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Effects of Caloric Restriction on Inflammatory Periodontal Disease

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Abstract

Objective—Dietary caloric restriction (CR) has been found to reduce systemic markers of inflammation and may attenuate the effects of chronic inflammatory conditions. The purpose of this study was to examine the effects of long-term CR on naturally occurring chronic inflammatory periodontal disease in a nonhuman primate model.

Methods—The effects of long-term CR on extent and severity of naturally occurring chronic periodontal disease, local inflammatory and immune responses, and periodontal microbiology, were evaluated in a cohort of 81 (35 female and 46 male; 13–40 years of age) rhesus monkeys (*M. mulatta*) with no previous exposure to routine oral hygiene. The CR monkeys had been subjected to 30% CR for 13–17 years relative to control-fed (CON) animals starting at 3–5 years of age. Clinical and laboratory parameters were submitted to analysis of covariance, including Tukey's test for post hoc comparisons, linear regression analysis, and nonparametric correlation analysis.

Results—Same sex CR and CON monkeys exhibited comparable mean scores for plaque index, calculus index, and bleeding on probing. Among CON animals, males showed significantly greater periodontal breakdown, as reflected by higher mean clinical attachment level (CAL) and periodontal probing depth (PD) scores, than females ($p \leq 0.05$). CR males had significantly less periodontal pocketing compared to CON males ($p \leq 0.05$). CR males demonstrated a significantly lower IgG antibody response and lower levels of IL-8 and β -glucuronidase in gingival crevicular fluid compared to control males. A similar but nonsignificant reduction was found for IL-1 β in CR male monkeys. In contrast, CR females exhibited mean PD and CAL scores comparable to CON females. CR females had a lower IgG antibody response but comparable levels of inflammatory markers in GCF compared

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to CON females. The CR diet had no demonstrable effects on the periodontal microbiota in male or female monkeys.

Conclusion—Males exhibited a greater risk for naturally occurring periodontal disease than females, consistent with findings from human populations. Moreover, long-term CR appears to differentially reduce the production of local inflammatory mediators and the risk for inflammatory periodontal disease among males but not females.

Keywords

monkey; caloric restriction; inflammation; periodontal disease; sex

INTRODUCTION

Oxidative stress and inflammation are considered important factors in the biology of aging and the etiology of many age-associated degenerative diseases. Recent studies have focused on the relationship of inflammatory mediators and markers, such as cytokines and acute phase proteins, to aging and age-related disease. [9,20,31] Circulating levels of interleukin-6 (IL-6), a pleiotropic inflammatory cytokine, have been consistently found to be elevated in association with low-grade chronic inflammation and advancing age. [7,8] Moreover, elevations in circulating IL-6 levels and chronic low-grade inflammation have been found closely associated with obesity and the metabolic syndrome of aging. [52,54,62]

Dietary CR is widely accepted as the only non-genetic method for altering physiological processes related to aging.[34] Adipocytes produce cytokines, including TNF- α and IL-6, which induces the synthesis of C-reactive protein (CRP) by the liver. [37,52] CR and weight loss lower serum levels of IL-6 and CRP in humans and nonhuman primates. [23] CR also appears to attenuate the age-associated up-regulation of NF- κ B, cyclooxygenase-2, and inducible nitric oxide synthase. [13,25,26,44,53,54,56] Therefore, CR may dampen the host inflammatory response and attenuate the effects of chronic inflammation. [23]

Periodontal disease is one of the few natural inflammatory conditions that can be readily visualized and studied in humans and other animals. Periodontal disease is characterized by inflammatory and immune responses to bacterial colonization involving the soft and hard tissues of the periodontium. Inflammatory periodontal disease is often chronic in nature and can result in connective tissue degradation and alveolar bone resorption in a susceptible host. Individual differences in immune responsiveness may explain differential susceptibility to periodontal breakdown. Inflammatory molecules, including cytokines and products of arachidonic acid metabolism, mediate the clinical manifestations of periodontal disease. Moreover, sustained elevations in circulating levels of inflammatory mediators have been postulated as the basis for the association between periodontal disease and other systemic inflammatory conditions, such as atherosclerotic heart disease and stroke. [4,5,24]

