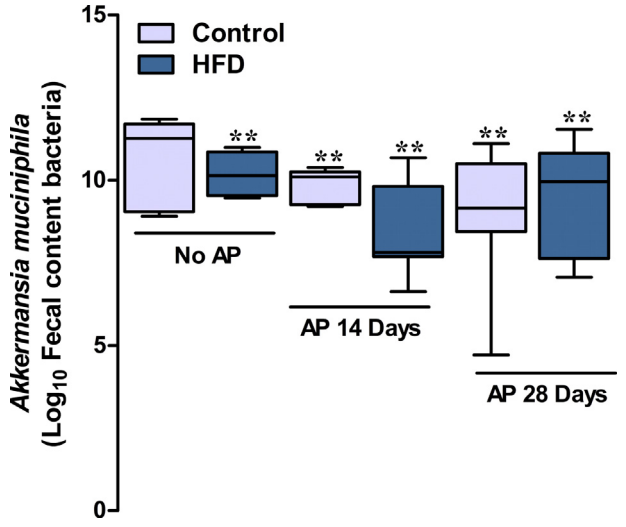

Figure 4. Fecal *Akkermansia muciniphila* expression is significantly reduced in all HFD and AP groups in relation to the control group (**$P < .01$). The columns represent the mean of 5−10 experiments, and the vertical lines indicate the standard error of mean.

oxidative stress is an early event when metabolic disease and AP are present, preceding the intestinal adipokine changes.

The present study also evaluated the possible influence of fructose-elicited metabolic syndrome on AP development. For this purpose, we used the classic AP model induced by the first molar tooth pulp exposure to the oral environment for 14 and 28 days (29). We opted to induce periapical lesion in a single tooth in order to evaluate the interaction among 2 variables (AP and HFD). The induction of periapical lesion in multiple elements is able to raise cytokine levels per se (30), which would be an interference to the analysis. We observed healthy pulp and periapical tissues in those groups without coronal opening despite the fructose supplementation. On the other hand, after 14 days of AP induction, it was possible to observe necrotic coronal pulp tissue and mild inflammation at the periapical area. After 28 days of AP induction, the pulp tissues were necrotic, with severe periapical inflammation and the presence of an abscess in most samples. The present set of results indicates a time-dependent progress of AP-related inflammation. HFD did not influence the extent of pulp or periapical inflammation at 14 or 28 days. It is tempting to conclude that AP leads to systemic alterations, but the induction of metabolic syndrome by fructose ingestion did not impair the progression of AP.

The predominant bacterial phyla in the bowel are *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* (31). Another phyla present in minor extent is *Verrucomicrobia,* which encompasses the gram-negative bacteria *A. muciniphila* (32). *A. muciniphila* resides at the gut mucus layer, and its levels are inversely correlated with the presence of metabolic diseases and chronic inflammation (33). Moreover, these bacteria are present in healthy subjects controlling fat storage, adipose tissue inflammation, and glucose metabolism, with overall favorable metabolic effects (9). Our results showed a reduction of fecal *A. muciniphila* levels in all of the experimental groups with HFD and/or AP induction. A decrease of *A. muciniphila* expression might be predictable in groups submitted to HFD because these bacteria are down-regulated under metabolic diseases and inflammatory alterations (9). However, as far as we know, this is the first experimental evidence showing the influence of AP on fecal *A. muciniphila* expression.

Conclusion

We provide evidence that AP is able to alter systemic parameters related to metabolic syndrome by showing that AP per se leads to intestinal leptinemia and dysbiosis. In contrast, HFD-induced metabolic changes did not appear to impair AP progression, at least in our experimental paradigm. Future animal studies are needed to investigate whether extended protocols of fructose supplementation might affect AP outcomes besides the influence of fructose-induced metabolic changes on periapical tissue healing.

Acknowledgments

The authors thank Mrs Janaína Pasetti Nunes for her excellent technical assistance.

C.O.T., R.B.M., and A.P.D. are PhD students supported by Coordenação de Aperfeiçoamento de Nível Superior, Brazil (Financial Code 001). M.M.C. is a researcher career awardee of the Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil (grant no. 303842-2014-8).

The authors deny any conflicts of interest related to this study.

Supplementary Material

Supplementary material associated with this article can be found in the online version at www.jendodon.com (https://doi.org/10.1016/j.joen.2018.10.013).

