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Original Research

Diet-induced Generalized Periodontitis in Lewis Rats

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Periodontitis is an important public health concern worldwide. Because rodents from the genus *Rattus* are resistant to spontaneous periodontitis, experimental periodontitis must be initiated by mechanical procedures and interventions. Due to their exacerbated Th1 response and imbalanced Th17 regulatory T-cell responses, Lewis rats are highly susceptible to inducible inflammatory and autoimmune diseases. We hypothesized that feeding Lewis rats a diet high in sucrose and casein (HSC) would alter the oral microenvironment and induce inflammation and the development of periodontitis lesions without mechanical intervention. A baseline group (BSL, *n* = 8) was euthanized at age 6 wk. Beginning at 6 wk of age, 2 groups of Lewis rats were fed standard (STD, *n* = 12) or HSC (*n* = 20) chow and euthanized at 29 wk of age. We evaluated the degree of periodontitis through histology and μCT of maxillae and mandibles. The HSC-induced inflammatory response of periodontal tissues was assessed by using immunohistochemistry. Gene expression analysis of inflammatory cytokines associated with Th1 and Th17 responses, innate immunity cytokines, and tissue damage in response to bacteria were assessed also. The potential systemic effects of HSC diet were evaluated by assessing body composition and bone densitometry endpoints; serum leptin and insulin concentrations; and gene expression of inflammatory cytokines in the liver. Placing Lewis rats on HSC diet for 24 wk induced a host Th1-immune response in periodontal tissues and mild to moderate, generalized periodontitis characterized by inflammatory cell infiltration (predominantly T cells and macrophages), osteoclast resorption of alveolar bone, and hyperplasia and migration of the gingival epithelium. HSC-fed Lewis rats developed periodontitis without mechanical intervention in the oral cavity and in the absence of any noteworthy metabolic abnormalities. Consequently, the rat model we described here may be a promising approach for modeling mild to moderate periodontitis that is similar in presentation to the human disease.

Abbreviations: ABC, alveolar bone crest; ABL, alveolar bone loss; ACH, alveolar crest height; BMD, bone mineral density; CEJ, cementoenamel junction; GE, gingival epithelium; HSC, high sugar and casein; LP, lamina propria; MMP, matrix metalloprotein; RANKL, receptor activator of NFκβ ligand; ROI, region of interest; STD, standard; TLR, toll-like receptor; vBMD, alveolar volumetric BMD; vTMD, volumetric tissue mineral density

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Periodontitis is a polymicrobial disease characterized by inflammation of the supporting tissues of the teeth, including the gingiva, periodontal ligament, and alveolar bone. Periodontitis is an important public health problem, affecting about 46% of dentate adults older than 30 y,[27,28,104] with about 9% of these cases considered severe.[27] Worldwide, periodontitis prevalence appears to be rising, with an increase of nearly 60% from 1990 to 2010.[53,55,78,84]

Rodent models have been used widely to examine the pathophysiology of periodontitis and to investigate preventive and therapeutic modalities.[1,2,5,18,26,30,35-37,40,41,44,57,69,93,97,103] Because of the relatively small size of the rodent oral cavity compared with human patients, routine clinical outcomes performed in humans (including oral visual inspection, evaluation of gingival bleeding, periodontal pocket probing, and so forth) are unreliable and lack sensitivity in rodents. This drawback can be overcome by using histologic methods to directly measure the loss of alveolar bone and assess alterations to periodontal soft tissues, μCT for analysis of mineralized jaw tissues, and analyses of gene and protein expression of inflammatory cells and inflammatory mediators of periodontal tissues. Rodents from the genera *Mus* and *Rattus* have clear advantages as experimental models because they produce measurable alterations in periodontal tissues within a timeframe of weeks, allow observation of the disease at a genetic level, and are readily available from commercial laboratory animal suppliers. However, mice and rats typically require some form of ongoing, local mechanical intervention to recreate features of mild to moderate periodontitis, given that these species are less prone to develop spontaneous, generalized periodontitis[37,93,103] in laboratory settings compared with other mammals.[31,40,82,97,103,108] These procedures include placement of a ligature around molars,[21,70] repeated injection of LPS into gingival tissues,[26,86] and repeated inoculation with bacterial pathogens into the oral tissues.[68,89] These interventions require

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specific skills that are best performed by persons with a dental background. A relevant rodent model of generalized periodontitis that does not require mechanical intervention could open opportunities for laboratories to examine periodontitis and bring new insights into the pathophysiology of periodontitis.

In humans, periodontitis results from tissue destruction due to both the host immune response and the pathogenic microorganisms present in oral biofilms.[9,14,51,85,102,114] Therefore, preclinical models with spontaneous accumulation of plaque and subsequent host-mediated tissue damage could address limitations in the mechanical intervention models. Currently, rice rats (*Oryzomys palustris*) are a rodent model that exhibits both spontaneous plaque accumulation and host-mediated, progressive, periodontal tissue damage without mechanical intervention. When fed a diet high in sucrose and casein (HSC), rice rats develop a spontaneous form of generalized periodontitis that eventually resembles moderate to severe periodontitis in humans, with plaque accumulation, a marked host immune response, destruction of the periodontal tissues, and loss of tooth attachment.[5,7,40,41,97,100] Although rice rat periodontitis is similar in many ways to that of humans, rice rats are an unconventional model in the laboratory setting. The current lack of well-characterized gene sequences and protein antigenic epitopes in rice rats makes mechanistic studies using conventional molecular biology techniques challenging. Furthermore, these animals are not commercially available, necessitating the maintenance of inhouse breeding colonies from wild stock.

Evidence that an HSC diet promotes accumulation of biofilm in rice rats suggests that dietary modification may produce an effective biofilm in some rodent species. A previous study in *Rattus* showed that a diet high in sucrose enhanced the total cultivable bacteria, particularly *Actinomyces naeslundii*, *Streptococcus rattus*, and *Streptococcus sui*s-like bacteria, but the study was not designed to assess periodontitis outcomes.[121] Given the well-established role of the host inflammatory response in the pathogenesis of periodontitis,[9,14,85,114] we hypothesized that rodent models prone to inducible inflammatory disease develop spontaneous periodontitis if combined with a dietary modification known to produce oral biofilms. Therefore, the primary purpose of the current study was to assess whether an HSC diet would induce periodontitis in the genus *Rattus* without mechanical intervention. We chose Lewis rats because of their well-characterized genetic susceptibility to various inducible T-cell mediated autoimmune diseases.[20,29,32,38,58,63,64,67,73,75,98,119,120] In addition, Th1 and Th17 effector responses associated with the development of autoimmune diseases are unique in Lewis rats.[73,74,109,118] Furthermore, accentuated Th1 responses and imbalances between Th17 and regulatory T cells have been shown to contribute to uveitis[20,118] and encephalomyelitis[73] in Lewis rats.

Lewis rats were fed an HSC diet for 24 wk to determine the extent to which they would develop periodontitis. Periodontitis was assessed by using histologic and µCT analysis of jaw quadrants and analysis of genes upregulated during tissue damage in response to the presence of bacteria. The inflammatory host response was assessed through immunocytochemistry and gene expression analysis of cytokines, particularly those associated with Th1 and Th17 responses. Potential systemic effects of the HSC diet were evaluated by assessing gene expression of proinflammatory liver cytokines, body composition measurement, bone densitometry, and serum leptin and insulin concentrations. We hypothesized that: 1) Lewis rats fed HSC diet would develop spontaneous periodontitis; 2) HSC diet would be associated with an inflammatory response characterized by upregulation of cytokine gene expression associated with Th1 and Th17 responses, and 3) HSC diet would not significantly affect systemic metabolic health outcomes.

Materials and Methods

Animals and diets. Female SPF Lewis rats (Lewis/SsNHsd; n = 40; age, 5 wk; weight, 125 ± 7 g) were purchased (Harlan Laboratories, Indianapolis, IN). Routine surveillance testing among the rats was performed quarterly. Rats were weighed biweekly and received either a pelleted standard (STD) rodent chow control diet (Envigo Teklad Global 18% protein diet; Teklad Diets, Madison, WI), or a pelleted HSC diet (Purina, Tampa, FL); the HSC diet is a modification of the AIN93G synthetic diet (Purina TestDiet, St Louis, MO), with 70% kcal from sucrose, 18% from casein, and 12% from fat, as previously described.[5,34,40] Rats were housed 2 per cage in polycarbonate cages (Lab Products, Seaford, DE), with hardwood chips as bedding material (7090 Teklad Sani-chips, Envigo, Dublin, VA) and continuous access to food and water. Municipal tap water (https://www.greensboro-nc.gov/departments/water-resources/water-system/monthly-water-quality-reports) was provided in water bottles. The housing room was maintained at 20 to 26 °C, with an average humidity of 30% to 70% and a 12:12-h light:dark cycle. All rats received cage enrichment including nesting material (Crink-l'Nest, The Andersons Lab Bedding, Maumee, OH, or cotton squares, Ancare, Bellmore, NY), certified irradiated Diamond Twists (7979C, Envigo, Dublin, VA), and plastic tunnels (Rat Retreats, BioServ, Flemington, NJ). Cageside observation of rats was performed daily. The bedding was changed twice weekly. Full cage changes (cage, bedding, water bottle, environmental enrichment) were performed every other week. Racks were changed monthly. Temperature and humidity in the animal room were recorded daily. The animal room was swept and mopped daily, except on weekends and holidays. Efforts were made at all times to minimize pain and discomfort in all animals. The protocol was approved by the University of North Carolina at Greensboro IACUC, which is assured through OLAW (assurance no. A3706-01); the University of North Carolina at Greensboro is not an AAALAC-accredited institution.