This study determined the effects of CR on the extent and severity of periodontal disease, local inflammatory and immune responses, and oral microbial colonization in a cohort of male and female non-human primates (rhesus macaques) that are part of a longitudinal study of CR and aging. [27] Given that CR attenuates age- and body weight-associated increases in systemic inflammation, we hypothesized that CR would decrease the prevalence and severity of local markers of inflammation and chronic periodontal disease.

MATERIAL AND METHODS

Rhesus monkeys and Diet

The study included 81 rhesus monkeys (*Macaca mulatta*) (35 female and 46 male) that are part of a longitudinal investigation of the effects of CR on aging conducted through the National Institute on Aging. [27] The protocol was approved by the Gerontology Research Center Animal Care and Use Committee and the National Institutes of Health Animal Center (Poolesville, MD), which is accredited by the Association for Accreditation of Laboratory Animal Care. The ages of monkeys ranged from 13–23 years for females and 16–33 years for males. The monkeys live in a controlled environment with a standard diet and are well characterized with regard to health status [27,35]. The CR monkeys have been subjected to a 30% reduction in dietary caloric intake relative to CON animals for periods of 13–17 years. [27] All animals received the same diet which was supplemented with minerals and vitamins up to 100% daily allowance for CR animals. The diet was supplemented once weekly with fresh fruit for all monkeys.

Clinical Examination

All animals were anesthetized and examined by the same periodontal investigator (MAR) in this cross-sectional study. The periodontal examination was conducted using a Maryland probe (William's markings) on the facial aspect of all teeth. Two proximal sites per tooth (mesio- and disto-buccal) were assessed for each tooth excluding the canines and 3rd molars. The periodontal examination included the following clinical measures: probing pocket depth (PD), clinical attachment level (CAL), plaque index (PII) [40], bleeding on probing (BOP) [51], and calculus index (CI). [48] The plaque index and calculus index characterize the extent and quantity of tooth-associated bacterial plaque and calculus (tartar), respectively. PD and CAL measures are made using a calibrated probe and characterize the depth of the gingival crevice/pocket and level of gingival attachment to the tooth, respectively. Periodontal breakdown is associated with increased pocketing and clinical attachment loss. The presence and degree of bleeding upon gentle periodontal probing provides a measure of the inflammatory changes in the lining of the gingival pocket.

Gingival Crevicular Fluid (GCF) Collection

Gingival sites were isolated and gently dried using cotton gauze. Absorbent filter strips (Periopaper strips, Oraflow, Inc.) were placed below the gingival margin at mesial sites of a premolar, 1st and 2nd molar teeth in the maxillary and mandibular quadrants on one side of the mouth. The strips were maintained, isolated from saliva, for a period of approximately 15–30 seconds. The filters were removed and the fluid volume determined using a Periotron 8000 that was calibrated, with a standard curve determined for each collection period. [11]

Inflammatory Mediators—IL-1 β TNF- α , IL-8 and PGE₂ levels in GCF were quantified using commercial ELISA kits as we have described previously [12,61]. β -glucuronidase level in GCF was determined colorimetrically. [33]

Subgingival Bacterial Plaque—Subgingival plaque specimens were obtained from mandibular molars, following isolation and supragingival debridement, using a Gracey curette that was inserted to the base of the periodontal sulcus prior to removal and plaque collection. Plaque specimens were pooled for each animal and placed into a pre-labeled vial containing Tris-EDTA buffer and stored at –80°C for subsequent DNA isolation.