References

1. World Health Organization (WHO). Global Health Observatory (GHO) – Overweight. Available at: http://www.who.int/gho/ncd/risk_factors/overweight_text/en/. Accessed July 10, 2017.
2. Abdelaal M, le Roux CW, Docherty NG. Morbidity and mortality associated with obesity. Ann Transl Med 2017;5:161.
3. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013;341:1241214.
4. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761–72.
5. Cani PD. Gut microbiota – at the intersection of everything? Nat Rev Gastroenterol Hepatol 2017;14:321–2.
6. Marchesi JR, Adams DH, Fava F, et al. The gut microbiota and host health: a new clinical frontier. Gut 2016;65:330–9.
7. Wen L, Duffy A. Factors influencing the gut microbiota, inflammation, and type 2 diabetes. J Nutr 2017;147:1468S–75S.
8. Schneeberger M, Everard A, Gómez-Valadés AG, et al. Akkermansia Muciniphila inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. Sci Rep 2015;5:16643.
9. Everard A, Belzer C, Geurts L, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A 2013;110:9066–71.
10. Cani P, De Vos WM. Next-generation beneficial microbes: the case of akkermansia muciniphila. Front Microbiol 2017;8:1765.
11. Sasaki H, Hirai K, Martins CM, et al. Interrelationship between periapical lesion and systemic metabolic disorders. Curr Pharm Des 2016;22:2204–15.
12. Kilkenny C, Browne W, Cuthill IC, et al. Animal research: reporting *in vivo* experiments–the ARRIVE guidelines. J Cereb Blood Flow Metab 2011;31:991–3.
13. Al-Rasheed N, Al-Rasheed N, Bassiouni Y, et al. Potential protective effects of Nigella sativa and Allium sativum against fructose-induced metabolic syndrome in rats. J Oleo Sci 2014;63:839–48.
14. Wolle CFB, Zollmann LA, Bairros PO, et al. Outcome of periapical lesions in a rat model of type 2 diabetes: refractoriness to systemic antioxidant therapy. J Endod 2013;39:643–7.
15. Scarparo RK, Dondoni L, Böetcher D, et al. Response to intracanal medication in immature teeth with pulp necrosis - an experimental model in rat molars. J Endod 2011;37:1069–73.
16. Shawkya NM, Shehatoua GSG, Rahimb MA, et al. Levocetirizine ameliorates high fructose diet-induced insulin resistance, vascular dysfunction and hepatic steatosis in rats. Eur J Pharmacol 2014;740:353–63.
17. Saad AF, Dickerson J, Kechichian TB, et al. High-fructose diet in pregnancy leads to fetal programming of hypertension, insulin resistance, and obesity in adult offspring. Am J Obstet Gynecol 2016;215:378.e1–6.

18. Pontes Andersen CC, Flyvbjerg A, Buschard K, Holmstrup P. Periodontitis is associated with aggravation of prediabetes in Zucker fatty rats. J Periodontol 2007;78:559–65.
19. Watanabe K, Petro BJ, Shlimon AE, et al. Effect of periodontitis on insulin resistance and the onset of type 2 diabetes mellitus in Zucker diabetic fatty rats. J Periodontol 2008;79:1208–16.
20. Sell H, Habich C, Eckel J. Adaptive immunity in obesity and insulin resistance. Nat Rev Endocrinol 2012;8:709–16.
21. Vielma SA, Klein RL, Levingston CA, et al. Adipocytes as immune-regulatory cells. Int Immunopharmacol 2013;16:224–31.
22. Yoo S, Ahn H, Park YK. High dietary fructose intake on cardiovascular disease related parameters in growing rats. Nutrients 2016;9:E11.
23. Matsushita K, Tajima T, Tomita K, et al. Inflammatory cytokine production and specific antibody responses against possible causative bacteria in patients with multilesional periapical periodontitis. J Endod 1998;24:817–21.
24. Lozano I, Van der Werf R, Bietiger W, et al. High-fructose and high-fat diet-induced disorders in rats: impact on diabetes risk, hepatic and vascular complications. Nutr Metab (Lond) 2016;13:15.
25. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 2004;25:4–7.
26. Castrogiovanni D, Alzamendi A, Ongaro L, et al. Fructose rich diet-induced high plasminogen activator inhibitor-1 (PAI-1) production in the adult female rat: protective effect of progesterone. Nutrients 2012;4:1137–50.
27. Luo S, Yang X, Wang D, et al. Periodontitis contributes to aberrant metabolism in type 2 diabetes mellitus rats by stimulating the expression of adipokines. J Periodontal Res 2016;51:453–61.
28. Glban AM, Vasiljević A, Veličković N, et al. The expression and activity of antioxidant enzymes in the liver of rats exposed to high-fructose diet in the period from weaning to adulthood. J Sci Food Agric 2015;95:2319–24.
29. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340–9.
30. Cintra LT, Samuel RO, Azuma MM, et al. Multiple apical periodontitis influences serum levels of cytokines and nitric oxide. J Endod 2016;42:747–51.
31. Barczynska R, Slizewska K, Litwin M, et al. Effects of dietary fiber preparations made from maize starch on the growth and activity of selected bacteria from the Firmicutes, Bacteroidetes, and Actinobacteria phyla in fecal samples from obese children. Acta Biochim Pol 2016;63:261–6.
32. Derrien M, Vaughan EE, Plugge CM, et al. Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. Int J Syst Evol Microbiol 2004;54:1469–76.
33. Karlsson CL, Onnerfalt J, Xu J, et al. The microbiota of the gut in preschool children with normal and excessive body weight. Obesity (Silver Spring) 2012;20:2257–61.