Study design. Rats (age, 5 wk) were randomly assigned to 1 of 3 groups: 1) the baseline (BSL) group (n = 8) was provided STD diet for 1 wk and necropsied at 6 wk of age; 2) the STD group (n = 12) received STD diet for 24 wk, and 3) the HSC group (n = 20) were provided HSC diet for 24 wk. The 24-wk dietary modification was based on previous studies in rice rats, which showed moderate to severe periodontitis after 24 wk of eating HSC diet.[5,7] Rats in the STD and HSC groups were necropsied at the end of the study period, at 29 wk of age.

Body composition and bone densitometry. At 5 d prior to necropsy, body composition, and whole-body bone mineral characteristics were assessed in vivo by using dual-energy X-ray absorptiometry (Lunar Prodigy, GE Healthcare, Madison, WI). Rats were anesthetized by using isoflurane inhalation, at concentrations of 4.5% v/v for induction and 2.5% v/v for maintenance, and scanned. Whole-body bone mineral density (BMD), bone mineral content, bone area, tissue mass, fat mass, and lean mass were measured. Percentage body fat was calculated by using the formula fat mass (g) / (fat mass [g] + lean mass [g] + bone mineral content) × 100%.

Euthanasia and tissue collection. Rats were fasted for 12 h prior to necropsy. All rats were euthanized by CO_2 inhalation followed by cervical dislocation. Blood samples were obtained through cardiocentesis. The liver was carefully dissected as an intact organ and weighed. Both maxillae and mandibles were

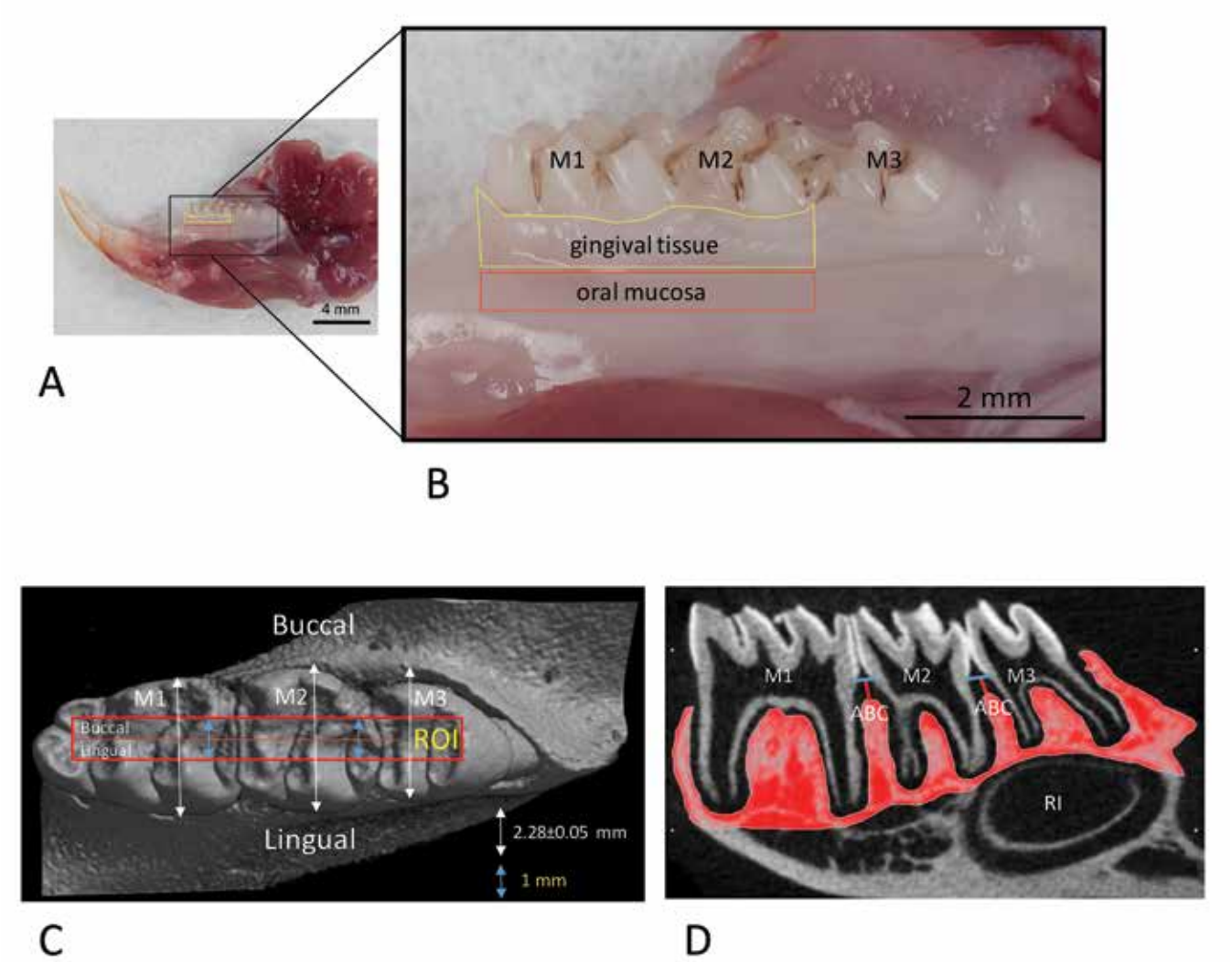

Figure 1. Mandibular anatomy and regions of interest for measurements. (A) Representative high-resolution photograph of the lingual aspect of a Lewis rat mandible, demonstrating gross features of the molars, incisors, and soft tissues; the excisional boundaries of gingiva (yellow) and oral mucosal tissues (red) used for gene expression analysis are shown also. (B) Close-up image demonstrating mandibular gingiva (yellow) and oral mucosa (red) excised for mRNA analysis. (C) µCT 3D reconstruction of the coronal aspect of the right mandible, showing the mean total buccolingual width of the alveolar process (white arrows) and the occlusal surfaces of the molars. A region of interest (ROI, blue arrows; width, 1 mm) within this area was designed to allow measurement of alveolar crest height (ACH) and volumetric µCT parameters. The total ROI was divided into buccal and lingual aspects to further investigate whether the HSC diet induced a specific pattern of alveolar bone loss (buccal or lingual pattern). (D) A representative slice from within the ROI oriented in the mesiodistal plane. ACH (red line) was defined as the distance between the cementoenamel junction (CEJ, blue line) and the alveolar bone crest (ABC) and was measured at both the M1M2 and M2M3 interdental regions. For µCT, the area selected for evaluating 3D alveolar bone volume, alveolar volumetric bone mineral density (vBMD), and volumetric tissue mineral density (vTMD) is shown in red. This area extended from a transverse point mesial to M1 to a point distal to M3 and included all of the alveolar bone. The inferior border of the region was drawn superior to the root of incisor (RI) and extended underneath the roots of all molars in a line roughly parallel to the surfaces of the crowns.

disarticulated and musculature was carefully trimmed, without disturbing oral mucosal or gingival tissues. High-resolution photos were taken of all jaws by using a digital camera (EOS 6D, Canon, Tokyo, Japan) attached to a macro lens (EF 100 mm 1:2.8, Canon), to examine potential gross features of periodontitis.

Left mandibles were used for gene expression analysis of gingiva and oral mucosa (described later). Two separate strips (length, 2 mm; width, 1 mm) of gingival tissue adjacent to the buccal and lingual surfaces of M1 and M2 were excised and collected from all rats. In addition, a strip of oral mucosa from the lingual plate was excised and collected for use as a tissue control (Figure 1 A and B). Tissue was excised by using a scalpel, and incision was made at full thickness into submucosa.

Right maxillae and mandibles were used for histologic and µCT analyses. Jaws were fixed in 10% phosphate-buffered formalin (pH 7.4) for 48 h at 4 °C and then transferred to 70% ethanol. Right mandibles were then scanned by using µCT (methods described later). After µCT, all jaws were decalcified in 5% formic acid for 4 wk and embedded in paraffin.[5-7] Sections (thickness, 5 µM) in the mesiodistal plane were obtained. Tissues were stained with hematoxylin and eosin and used to assess periodontitis severity (periodontitis score) and alveolar crest height (ACH), as described later.

Serum ELISA. Whole blood collected through cardiocentesis was allowed to clot at room temperature for at least 1 h. Serum was obtained by centrifuging at 1400 × *g* for 15 min and stored at –20 °C. Insulin levels were evaluated by using Rat/Mouse

Score	Degree	Description
0	none	No signs of inflammation or periodontitis
1	slight	Gingivitis: slight hyperplasia of gingival epithelium (GE), intraepithelial inflammatory cell infiltration. Bacterial plaque accumulation. Normal lamina propria (LP), periodontal ligament (PDL), and alveolar bone (AB).
2	mild	Gingival hyperplasia, inflammatory cell infiltration of the GE and LP. Bacterial plaque accumulation. Normal PDL and AB.
3	moderate	Erosion or ulceration and hyperplasia of the GE and marked bacterial plaque accumulation. Moderate inflammatory cell infiltration of LP, disruption of PDL, migration of the junctional epithelium, and mild AB resorption.
4	severe	Ulceration or hyperplasia of the GE with marked bacterial plaque accumulation. Severe inflammatory cell infiltration of LP, disruption of PDL, migration of the junctional epithelium, and marked AB resorption.

Figure 2. Periodontitis (inflammation) scoring system used to characterize periodontal lesions in maxillae and mandibles of rice rats. AB, alveolar bone; GE, gingival epithelium; LP, lamina propria; PDL, periodontal ligament.

Insulin ELISA (Millipore, Billerica, MA), and leptin was quantified by using Rat Leptin ELISA (Millipore).