Each pooled plaque sample was thawed and thoroughly dispersed by vortexing. Sodium hydroxide (0.5 M) was added and each sample was boiled for 5 minutes then neutralized using 0.8ml of 5M ammonium acetate. The resulting isolated DNA samples were separated into two

aliquots and stored at -20°C for characterization by checkerboard DNA-DNA hybridization and quantitative polymerase chain reaction (qPCR).

One aliquot of the DNA sample collected for the cross-sectional analysis was placed into the extended slots of a Minislot (Immunetics, Cambridge, MA), and immobilized on a nylon membrane (Roche Diagnostics/Boehringer Mannheim Corp., Indianapolis, IN) by exposure to UV light followed by baking at 120°C for 20min. The plaque samples were evaluated by “checkerboard hybridization” using digoxigenin-labeled whole genomic probes DNA probes to a panel 40 different bacteria found in the oral cavity that are representative of the predominant bacterial species presumed to be associated with pathogenic plaque. Following a two-hour prehybridization, the DNA probes were allowed to hybridize overnight with the sample at 42°C . After a series of stringent washes, hybridization was detected using anti-digoxigenin antibody and enhanced chemiluminescence. Sample evaluation was performed by comparison of the signals obtained with those from controls containing 10^4 – 10^7 of each species. [60]

DNA samples were also evaluated by quantitative real-time polymerase chain reaction (qPCR) using a LightCycler 2.0, as previously described [29], for the following target periodontal pathogens: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Campylobacter rectus*. Briefly, each PCR was performed in a total volume of 20 μl containing 2 μl of 10X LightCycler FastStart DNA Master SYBR Green I 0.5 μM each of HPLC purified forward and reverse primers (Table 1), 4mM MgCl_2 , and 1ml of template DNA. Real-time PCR was carried out with an initial incubation of 10 min at 95°C followed by 45 cycles consisting of denaturing for 95°C for 10 sec; annealing for 5 sec (Table 1) followed by amplification at 72°C for X sec, where X is the amplification time in seconds (product size/25). At the end of each cycle, fluorescent products were detected by the LightCycler 2.0 (Roche Diagnostics/Boehringer Mannheim Corp., Indianapolis, IN).

Quantification analysis was performed using LightCycler 4.0 software (Roche Diagnostics/Boehringer Mannheim Corp., Indianapolis, IN). Standard curves were generated for all the target bacteria using DNA from pure cultures and species specific DNA primers. DNA isolated from a pure culture of *P. gingivalis* was used in generating a standard curve for the universal primers. [17,42] The number of copies of each microorganism in a given plaque DNA was calculated using the following formula: (Avogadro constant $\times$ amount of DNA in $\mu\text{g}/\mu\text{l}$) / (genome size $\times$ mw), where mw is the molecular weight per base pairs or nucleotide which is 660. [6,19]

Statistical Analysis

Clinical Measures—In the primary analysis, the effects of age and CR were analyzed separately by sex due to different age distributions. Data were submitted to analysis of covariance (ANCOVA) using age (modeled both as a linear variable and as a grouping variable) and subspecies as covariates. In secondary analyses, data were submitted to a linear regression analysis in which sex was included in the model. The purpose of the secondary analysis was to determine the robustness of the results. In the case of CAL and PD measurements, the data were analyzed with both PI in the model and out of the model, to determine if it would explain any diet effects. A subgroup analysis was conducted to examine the effects of sex on the clinical measures in which the age ranges for males and females were corrected by censoring males over the age of 30 years in the analysis. The latter analysis provided a more stringent assessment of the effects of sex, given that upper age for males exceeded that of females. Finally, a subgroup analysis was conducted on the number of monkeys exhibiting signs of periodontitis, defined on the basis of mean CAL (≥ 2.0 mm) and PD (≥ 3.0 mm), using logistic regression analysis. PD was included in the analysis because of the higher error of measurement for CAL

(measured as difference from the cemento-enamel junction to the base of the sulcus/pocket) than for PD in human trials. [63,64]

Inflammatory Markers and Target Bacteria—Primary statistical analysis assessed the univariate relationships of GCF measures with diet by gender and relationships adjusting for age within each gender. Secondary analyses included nonparametric correlations (Spearman Coefficient) among measures and linear regression to model each laboratory parameter with gender, diet, and interaction between gender and diet with age as a covariate. The statistical analysis was performed using JMP 6 (SAS Institute, Cary, NC), with a significance level set at 0.05.

RESULTS

Clinical Measures

The mean age of the females was approximately 4 years younger than the males ($p \leq 0.0005$); however, there were no statistically significant differences in mean age of CON and CR monkeys within sex groups (Table 2); however, the distribution of ages for each sex were dissimilar. Therefore, due to this difference in distribution, the primary analysis of CR effects was limited to testing within each sex.

Among animals in the CON groups, PD and CAL exhibited a significant correlation with age in males ($r = 0.67$ and 0.63 , respectively, $p \leq 0.01$) and females ($r = 0.44$ and 0.46 , respectively, $p \leq 0.05$). The association of pocketing and attachment level with age remained significant when the plaque index was included as a covariate in regression analysis. Among CON animals, the plaque index exhibited a significant correlation with age in males ($r = 0.39$, $p \leq 0.05$) but not females ($r = 0.17$, $p > 0.05$), whereas the calculus index demonstrated a positive correlation with age in males ($r = 0.47$, $p \leq 0.05$) and females ($r = 0.55$, $p \leq 0.01$).

CON males exhibited significantly greater pocketing and attachment loss than CON females. The difference between CON males and females in mean PD (2.74 ± 0.12 and 2.28 ± 0.05 , respectively) and CAL (1.56 ± 0.09 and 1.29 ± 0.04 , respectively) remained significant after correcting for differences in the age ranges for males and females by censoring males over the age of 30 years in the analysis. In contrast, CR males and females were found to have comparable mean PD (2.49 ± 0.12 and 2.51 ± 0.11 , respectively) and CAL (1.43 ± 0.07 and 1.42 ± 0.07 , respectively), after correcting for sex differences in age distribution.

Table 3 summarizes the effects of CR on the clinical measures as a function of sex. For both males and females, CON and CR animals were found to have comparable mean PII, CAL, and BOP scores. In contrast, CR males exhibited significantly less periodontal pocketing, as reflected in mean PD, compared to CON males. CR males also showed evidence of a similar but nonsignificant trend towards lower mean CAL compared to CON males (1.55 ± 0.09 and 1.76 ± 0.11 , respectively, $p \leq 0.10$). CR females, however, exhibited comparable levels of pocketing and clinical attachment loss as CON females.

In the complete factorial model, males were found to have significantly higher mean scores for PII, CAL, PD, and CI compared to females, when collapsing across age groups; however, when age was added to the analysis, the gender effects became nonsignificant. A significant interaction between sex and diet emerged for PD and CAL in the analysis. CR males exhibited a significantly lower mean PD and CAL than observed for CON males ($p < 0.05$), whereas no dietary effects were observed for any clinical measure between CR and CON females. The subgroup differences in CAL became nonsignificant when correcting for multiple comparisons. The effect of CR on pocket depth in males could not be explained by the addition of plaque or calculus indices as covariates in the analysis.

Table 4 summarizes the number of monkeys exhibiting signs of periodontitis, defined on the basis of mean CAL (≥ 2.0 mm) and PD (≥ 3.0 mm). The proportion of females with evidence of periodontal breakdown based on CAL and PD was low and comparable between diet groups. In contrast, the proportion of males exhibiting PD scores consistent with periodontal breakdown was non-significantly higher ($p = 0.08$) in the CON group (34.6 %) compared to the CR group (31.5 %). No difference in the proportion of males with periodontitis was found based on CAL between diet groups.