Assessment of periodontitis in rodent models. Periodontitis is a polymicrobial inflammatory disease, characterized by inflammation of the supporting tissues of teeth, including the gingiva, periodontal ligament, and the alveolar bone. Due to the relatively small oral cavity of laboratory rodent species, periodontal evaluation in laboratory rodents strongly relies on histologic assessments and analysis of protein or gene expression of inflammatory cells and inflammatory mediators of periodontal tissues. In light of these constraints, we used standard methods previously used in small rodent models of periodontitis[5,7,19,65,69] including: 1) assessment of alveolar bone loss (ABL) severity by histologic and/or µCT methods; 2) histopathologic evaluation of the inflammatory responses of periodontal tissues in histologic sections; and 3) quantification of inflammatory cells and gene expression analysis of inflammatory markers in periodontal tissues by using immunohistochemistry and real-time PCR analysis, respectively. ABL is an irreversible event and one of the most important hallmarks of periodontitis and therefore is used as a strong indicator of severity progression of the disease.[25,48,99] Consequently, ABL in both maxillae and mandibles was evaluated by using histomorphometry and µCT methods, respectively (described later).

µCT. Mandibles were trimmed by using a slow saw or dremel rotary tool to remove the ramus, making a final mesiodistal length of approximately 7.5 mm. Mandibles were scanned on a µCT system (Skyscan 1172, Bruker, Kontich, Belgium) by using methods that are standard in our laboratory.[11,12,92,115-117] These methodologies are in accordance with the American Society of Bone and Mineral Research.[13] Briefly, samples were scanned from the lingual to buccal plane at 80 kVP, 120 µA with a 0.5-mm aluminum filter, 1 K camera resolution, 19.1-µm voxel, 0.5° rotation step, and 360° tomographic rotation. Cross-sectional images were reconstructed by using a filtered back-projection algorithm (NRecon, Bruker). To allow assessment of alveolar bone, images were then rotated identically by using DataViewer software (Bruker). A 1.0-mm region of interest (ROI) was then selected for consistent evaluation of both alveolar bone loss and µCT volumetric bone parameters in all mandibles (Figure 1 C and 1 D). CTan software (Bruker) was used to select the ROI, which was situated at the midpoint of the mandibular molars (Figure 1 C) and encompassed all alveolar bone superior to the incisor root (Figure 1 D**)**. The ROI was placed at 610 ± 77 µm from the lingual plate of the alveolar process and 637 ± 64 µm from the buccal plate of the alveolar process, with a mean total buccolingual width of 2280 ±54 µm (Figure 1 C). The location and dimensions of the ROI were specifically designed so that the cementoenamel junction (CEJ) and alveolar bone crest (ABC) were clearly visible in each of the slices throughout the mandibular buccolingual dimension from molar M1 to M3, for measurement of ABL (Figure 1 D). For evaluating volumetric parameters, an area that included alveolar bone from the mesial aspect of M1 to the distal aspect of M3 was included, and the inferior border was drawn as a longitudinal line roughly parallel to the crowns of the molars at the level of the apical region of the roots, superior to the coronal border of the root of the incisor (RI; Figure 1 D).

2D slices spaced equidistantly throughout the width of the ROI were evaluated in the mesiodistal plane (Figure 1 D) to determine mandibular ABL (described later) by using CTan software. To determine additional effects of the HSC diet, 3D alveolar bone volume was determined in slices of the mandibles within the ROI described earlier. Alveolar volumetric BMD (vBMD) and volumetric tissue mineral density (vTMD) were determined after calibration by using hydroxyapatite phantoms.

Histopathologic characterization and lesion scoring. Right maxillae and mandibles were sectioned (width, 5 µm) in the mesiodistal plane by using a microtome (Microm HM 325, GMI, Ramsey, MN) and stained with hematoxylin and eosin. Sectioning was performed from the lingual to buccal surfaces. Sections from near the lingual and buccal surfaces were used to determine periodontitis score.[7] Periodontitis scoring of right jaw quadrants (right maxilla and right mandible) was completed at M1M2 and M2M3 in a randomized, blinded manner by a single operator (JIA), who used a 5-point scoring system (periodontitis score) as previously described[5,7,79,80] (Figure 2). A higher periodontitis score indicates more severe periodontitis.

ABL. To determine ABL, we measured the alveolar crest height (ACH) in maxillae by using sections stained with hematoxylin and eosin and in mandibles using µCT. ACH was defined as the distance between the CEJ and ABC for both maxillae and mandibles (Figure 1 D) and was measured at both the M1M2 and M2M3 interdental spaces.[5-7,80] ABL is represented as an increased CEJ–ABC distance, as is indicated by increased ACH values.

For µCT, ACH measurements across the width of the whole ROI were made on 18 equidistant 2D slices spaced throughout the 1.0-mm ROI of mandibles oriented in the mesiodistal plane (Figure 1 D). To further investigate whether the HSC diet induced a location-specific pattern of buccal or lingual ABL, the ROI was subdivided into 2 halves (Figure 1 C): 1) the lingual half, corresponding to the 9 consecutive slices situated most adjacent to the lingual site, and 2) the buccal half, corresponding to the 9 consecutive slices most adjacent to the buccal site.

For stained sections, ACH was measured by using the OsteoMeasure System (OsteoMetrics, Decatur, GA) at a magnification of 100×.[5-7,80] As with µCT, in the histometric evaluation of jawbones, an increased CEJ–ABC distance at M1M2 and M2M3 indicates a reduction in ACH and thus increased ABL. All histometric ACH measurements were done in a randomized and blinded fashion.

Table 1. Bone density, body composition, and serum leptin and insulin levels

Diet group	Baseline	STD diet	HSC diet
Number of animals	8	12	20
Age (wk)	6	29	29
Initial body weight (g)	123.0 ± 3.0	123.8 ± 5.4	125.4 ± 7.7
Final body weight (g)		234 ± 21[a]	238 ± 15[a]
Bone densitometry			
Bone mineral density (mg/cm^2)	109 ± 3	183 ± 5[b]	193 ± 6[b,c]
Bone mineral content (mg)	2919 ± 107	8329 ± 651[b]	8776 ± 1932[b]
Bone area (cm^2)	26 ± 2	46 ± 3[b]	48 ± 4[b]
Body composition			
Fat mass (g)	20 ± 1	82 ± 16[b]	88 ± 15[b]
Lean mass (g)	94 ± 3	148 ± 7[b]	149 ± 8[B]
Percentage fat (%)	17 ± 1	34 ± 4[b]	36 ± 5[b]
Liver mass (g)	4.37 ± 0.32	4.99 ± 0.67[a]	5.51 ± 0.59[a,c]
Serum levels			
Leptin (ng/mL)	ND	6.08 ± 2.23	6.64 ± 3.30
Insulin (ng/mL)	0.69 ± 0.05	1.19 ± 0.20[b]	1.08 ± 0.27[b]

Where appropriate, data are given as mean ± 1 SD.
[a,b]Value is significantly ([a]$P < 0.05$; [b]$P < 0.001$) different from baseline value.
[c]Value is significantly ([c]$P < 0.05$) different from that for STD diet.

Immunohistochemistry. Paraffin-embedded sections (5 µm) of decalcified mandibles were deparaffinized and rehydrated through graded alcohols. Endogenous peroxidases were quenched by using 3% hydrogen peroxide in methanol for 10 min. A heat antigen retrieval method using Triology solution (Cell Marque, Rocklin, CA) was performed on sections for 25 min at 95 °C. Sections were serum-blocked for 20 min and incubated overnight at 4 °C in a humidified chamber with primary antibody diluted with antibody diluent (Zymed Laboratories, South San Francisco, CA). Antibodies for various immune cell markers included 1) a mouse monoclonal antibody to CD68, which is highly expressed by cells in the monocyte lineage, including macrophages, pericytes, and osteoclasts (3.3µg/mL; ab31630, Abcam, Cambridge, MA); 2) a rabbit polyclonal antibody to CD3, which is present in the T-cell coreceptor for CD4$^+$ and CD8$^+$ cells (1 µg/mL; A0452, Dako, Carpinteria, CA); 3) a rabbit polyclonal antibody to cathepsin K, a specific protease present in osteoclast lysosomes (0.62 µg/mL; ab19027, Abcam, Cambridge, MA;); and 4) a rabbit monoclonal antibody to CD15 to detect neutrophils (1 µg/mL; FUT4/1478R, Novus Biologicals, Littleton, CO). Antigens were visualized by using VectaStain ABC Elite kit (Vector Laboratories, Burlingame, CA). Diaminobenzidine was used as the chromogen. Negative controls in sections incubated without primary antibody or with isotype-matched IgG (Vector Laboratories) demonstrated absence of signal. Slides were counterstained with hematoxylin (QS, Vector Laboratories), dehydrated, cleared in xylene, mounted in Permount (Fisher Scientific, Waltham, MA), and examined by light microscopy.

Gene expression. At necropsy, excised gingiva and oral mucosa tissues and thin slices of liver were placed in RNAlater (Applied Biosystems, Foster City, CA) in microfuge tubes (150 µL and 650 µL, respectively) for immediate stabilization of RNA. RNA was extracted by using RNeasy Plus Universal Mini Kit (Qiagen, Venlo, Netherlands), and 1 µg RNA was reverse-transcribed by using High Capacity cDNA RT kit with RNase inhibitor (Applied Biosystems), after which 50 ng cDNA was amplified in duplicate reactions by using *Taq*Man Fast Universal Mastermix and *Taq*Man Gene Expression Assays (Applied Biosystems).