Inflammatory Markers

Figure 1 shows the GCF levels of IL-1 β TNF- α , PGE₂, IL-8 and β -glucuronidase. The IL-1 β levels in GCF were nearly 50% lower in the CR compared to the CON male monkeys; however, this difference failed to reach statistical significance given the variability in GCF levels within each group. No differences in levels of TNF- α or PGE₂ were observed in relation to diet or sex. IL-8 levels in GCF were significantly higher in control males compared to control females ($p < 0.05$), with levels in the CR males comparable with those seen in the control female group. CR males exhibited significantly lower GCF levels of β -glucuronidase. A similar but not significant reduction in β -glucuronidase was found with the CR females compared to CON females.

Figure 2 presents the levels of IgA and IgG in GCF by gender and diet. No significant overall differences were found in GCF levels of IgA or IgG between male and female monkeys. However, CR was associated with significant reductions in the levels of IgG for both females and males compared to same-sex CON animals ($p < 0.05$).

Periodontal Microbiology

The distribution of the 40 target bacteria within the entire cross-sectional sample population was assessed by checkerboard hybridization. [60] Although the levels varied, all 40 bacterial species were present in this animal population (data not shown). Each individual species represented $< 6\%$ of the total DNA, with the exception of *Porphyromonas gingivalis* ($> 10\%$), *Fusobacterium nucleatum* ss. *polymorphum* (6–8%) and *Eikenella corrodens* (6–8%). Analysis of these data relative to age, sex, and diet demonstrated that there were no significant differences in bacterial profiles based upon these three variables (data not shown).

Similar results were obtained when evaluating the data obtained from qPCR analysis of seven selected target periodontal bacteria. Varying levels of all seven bacterial species were detected by qPCR. Again, analysis of these data relative to age, gender and diet demonstrated that there were no significant differences in either log counts or % total DNA of any of the seven species under evaluation (Figure 3).

A comparison of the two methods (checkerboard hybridization and qPCR) for detecting and enumerating the seven target bacterial species revealed statistically significant positive correlations for six of the seven target bacterial species: *A. actinomycetemcomitans* ($r = .33$), *P. gingivalis* ($r = .69$), *T. denticola* ($r = .41$), *T. forsythia* ($r = .55$), *F. nucleatum* ($r = .41$), and *C. rectus* ($r = .30$). Only levels of *P. intermedia* failed to exhibit a significant concordance using the two methods of evaluation ($r = -.01$).

In evaluating the impact of the microflora on clinical parameters, the PII score exhibited a significant inverse relationship with *F. nucleatum* levels ($r = -.40$, $p \leq 0.05$), reflecting a lower proportional recovery of this microorganism with increasing biomass formation and complexity. *P. gingivalis* levels were significantly correlated with PD ($r = .25$, $p \leq 0.05$), CAL ($r = .31$, $p \leq 0.005$), and bleeding on probing ($r = .25$, $p \leq 0.05$). Although the percentage of *P. intermedia* was low in all animal groups (Figure 3), levels of this bacterium were

significantly correlated with probing depth ($r = .24$, $p \leq 0.05$) and attachment loss ($r = .26$, $p \leq 0.05$).

DISCUSSION

Efforts to study the incidence, prevalence, and natural progression of inflammatory periodontal diseases in humans have been hampered by the complex and multi-factorial nature of the disease, including confounding factors, such as smoking and medications. Prevalence studies in the U.S. suggest that approximately one-third of the dentate adults aged 30 to 90 have periodontitis, with 10% or more having moderate or severe disease. [1,2] Nevertheless, the role of diet and age-related processes in periodontal disease susceptibility and progression remains unclear in humans. [39,49,58] Nonhuman primates, such as the rhesus monkey, provide an important model for studying inflammatory periodontal disease and oral host-parasite interactions. [10,18,38,57]