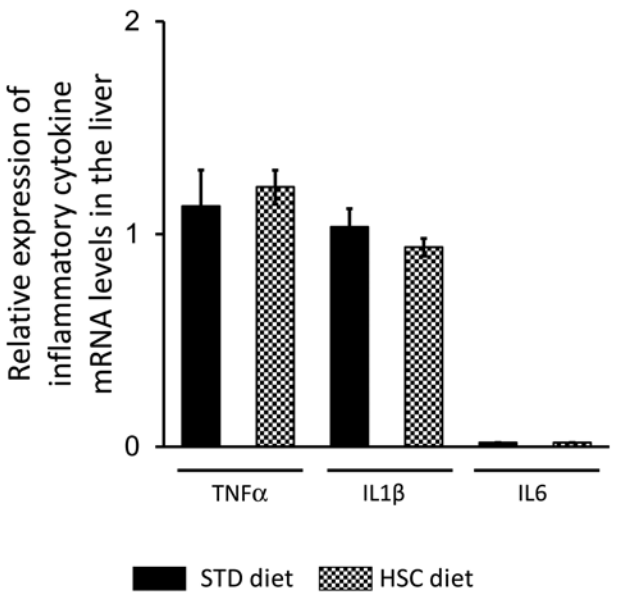

Figure 3. mRNA levels (mean ± SEM) of inflammatory cytokines in the livers of Lewis rats. TNFα, IL1β, and IL6 mRNA levels in HSC rat livers were not significantly different from those in STD t rat livers.

Given the importance of T-helper cell alterations in the phenotype of Lewis rats, the expression of genes associated with Th1- and Th17-mediated inflammation[20,73,118] was analyzed. Th1-associated genes included IL12A, IL2, and IFNγ, and Th17-associated genes included IL1β, IL6, IL17A, IL23A, and IL23B (IL12B).[83]

In addition, genes associated with activation of the innate immune response and host-mediated tissue damage were assessed. Toll-like receptors 2 (TLR2) and 4 (TLR4) and TNFα are involved in mediating the innate immune response in the presence of bacteria.[3,54,76,94,105] Receptor activator of NFκβ ligand (RANKL) and matrix metalloproteinase 9 (MMP9) are directly involved in bone resorption and tissue matrix degradation/

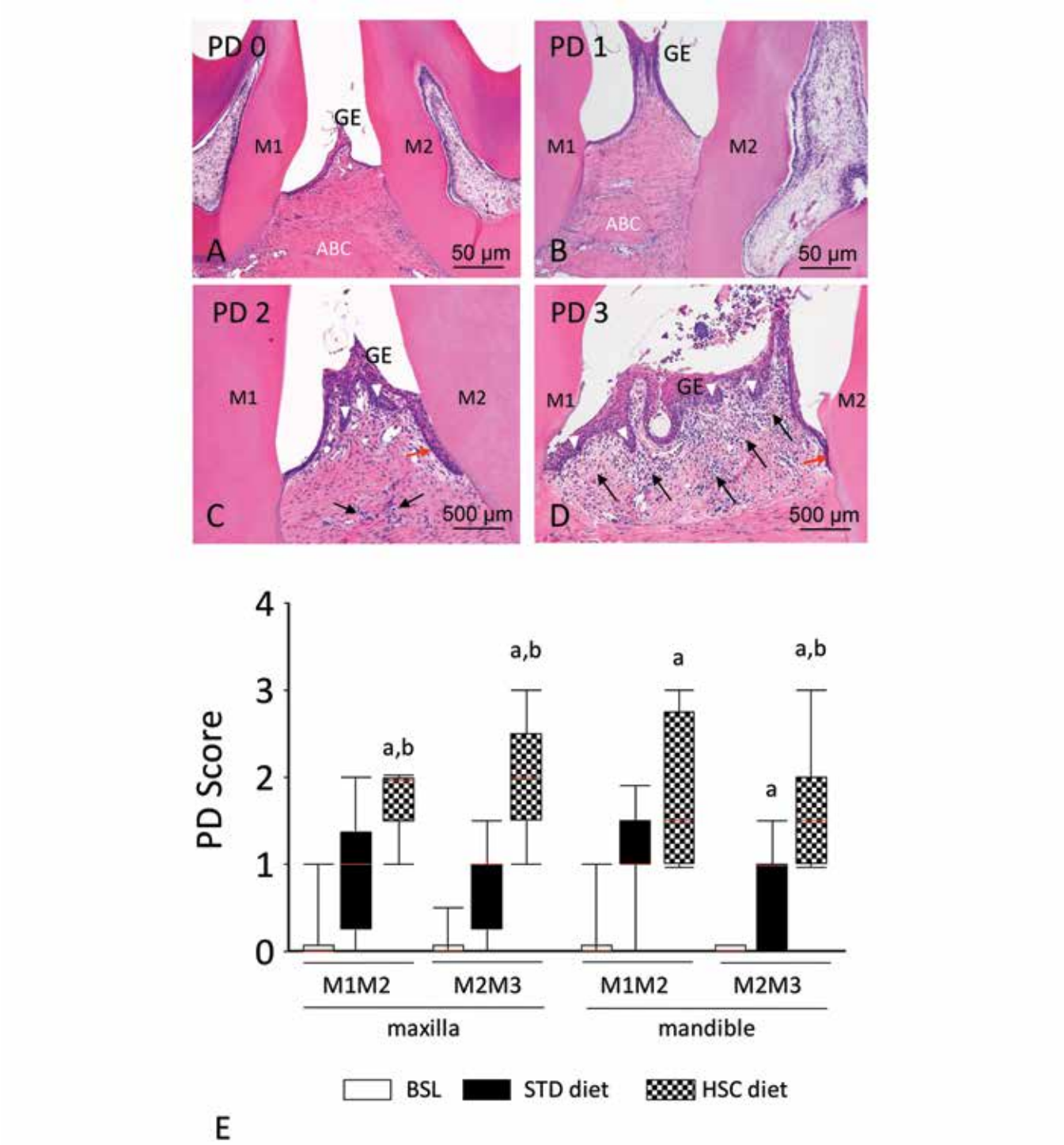

Figure 4. Periodontitis scores were higher in Lewis rats fed HSC diet compared with STD diet. (A through D) Representative photomicrographs associated with the periodontitis scores at M1M2 interdental regions of the mandible in the mesiodistal plane of experimental Lewis rats. Compared with the rat in panel A (periodontitis score [PD], 0) and that in panel B (periodontitis score 1), rats with a periodontitis scores of (C) 2 or (D) 3 had marked gingival epithelial (GE) hyperplasia (white arrowheads), migration of the junctional epithelium (red arrows), and increased mononuclear inflammatory cell (lymphocytes and macrophages) infiltrate in the lamina propria (LP, black arrows). In addition, increased mononuclear inflammatory cell infiltrate was present in the periodontal ligament (PDL) area of rats with periodontitis score 3. (E) Median maxillary and mandibular periodontitis scores at M1M2 and M2M3 interdental spaces are represented by red lines on box-and-whisker plots. HSC rats had higher maxillary periodontitis scores at M1M2 and M2M3 compared with both baseline (BSL; $^{a}P < 0.05$) and STD ($^{b}P < 0.05$) groups. HSC rats had higher mandibular periodontitis scores at M1M2 and M2M3 compared with BSL rats ($^{a}P < 0.05$). Furthermore, STD rats had mandibular higher periodontitis scores compared with BSL rats, but only at M2M3 ($^{a}P < 0.05$).

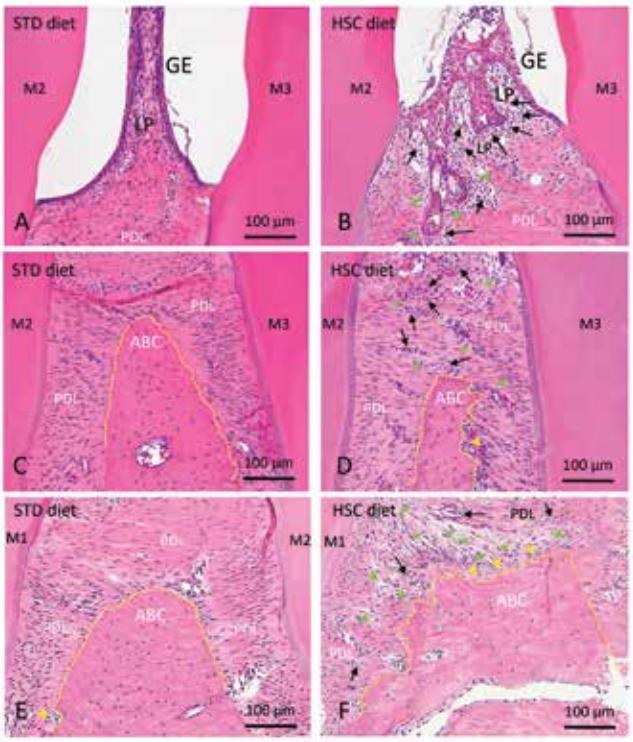

Figure 5. Histopathologic findings in periodontitis lesions of Lewis rats. Representative microphotographs of longitudinal sections of (A through D) maxillae and (E and F) mandibles at the interdental space in (A, C, and E) STD and (B, D, and F) HSC rats. (B) Histopathologic findings of periodontal tissues in HSC rats included hyperplasia of the gingival epithelium (GE) with formation of rete pegs (white arrowheads) and moderate mononuclear inflammatory cell infiltrate (lymphocytes and macrophages) in the lamina propria (LP; black arrow). (B, D, and F) Pathologic changes were seen in the periodontal ligament (PDL) of HSC rats compared with STD diet rats. Specifically, disruption, fragmentation, and separation—particularly of transeptal fibers and alveolar crest fibers (green arrowheads)—as well as (D and F) interfibrillar mononuclear inflammatory cell infiltrate (lymphocytes and macrophages, black arrows) and (F) edema separating the normal arrangement of transeptal fibers and alveolar crest fibers. (C and E) The arrangement pattern of the periodontal ligament fibers in STD rats appears histologically unaffected. In addition, alveolar bone resorption, characterized by the presence of eroded surfaces (yellow arrowheads) on alveolar bone surfaces (yellow dotted lines) of the alveolar bone crest (ABC), were more frequent in (D and F) HSC rats compared with (C and E) STD rats. Hematoxylin and eosin stain.

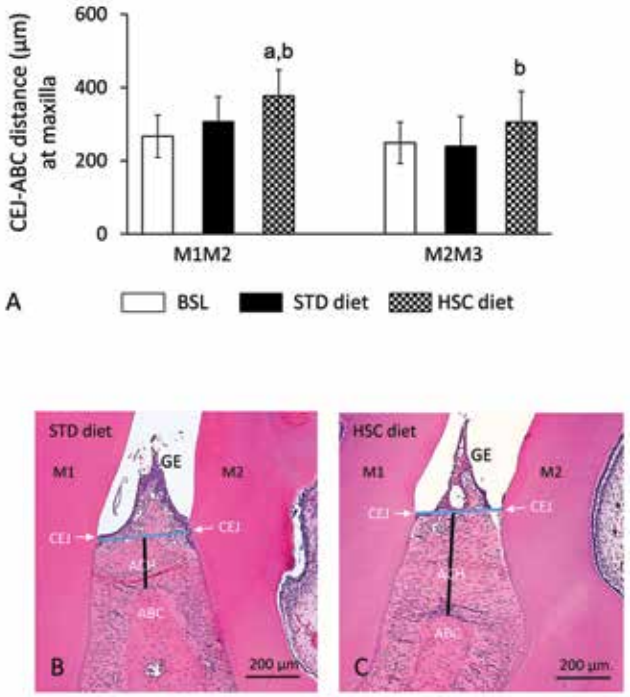

Figure 6. HSC rats had greater maxillary alveolar bone loss (mean ± 1 SD) according to histology. (A) HSC rats had significantly greater maxillary alveolar bone loss, as indicated by a greater cementoenamel junction [CEJ]–alveolar bone crest [ABC] distance at M1M2 compared with BSL ($^{a}P < 0.05$) and STD ($^{b}P < 0.05$), and at M2M3 compared with STD ($^{b}P < 0.05$). Representative microphotographs of maxillary sections at interdental spaces M1M2 of rats from the (B) STD diet group and (C) from the HSC diet group. Note the greater distance between the CEJ–ABC (black vertical line) in HSC rats compared with STD rat (B), indicating greater alveolar bone loss and higher ACH values. Hematoxylin and eosin stain.

remodeling, respectively.[23,48,77,90] Liver tissue samples were evaluated for indications of systemic inflammation by analyzing TNFα, IL1β, and IL6. Gene expression in liver and oral tissues was normalized by using 18S and β-actin as loading controls, respectively. Gene expression was calibrated to the mucosal STD values, except for RANKL and TNFα expression. RANKL and TNFα were not detected in oral mucosal tissue, so gene expression was calibrated to the values from the gingival HSC group. For liver analysis, gene expression was calibrated to STD values.

Statistical analysis. Data are expressed as mean ± 1 SD, except for gene expression values, which are expressed as mean ± SEM. One-way ANOVA with Holm–Sidak posthoc tests was used to assess intergroup differences. When assumptions of data normality were not met, Kruskal–Wallis ANOVA followed by Dunn multiple comparisons was applied. 2-way ANOVA was used to test the main effects of diet and tissue type on gene expression analysis in the oral cavity. Differences between STD and HSC groups in regard to gene expression analysis within oral tissue type (mucosa and gingiva) were assessed by using pairwise Holm–Sidak posthoc tests. A P value less than 0.05 was considered significant.

Results

Body weight; anatomic, metabolic, and serologic endpoints; and liver inflammation. No animal welfare concerns occurred at any time. Endpoints assessed in 29-wk-old Lewis rats on STD and HSC diets are shown in Table 1. Rats in both STD and HSC groups gained significant weight between 6 and 29 wk of age, but no significant differences in weight were observed between these groups throughout the study. All anatomic, metabolic, and serologic endpoints parameters were significantly higher in STD and HSC rats compared with BSL rats (liver mass, $P < 0.05$; all other parameters and intergroup comparisons, $P < 0.001$; Table 1). Total BMD was 5% higher ($P < 0.001$) in HSC rats compared with STD rats, but no significant differences in bone mineral content or bone area were observed between these groups. Other than a 10% greater ($P < 0.05$) liver mass in HSC compared with STD rats, other body composition parameters including fat mass, lean mass, and percentage fat did not differ between these groups (Table 1). In addition, serum levels of leptin and insulin did not differ between STD and HSC diet groups. Expression of

TNFα, IL1β, and IL6 mRNA in the liver was not altered by HSC diet (Figure 3).

Histopathologic characterization and scoring of periodontitis lesions. No gross alterations in maxillae or mandibles were detectable in high-resolution photographs (data not shown). Maxillary and mandibular histologic periodontitis scores at the M1M2 and M2M3 interdental regions ranged from 0 to 3 in experimental rats (Figure 4 A through D). Periodontal tissues with periodontitis scores of 2 to 3 were present in HSC rats only. Periodontitis scores at the maxillary M1M2 and M2M3 interdental regions both were higher ($P < 0.05$) in HSC rats compared with STD and BSL animals (Figure 4 E). Maxillary periodontitis scores at M1M2 and M2M3 were not significantly different in STD rats compared with BSL rats. Periodontitis scores at mandibular M2M3 were significantly higher in HSC rats ($P < 0.05$) compared with both STD and BSL groups, but periodontitis score at mandibular M1M2 in HSC rats was significantly different from BSL only and not STD (Figure 4 E).

Maxillary and mandibular periodontal lesions at M1M2 and M2M3 interdental spaces were evaluated histologically. Histopathologic findings consistent with periodontitis[91] included hyperplasia of the gingival epithelium (GE) with formation of rete pegs, moderate mononuclear inflammatory cell infiltrate (lymphocytes and macrophages) in the lamina propria (LP), and migration of the junctional epithelium (Figure 5 B through D, Figure 6 B). In addition, pathologic changes were frequently observed in the periodontal ligament area of HSC rats (Figure 5 B, D, and F) compared with STD diet rats (Figure 5 A, C, and E). Specifically, disruption, fragmentation, and separation of fibers in the periodontal ligament, particularly of transeptal fibers and alveolar crest fibers (Figure 5 B and D), was present, as well as interfibrillar mononuclear inflammatory cell infiltrate (lymphocytes and macrophages; Figures 5 D and F) and edema separating the normal arrangement of transeptal fibers and alveolar crest fibers (Figure 5 F). In addition, alveolar bone resorption, characterized by abundant eroded surfaces and the presence of osteoclasts on alveolar bone surfaces of the ABC occurred more frequently observed in HSC rats (Figure 4 D and F) compared with STD rats (Figure 4 C and E).

Maxillary ACH according to histology. Maxillary ABL was greater in HSC diet groups at both interdental regions. Maxillary ACH at M1M2 was greater (indicated as increased CEJ–ABC distance) in rats fed HSC diet compared with STD diet (74 µm difference, $P < 0.05$) and BSL groups (109 µm difference, $P < 0.05$) (Figure 6 A). At M2M3, maxillary ACH was greater in HSC rats compared with STD rats only (65 µm difference, $P < 0.05$; Figure 6 A). Representative microphotographs of the ACH in a STD rat (Figure 6 B) and a HSC rat (Figure 6 C) are shown.

Mandibular ACH according to µCT. Mandibular ABL throughout the ROI was greatest in HSC-fed rats compared with BSL and STD rats; this difference is indicated as the increased ACH (that is, greater CEJ–ABC distance) in HSC-fed rats at M1M2 (272 and 201 µm differences, respectively; $P < 0.05$) and M2M3 (377 and 201 µm, differences respectively; $P < 0.05$; Figure 7 A). To further investigate the ABL pattern observed in experimental rats, we separated the ROI into 2 halves across the buccolingual dimension: 9 µCT 2D slices on the buccal region, and the other 9 on the lingual region. We observed that, overall, ABL was more pronounced in the HSC rats at both the buccal and lingual regions (Figure 6 B and C). At the buccal region, ACH at M1M2 was greater (increased CEJ–ABC distance) in HSC rats compared with BSL (361 µm difference, $P < 0.05$) and STD rats (174 µm difference, $P < 0.05$; Figure 7 B). At the buccal region of M2M3, ACH again was greater in HSC rats compared with BSL