In this cross-sectional study, the extent and severity of the pathologic changes associated with inflammatory disease, measured clinically by periodontal pocketing and attachment loss, were shown to increase with advancing age among males and females on a controlled laboratory diet. Although older males exhibited greater bacterial plaque and calculus deposits than younger monkeys, individual levels in tooth-associated deposits failed to explain overall age-related differences in periodontal breakdown in the multivariate analysis. No sex differences were found in the target bacterial pathogens. The more modest age-associated increase in periodontal disease in females, when compared to males, may reflect an insufficient age distribution with which to characterize age-related differences in severity of disease. The age-associated increase in severity of periodontal disease is consistent with human studies documenting moderate to severe levels of attachment loss in a high percentage of middle-aged and elderly individuals. [39]

Epidemiological studies generally report a higher prevalence of periodontitis in men than women; however, it remains unclear whether this sex variation in disease reflects differences between men and women in exposure to risk factors, such as cigarette smoking. [2,30] In this study, CON males were found to have significantly deeper periodontal pockets and greater clinical attachment loss than females, when controlling for age and levels of bacterial plaque and calculus. This difference in severity of periodontal breakdown is important and consistent with an underlying sex difference in host-parasite interaction. CR males also exhibited significantly deeper periodontal pockets than CON females.

An analysis of selected periodontal pathogens revealed no significant differences in either log counts or percent total DNA of any of the target bacterial species, suggesting that the effects of CR were not attributable to shifts in oral bacterial colonization. *P. gingivalis* and *P. intermedia* exhibited significant correlations with clinical measures of periodontal disease, as in humans, consistent with an important role for these bacteria in periodontal disease in this nonhuman primate. The results of the present study, therefore, suggest that men are at greater risk for periodontal breakdown than women secondary to differences in host-parasite interactions, such as immunologic and inflammatory responsiveness, rather than resulting solely from behavioral and environmental factors.

Given that CR reduces age-associated increases in markers of systemic inflammation, we hypothesized that CR would dampen the local inflammatory response to chronic bacterial biofilm contact, as reflected in reductions in inflammatory markers and measures of periodontal destruction. In this study, CR males were found to exhibit significantly less periodontal pocket formation than CON males. The proportion of males exhibiting PD scores consistent with periodontal breakdown was higher in the CON group (34.6 %) compared to the CR group (15.0 %).

%), although this difference failed to reach statistical significance. CR males also showed evidence of a similar but nonsignificant trend towards less clinical attachment loss when compared to CON males. No difference in the prevalence of disease was found based on CAL among males between diet groups. In the absence of differences the height of marginal gingiva, PD serves as an accurate proxy measure of clinical attachment loss, given the larger error in measurement for CAL compared to PD. [63,64] In contrast, CR females exhibited comparable levels of pocketing and clinical attachment loss to CON females. The proportion of females with evidence of periodontal breakdown based on CAL and PD was low and similar between diet groups.

The differential effect of CR on severity of periodontal pocket formation in relation to sex is consistent with recent studies suggesting that men and women differ in their response to chronic inflammation. In humans, chronic low-grade inflammation in aging appears to promote an atherogenic profile that is related to other age-associated disorders (*e.g.*, Alzheimer disease, atherosclerosis, type 2 diabetes) and enhanced mortality risk. [8,46] Of particular interest is that males carry a disproportionate burden of both periodontal disease and cardiovascular disease. Desvarieux and coworkers reported evidence that long-term periodontitis was related to subclinical atherosclerosis in men but not women. [16] Moreover, periodontal disease has been shown to be an independent risk factor for cerebral ischemia in men. [22] The results of the present study suggest that CR may result in differential reductions in inflammatory responsiveness in men and women.