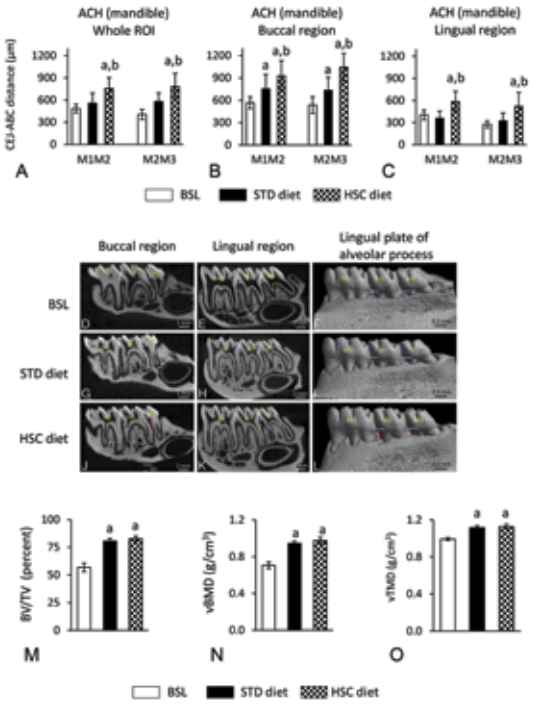

Figure 7. According to µCT, HSC rats had greater mandibular alveolar bone loss, but no differences in alveolar BMD compared with STD rats. (A) HSC rats had greater alveolar bone loss (ABL), as indicated by higher values for alveolar crest height (ACH; that is, greater CEJ–ABC distance) at M1M2 and M2M3 compared with baseline (BSL; $^aP < 0.05$) and STD ($^bP < 0.05$) rats throughout the ROI. No differences in ACH were found between STD diet and BSL control groups ($P > 0.05$). ACH values in HSC rats were significantly greater, indicating ABL, at both the buccal B) and lingual regions (C) compared with BSL ($^aP < 0.05$) and STD ($^bP < 0.05$) rats. (B) At the buccal region, significantly greater ACH values (greater CEJ–ABC distance) at M1M2 and M2M3 were present in STD rats compared with BSL ($^aP < 0.05$). Representative 2D µCT slices showing the ABL pattern at the (D, G, and J) buccal and (E, H, and K) lingual regions in mandibles of (D and E) BSL, (G and H) STD, and (J and K) HSC rats are shown. Note the distance between the CEJ of 2 adjacent molars (horizontal blue line) and the alveolar bone crest (ABC; dotted red line) is greater in (J and K) HSC rats, particularly at the buccal region, and less pronounced at the lingual region, compared with those observed (D and E) at BSL and (G and H) in STD rats. 3D reconstructed µCT images from the lingual surface of representative mandibles from (F) BSL, (I) STD, and (L) HSC rats show a moderate increase in ABL at the lingual plate of the alveolar process observed at M1M2 and M2M3 in a rat from the (L) HSC diet group compared with rats from the (F) BSL and (I) STD groups. (M through O) STD and HSC rats had greater alveolar bone volume (bone volume relative to total volume [BV/TV]), volumetric bone mineral density (vBMD), and volumetric tissue mineral density (vTMD) compared with BSL rats ($^aP < 0.05$). However, no differences in any of these parameters were found between STD and HSC rats ($P > 0.05$).

(508 µm, $P < 0.05$) and STD groups (310 µm difference, $P < 0.05$) groups (Figures 6 B). At the lingual region, ACH at M1M2 was greater (increased CEJ–ABC distance) in HSC rats compared with BSL (181 µm difference, $P < 0.05$) and STD (227 µm difference, $P < 0.05$) groups. At the lingual M2M3 region, HSC-fed rats again showed a greater ACH (increased CEJ–ABC distance) compared with BSL (245 µm difference, $P < 0.05$) and STD (196 µm difference, $P < 0.05$) rats (Figure 7 C). µCT analysis further

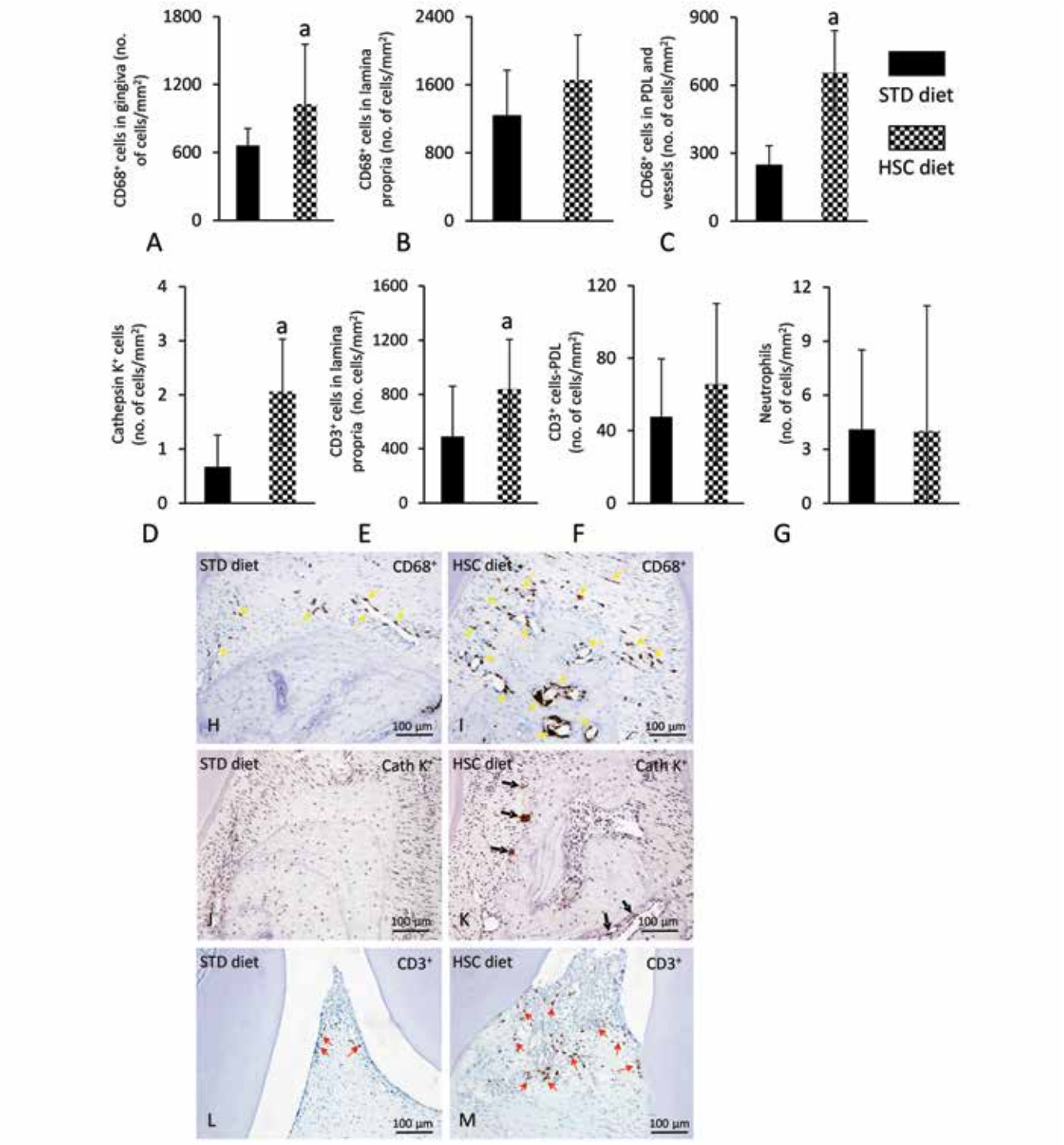

Figure 8. Numbers (mean ± 1 SD) of macrophages, T cells, and osteoclasts were higher in the periodontal tissues of HSC rats. Increased numbers of CD68+ cells were found in the (A) gingiva, (C) periodontal ligament (PDL) and vessels, but not in the (B) lamina propria of HSC diet rats compared with STD diet rats. (H and I) CD68+ cells (yellow arrows) were localized with blood vessels (pericytes) and in the periodontal ligament (macrophages), as well as at alveolar bone surfaces (osteoclasts) yellow arrows) particularly in HSC diet rats. The number of cathepsin K+ cells (black arrows) on bone surfaces was greater in (D, H, and K) HSC rats compared with STD diet rats. More CD3+ T cells (red arrows) were present in the (E and F) lamina propria, but not in the (L and M) periodontal ligament, of HSC rats compared with STD rats. (G) No significant differences in the number of neutrophils in the periodontal tissues between HSC and STD rats.

revealed that ACH values in STD rats were greater (increased CEJ–ABC values) at M1M2 ($P < 0.05$) and M2M3 ($P < 0.05$) at the buccal region, but not at the lingual region, compared with BSL rats (Figure 6 B and C). Representative 2D μCT slices of the ABL pattern at the buccal and lingual regions in the mandibles of the BSL (Figure 7 D and E), STD (Figure 7 G and H), and HSC (Figure 7 J and K) groups are shown.

Representative 3D μCT reconstructed images at the lingual surface of mandibles from BSL, STD, and HSC rats are shown in Figure 6 F, I, and 6 L, respectively. A moderate, qualitative increase in ABL at the lingual plate of the alveolar process is observed at M1M2 and M2M3, as well as mesial to M1, in a rat from the HSC group (Figure 7 L) compared with animals from the BSL (Figure 7 F) and STD (Figure 7 I) groups.

Alveolar bone volume, vBMD, and vTMD according to μCT. To determine whether the HSC diet had effects on alveolar bone in addition to the alterations to ACH, we determined alveolar bone volume, vBMD, and vTMD also. We found that STD and HSC rats had significantly greater alveolar bone volume, vBMD, and vTMD ($P < 0.05$) than BSL rats (Figure 7 M through O). However, none of these parameters differed between STD and HSC animals ($P > 0.05$; Figure 7 M through O).

Inflammatory infiltrate and osteoclast count. In general, HSC rats had a greater number of $CD68^+$ macrophage-lineage cells (Figure 8 A through C), cathepsin K^+ osteoclasts (Figure 8 D), and $CD3^+$ T cells (Figure 8 E and F) than STD rats. There were significantly more $CD68^+$ cells in the GE, periodontal ligament, and blood vessels—but not LP—of HSC rats compared with STD rats (Figure 8 A through C, H, and I). These findings are consistent with the known distribution of CD68, a marker for mononuclear-lineage cells, including macrophages, histiocytes, and osteoclasts.[39,95] In addition, increased numbers of $CD68^+$ cells consistent with osteoclasts were found on alveolar bone surfaces in HSC (Figure 8I) compared with STD (Figure 8 H) rats. Consistent with the increased number of $CD68^+$ cells on alveolar bone surfaces in HSC rats, the number of cathepsin K^+ cells was significantly ($P < 0.05$) greater in HSC rats compared with STD rats (Figure 8 D, J, and K). More ($P < 0.05$) $CD3^+$ T cells were present in the LP but not the periodontal ligament of HSC rats compared with STD rats (Figure 8 E, F, L, and M). The number of neutrophils in the periodontal tissues did not differ between HSC and STD rats (Figure 8 G).

Gene expression in gingiva and oral mucosa. *Th1-associated genes.* mRNA levels of genes associated with the Th1 immune response were assessed, including IFNγ, IL12A, and IL2.[101] IFNγ expression was significantly ($P < 0.05$) higher in the gingiva of HSC rats compared with STD rats (Figure 9 A). In addition, gingival IFNγ expression was significantly ($P < 0.05$) greater than its expression in oral mucosa in HSC rats. IL12A (Figure 9 B) and IL2 (Figure 9 C) did not differ regardless of tissue type or diet.

Th17-associated genes. mRNA expression of genes associated with the Th17 immune response were assessed, including IL23A, IL23B, IL17A, IL1β, and IL6. IL23A was significantly ($P < 0.05$) higher in HSC gingival tissue compared with HSC mucosa ($P < 0.05$), but there was no significant effect of diet (Figure 9 D). Expression levels of IL23B (Figure 9 E), IL17A (Figure 9 F), and IL1β (Figure 9 G) were all significantly higher ($P < 0.05$) in gingival tissues compared with mucosal tissues, but there was no significant effect of diet ($P > 0.05$). IL6 expression in gingival tissue was significantly ($P < 0.05$) greater in HSC compared with STD rats and was significantly ($P < 0.05$) greater than in mucosal tissue (Figure 9 H).

Expression of genes associated with innate immunity and tissue damage. TLR2 and TLR4, which are involved in activation of the innate immune response to recognition of bacterial plaque, were assessed.[88] TLR2 expression was significantly ($P < 0.05$) greater in gingival than mucosal tissues, but there was no effect of diet (Figure 10 A). TLR4 expression did not differ according to diet or tissue type (Figure 10 B). TNFα, another cytokine involved in the regulation of innate immunity,[106] was not altered by diet in gingival tissues and was undetectable in the mucosa (Figure 10 C). The expression of MMP9, which degrades protein matrix,[112] was significantly ($P < 0.05$) greater in gingival tissues compared with mucosa, but there was no dietary effect (Figure 10 D). RANKL, which is required for the differentiation of osteoclasts,[52] was not significantly altered by diet in the gingival tissues and was undetectable in the mucosa (Figure 10 E).

Discussion

In the current study, Lewis rats developed mild-to-moderate generalized periodontitis when fed an HSC diet for 24 wk. The periodontitis involved both maxillae and mandibles, and lesions were characterized by hyperplasia and migration of the GE, inflammatory cell infiltration of the GE and periodontal ligament, disruption of the periodontal ligament, and increased ABL. Slowly progressing, mild-to-moderate periodontitis occurs in approximately 1/3 of adults in the United States.[27,28] Therefore, a rodent model of mild-to-moderate periodontitis that does not require local oral manipulation and that occurs in a readily available laboratory rat species may provide a convenient alternative for organizations and investigators that desire to study periodontitis without the necessity of labor-intensive mechanical induction.

The predominant cell types present in the inflammatory infiltrate were $CD3^+$ T cells, observed in the LP, and $CD68^+$ mononuclear cells, which were observed in the GE, periodontal ligament, and surrounding blood vessels and on alveolar bone surfaces. CD68 is a marker of inflammatory cells of the mononuclear lineage, including macrophages and osteoclasts.[49,50] In addition, CD68 is constitutively expressed in NK and γδ-T cells, and its expression is strongly induced in activated $CD4^+$ and $CD8^+$ T lymphocytes.[46] Furthermore, numerous cathepsin K^+ cells and eroded surfaces were present on the alveolar bone of HSC rats compared with STD rats. Together, these findings and the increased ABL confirm that HSC produced an inflammatory environment in the periodontal tissues and increased osteoclastic bone resorption activity, leading to reduced alveolar bone height. Neutrophils were not a typical inflammatory cell present in the periodontal infiltrates in this model, given that only a few of these cells were identified in periodontal tissues of both HSC and STD rats. Altogether, HSC diet appears to produce a chronic inflammatory response in Lewis rats that leads to lesions in periodontal tissues that are characteristic of periodontitis.

In line with the histologic and immunohistochemical findings, gene expression analyses of gingival and mucosal tissues from Lewis rats revealed that HSC diet rats had greater expression of IFNγ, IL6, and RANKL in the gingiva—but not mucosa—compared with STD rats. Although mRNA expression of TNFα and IL1β in gingiva did not differ between the dietary groups, mRNA levels of these genes were significantly higher in the gingiva compared with the oral mucosa. No significant differences in the mRNA expression of IL2, IL12A, IL12B (IL23B), MMP9, IL17A, and IL23A occurred between dietary groups or tissue locations (gingiva compared with oral mucosa). Overall, the gene expression analysis suggests that the HSC diet induced a

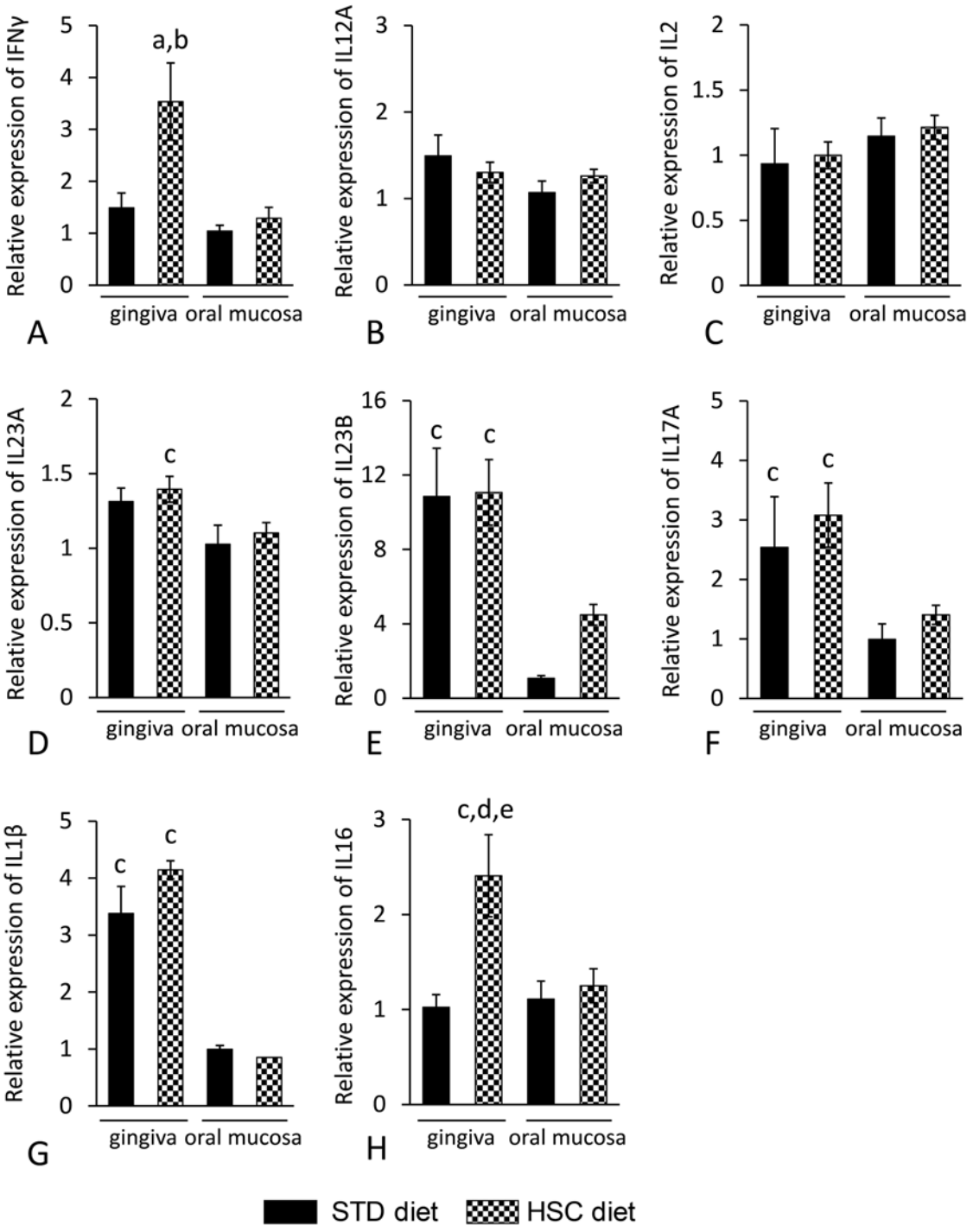

Figure 9. Th1- and Th17-mediated inflammatory mRNA levels (mean ± SEM) in Lewis rats fed HSC diet. (A) IFNγ, (B) IL12A, and (C) IL2, which are all involved in the Th1 response, were assessed. IFNγ expression was higher in HSC gingiva compared with STD gingiva ($^{a}P < 0.05$) and in HSC gingiva compared with HSC mucosal tissues ($^{b}P < 0.05$). IL-12A and IL2 mRNA expression levels were not significantly different within diet or tissue type. (D) IL23A, (E) IL23B, (F) IL17A, (G) IL1β, and (H) IL6, which are all associated with a Th17 response, were assessed. There was significantly higher expression of IL23B, IL17A, and IL1β in gingival tissues compared with mucosal tissues ($^{c}P < 0.05$), but there was not a significant dietary effect. IL6 mRNA expression was significantly higher in HSC gingiva compared with STD gingiva ($^{d}P < 0.05$) or mucosal tissues ($^{e}P < 0.05$). Tissues were normalized to the β-actin loading control and calibrated to STD mucosa. ND, not detectable.