The beneficial effects of CR on periodontal health in males may be attributable to the potential attenuation of both oxidative stress and pro-inflammatory cytokines [14], perhaps secondary to reductions in body weight, as shown across the lifespan in the CR monkeys. [45] In the present study, IL-8 levels were significantly higher in control males compared to control females, and the higher levels in the fed control males were significantly reduced by CR to levels that were comparable with those seen in the control female group. In addition, IL-1 β levels in GCF were nearly 50% lower in the CR males compared to the fed control monkeys, although this difference failed to reach statistical significance due to variability in GCF levels in both dietary groups. No differences, however, were observed in levels of TNF- α or PGE₂ in relation to diet. The observed differences in IL-8 levels would suggest a more exuberant local innate immune response in control males compared to females, consistent with clinical findings of more severe periodontal disease. The effect of CR on reducing IL-8 levels in the GCF of males was consistent with the ability of CR to reduce the clinical manifestation of periodontal disease in the same group. CR was also found to significantly reduce levels of β -glucuronidase in males, suggesting a dietary effect on the number and/or function of polymorphonuclear leukocytes in the gingival lesions. While a similar trend was found with the females, this difference was not statistically significant. Collectively, these findings point to potentially important sex-related differences in both local innate immune response and modulatory effects of dietary restriction, concordant with the observed differences in periodontal disease.

In humans, both obesity and metabolic syndrome are associated with increased markers of systemic inflammation as well as periodontal disease. [7,32,52,54,65] Dietary CR and weight loss lower circulating levels of IL-6 and CRP in humans and nonhuman primates. [23] In vitro studies, however, have yielded inconsistent results with respect to peripheral blood mononuclear cells [28,43], consistent with a potential role of adipocytes and other cells in circulating levels of inflammatory mediators. [37,62] The differential effects of CR on reducing periodontal pocketing parallel the effects of CR on reducing body weight, which has been shown to be consistently more pronounced in males compared to females in these monkeys. [45]

Other effects attributable to CR may have contributed to the observed dietary effects on periodontal breakdown in the male monkeys. Diabetes mellitus, for example, is associated with increased risk of periodontal disease in the rhesus monkey [50], and CR has been shown to result in reductions in plasma insulin levels and greater sensitivity in these animals. [36] Oral bacterial colonization patterns appear to change with advancing age in humans, which may influence the risk of periodontal disease. [47] In this study, although greater accumulation of tooth-associated materials were found in male than female monkeys, no differences were found in the periodontal bacterial species relative to age, gender and diet. Similarly, no significant overall sex-related differences were found in local antibody responses. Interestingly, however, CR was associated with significant reductions in the levels of IgG for both females and males compared to same-sex ad libitum-fed animals. The latter findings suggest that CR modulates the antibody response to biofilm exposure, since no differences were evidenced in the distribution or bacterial profiles of 40 target bacteria in relation to diet.

The results of this cross-sectional study revealed that males exhibit greater risk for inflammatory periodontal breakdown than females. Moreover, the findings clearly suggest that CR differentially reduces the risk for inflammatory periodontal disease in males but not females and these differences may be due to the modulating effects of CR on the immune-inflammatory response.

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Fig. 1.

Levels of inflammatory mediators (mean $\pm$ standard error) in gingival crevicular fluid (GCF) for male and female rhesus monkeys in the control (CON) and caloric restriction (CR) diet groups. (A) IL-1 β (interleukin-1 β); TNF α (tumor necrosis factor α); (B) PGE₂ (prostaglandin E₂), IL-8 (interleukin-8); (C) β -glucuronidase.

Fig. 2. Levels of IgA and IgG (mean $\pm$ standard error) in gingival crevicular fluid (GCF) for male and female rhesus monkeys in the control (CON) and caloric restriction (CR) diet groups.

Fig. 3. Percentage of target bacterial species (mean $\pm$ standard error) recovered in subgingival plaque from male and female rhesus monkeys in the control (CON) and caloric restriction (CR) diet groups. Data were generated using quantitative polymerase chain reaction for individual and the total of all seven target bacterial species (SUM). *Aa*, *Aggregatibacter actinomycetemcomitans*; *Pg*, *Porphyromonas gingivalis*; *Td*, *Treponema denticola*; *Tf*, *Tannerella forsythia*; *Pi*, *Prevotella intermedia*; *Cr*, *Campylobacter rectus*; *Fn*, *Fusobacterium nucleatum*.