Th1-associated, rather than Th17-associated, immune response in the gingiva, which is closest to the polymicrobial biofilm on the molar surface or in the gingival sulcus. These data are in agreement with previous studies, which demonstrated that specific Th1 responses promote infection-stimulated ABL.[56,102] Indeed, mice that were immunized to develop strong, polarized Th1-based responses to the oral pathogen *Porphyromonas gingivalis* had alveolar bone destruction, numerous osteoclasts, and high local expression of IFNγ, IL1α, and IL1β.[102]

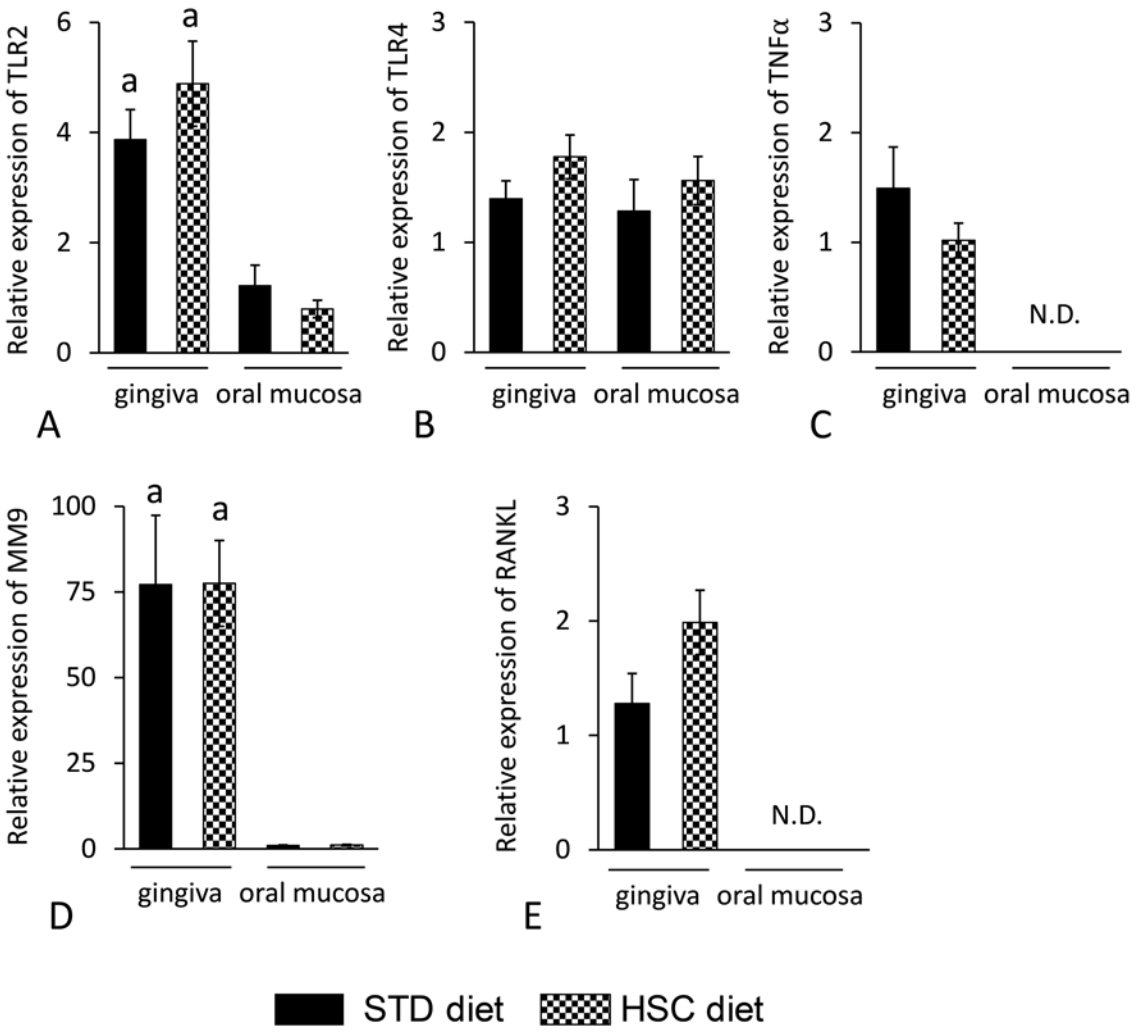

Figure 10. mRNA levels (mean ± SEM) of genes associated with innate immunity and tissue damage in Lewis rats fed HSC diet. Transcription of (A) TLR2 and (D) MMP9 was significantly ($^{a}P < 0.05$) upregulated in gingiva compared with mucosal tissue, but diet had no effect. (B) Neither tissue type nor diet influenced TLR4 expression. (C) TNFα and (E) RANKL were expressed in gingival tissue only and were not detectable (ND) in mucosa. Expression was normalized to the β-actin loading control and calibrated to STD mucosa, except for TNFα and RANKL, which were calibrated to STD gingiva.

Lewis rats are susceptible to several (inducible) autoimmune diseases including uveitis,[29] valvulitis,[32,75] encephalomyelitis,[63,64] and inflammatory arthritis,[38,58,110,120] due to genetic alterations that are characterized by reactivity of the HPA axis. This alteration results in an abnormally higher and prolonged release of glucocorticoids than in other rat strains,[33,109] which thus makes the Lewis rat prone to infectious diseases including periodontitis.[15-17] Periodontitis has previously been proposed to have an autoimmune component,[14] and this association has been supported in several studies.[9,51,85,114] Furthermore, the Th1—and, more recently, Th17—effector responses are associated with the development of autoimmune diseases.[24,61,73,74,118] The upregulation of Th1 response genes and proteins in our current study is supported by other disease mechanisms in the Lewis rat strain, in which Th1 responses[20,30,98] occur after induced autoimmune uveitis[118] and encephalomyelitis.[73]

Periodontitis lesions in our Lewis rats were less severe than reported previously for rice rats (*O. palustris*) under similar laboratory conditions and after the same duration of exposure to HSC diet.[40,41,97,100] Periodontitis in rice rats fed HSC diet appears to be driven by the accumulation of microbial biofilm,[5,7,40] and early studies in rice rats have shown the pivotal roles of a HSC diet and the oral microbial flora in the induction of periodontitis.[42] However, rice rats fed HSC diet appear to develop lesions that rapidly progress to a level of severity that resembles severe human periodontitis, which is clinically observed less frequently than the more frequent, milder cases.[5,7,10,34,40,41,47,97] In contrast to rice rats, *Rattus* rodents do not develop spontaneous periodontitis[37,93,103] under standard laboratory conditions. Typically, ligatures are used to induce the host immune response necessary to produce inflammation-mediated damage to the periodontium. Recent studies using ligature models in *Rattus* show soft tissue alterations (GE hyperplasia, inflammatory infiltrate, and junctional epithelial migration) and ABL in the range of 200 to 350 µm that is localized to alveolar bone proximal to the ligatured molar.[59,66,111] Our Lewis rats developed similar soft tissue alterations and had maximal ABL within a similar range as seen for ligature models, but in contrast to those models Lewis rats appeared to develop a more generalized form of periodontitis that affected all jaw quadrants. This finding is supported by the loss of alveolar bone in both interdental regions (M1M2 and M2M3) in both maxillae and mandibles. Therefore, the current findings

suggest that Lewis rats fed HSC diet may be promising as a model of generalized periodontitis in rats (*Rattus* spp.) without the need to perform additional mechanical oral intervention. These findings also suggest that prolonging exposure to HSC diet may produce more severe levels of periodontitis.

Although the HSC diet produced periodontitis in Lewis rats, metabolic alterations due to the HSC diet might arise, including hyperglycemia and glucose intolerance, insulin resistance, hyperinsulinemia or hypertriglyceridemia, and hypertension might occur in the absence of obesity or changes in fasting plasma glucose and leptin levels.[4,22,43,45,71,72] Diabetes alters wound healing[81,87] and is associated with increased periodontitis prevalence and increased severity in humans, which may produce a confounding effect in diseased tissues.[62,107] However, HSC feeding to rice rats did not induce significant alterations in weight gain or metabolic disturbances characteristic of diabetes, even after 26 wk.[7] Likewise, in the current study, there were no significant alterations in body composition, body weight, leptin and insulin serum levels, or liver cytokine gene expression, suggesting that our Lewis rats did not develop alterations in metabolic outcomes and that the HSC diet formula we used here poses no systemic health risk during 24 wk of exposure.

In conclusion, administration of a diet high in sucrose and casein induced mild-to-moderate generalized periodontitis in Lewis rats that was characterized by a Th1 immune response in periodontal tissues but without producing significant metabolic abnormalities. Importantly, no mechanical interventions were needed to induce periodontitis. Taken together, these findings suggest that feeding an HSC diet to Lewis rats produces a viable alternative for inducing mild periodontitis. Further studies are necessary to better characterize the model, including assessment of the oral polymicrobial community, investigation of the host–bacteria interrelationship, and more comprehensive analysis of the immune response of the rats to the diet.

Acknowledgments

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