Table 1

16S rDNA Bacterial Primers and Annealing Temperatures Used in Quantitative Real Time Polymerase Chain Reaction

Organism	Sequence	Size (bp) *	Annealing temperature	Reference
Universal	TCCTACGGGAGGCAGCAGT	466	58	[41]
	GGACTACCAGGGTATCTAATCCTGTT			
<i>Aggregatibacter actinomycetemcomitans</i>	CTAGGTATTGCGAAACAATTG	262	55	[21]
	CCTGAAATTAAGCTGGTAATC			
<i>Porphyromonas gingivalis</i>	AGGCAGCTTGCCATACTGCG	404	60	[55]
	ACTGTTAGCAACTACCGATGT			
<i>Treponema denticola</i>	TAATACCGAATGTGCTCATTTACAT	316	59	[55]
	TCAAAGAAGCATTCCTCTTCTCTTA			
<i>Tannerella forsythia</i>	GCGTATGTAACCTGCCCCGA	641	62	[55]
	TGCTTCAGTGTCAATTATACCT			
<i>Prevotella intermedia</i>	CGTGGACCAAAGATTCATCGGTGGA	259	64	[3]
	CCGCTTACTCCCCAACAAA			
<i>Fusobacterium nucleatum</i>	AGAGTTGATCCTGGCTCAG	360	60	[15]
	GTCATCGTGCACACAGAATTGCTG			
<i>Campylobacter rectus</i>	TTTCGGAGCGTAAACTCCTTTTC	598	60	[59]
	TTTCTGCAAGCAGACACTCTT			

* Expected product size

Table 2
Age of Male and Female Monkeys within Dietary Groups

Sex	Group	N	Age
Female	CON	19	18.7 ± 5.6
	CR	16	16.9 ± 4.9
Male	CON	26	22.4 ± 6.1
	CR	20	22.7 ± 6.8

Note: Values for age (years) represent mean ± standard deviation.

Table 3
Clinical Measures for Male and Female Monkeys within Dietary Groups

Sex	Diet	N	PI	PD	CAL	BOP	CI
Female	CON	19	1.44 ± 0.11	2.37 ± 0.07	1.36 ± 0.05	1.74 ± 0.12	0.94 ± 0.10
	CR	16	1.25 ± 0.11	2.49 ± 0.11	1.41 ± 0.07	1.77 ± 0.09	1.09 ± 0.09
Male	CON	26	1.73 ± 0.12	2.93 ± 0.12 [*]	1.76 ± 0.11	1.89 ± 0.12	1.15 ± 0.06
	CR	20	1.67 ± 0.16	2.60 ± 0.11	1.55 ± 0.09	1.78 ± 0.12	1.23 ± 0.12

Note. Values represent mean ± standard error. Treatment effects held whether age was modeled by a group variable or by a linear variable. CON (Control); CR (Caloric Restriction); PI (Plaque Index); PD (Probing Depth); CAL (Clinical Attachment Level); BOP (Bleeding on Probing); CI (Calculus Index)

^{*} p < 0.05 significantly different from CR

Table 4
Frequency of Periodontal Sites with Probing Depths ≥ 3 mm or Clinical Attachment Loss ≥ 2 mm

Sex	Diet	PD		CAL	
		≥ 3 mm	< 3 mm	≥ 2 mm	< 2 mm
Female	CON	1	18	1	17
	CR	3	13	0	20
Male	CON	9*	17	6	17
	CR	3	17	3	19

Note. CON (Control); CR (Caloric Restriction); PD (Probing Depth); CAL (Clinical Attachment Level).

* $p \leq 0.08$ CON versus CR