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The Effects of a Calorie Reduced Diet on Periodontal Inflammation and Disease in a Non Human Primate Model

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Abstract

Background—Low calorie diets are commonplace for reducing body weight. However, no information is available on the effects of a reduced calorie diet on periodontal inflammation and disease. The purpose of this study was to evaluate the clinical effects of a long term calorie restricted diet (CR) on periodontitis in an animal model of periodontitis.

Methods—Periodontitis was induced in 55 young, healthy, adult rhesus monkeys (*Macaca mulatta*) by tying 2.0 silk ligatures at the gingival margins of maxillary premolar/molar teeth. Animals on a CR diet (30% CR; n=23) were compared to ad libitum diet controls (n=32). Clinical measures including plaque (PLI), probing pocket depth (PD), clinical attachment level (CAL), modified Gingival Index (GI) and bleeding on probing (BOP) were taken at baseline and 1, 2, and 3 months after ligature placement.

Results—Significant effects of CR were observed on the development of inflammation and the progression of periodontal destruction in this model. When compared to controls, CR resulted in a significant reduction in ligature induced GI (p<0.0001), BOP (p<0.0015), PD (p<0.0016), and CAL (p<0.0038). When viewed over time, periodontal destruction, as measured by CAL, progressed significantly more slowly in the CR animals than in the controls (p<0.001).

Conclusions—These clinical findings are consistent with available evidence that CR has anti-inflammatory effects. Moreover, these experimental findings are the first observations that CR dampens the inflammatory response and reduces active periodontal breakdown associated with an acute microbial challenge.

Keywords

Caloric restriction; periodontitis; ligature model; inflammation; non human primate

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None of the investigators in this study have a conflict of interest.

INTRODUCTION

The deleterious effects of obesity and type 2 diabetes mellitus on the severity and extent of periodontal disease have been reported previously.^{1–3} In contrast, cross sectional studies suggest that maintenance of normal body weight by a healthy diet and exercise, significantly reduces the prevalence and severity of periodontitis.¹ For individuals needing to lose weight, calorie restricted diet programs have become extremely popular and successful. However, nothing is known of the effects of a calorie restricted diet on the initiation and progression of periodontal disease. Dietary caloric restriction (CR) has been found to alter gene expression, attenuate free-radical induced cell damage, and sustain more robust host responses that protect against deleterious extrinsic and intrinsic challenges to normal cell, tissue, and organ function.^{4–10} In addition to host resistance mechanisms, CR appears to preserve hormonal balance^{11–14} and general physiologic functions.^{6,11–17} Dietary CR has been found to attenuate the up-regulation of reactive oxygen species and the expression of inflammatory cytokines. CR can also decrease the expression of inflammatory cytokines, such as TNF α and IL-6, and increase the expression of immune-suppressive mediators, such as TGF- β .¹⁸ Alveolar macrophages from CR rats exhibit a reduced endotoxin-stimulated expression of TNF α and IL-6.¹⁹ Collectively, these and other findings suggest that CR may dampen inflammation and concomitant tissue damage in inflammatory conditions, such as periodontal disease. Recently, reports have also emerged regarding the potential for long term dietary CR to alter aging parameters in animal models.^{20–30}

Periodontitis is a chronic inflammatory disease that is characterized by inflammatory and immune responses to bacterial colonization of teeth and periodontal tissues that often lead to the destruction of periodontal connective tissues and resorption of alveolar bone. The progression of periodontal destruction is chronic, and unpredictably intermittent making studies of disease progression extremely difficult. For this reason, experimental animal models of active periodontal destruction have been used to study the clinical, inflammatory, and immunologic kinetics of disease initiation and progression.^{31,32}

To gain insight into the impact of a CR diet on the progression of active periodontal destruction, this study was designed to evaluate the clinical effects of dietary CR on the development and progression of ligature induced periodontitis in a well characterized animal model of active periodontal inflammation and disease.

MATERIAL AND METHODS

We examined 62 young adult male and female rhesus monkeys (*Macaca mulatta*), that are subjects in a longitudinal study of the effects of long-term CR on aging being conducted by the National Institute on Aging. These monkeys are well characterized with regard to health status, and were subject to additional health screens for use in this study.^{33,34} Of the 62 screened animals, 7 were exited due to poor health or advanced periodontal disease. The remaining 55 animals were balanced across study groups for gender and age (Table 1), were selected for having minimal baseline measures of periodontal disease (Table 2), were individually housed under controlled environmental conditions, and fed a specially formulated diet enriched with vitamins and minerals at the National Institutes of Health Animal Center (Poolesville, MD). All monkeys received water *ad libitum* and were on a 12 hour light/dark cycle. Monkeys were fed two meals per day at 6:30am and 1pm. Those in the control (CON) group (n = 32) were fed allotments approximating their *ad libitum* intake whereas, monkeys in the caloric restriction (CR) group (n=23) received 30% less food than gender-, age-, and weight-matched controls. At the time of the present study initiation, monkeys had been on this feeding regimen for periods of 13–17 years. All monkeys received fresh fruit weekly to supplement the diet.³³

Ligature-induced Periodontitis

The effects of ligature-induced inflammation on periodontal disease were evaluated using the maxillary right second premolar, first molar, and second molar in all animals. All animals were examined under sedation, provided by the NIH veterinary staff, by the same investigator (MJN) who was blinded as to the diet assignment of the animal. The proximal sites (mesio- and disto-buccal) of the maxillary second premolar and the maxillary first and second molars were assessed using the following clinical measures: probing pocket depth (PD), clinical attachment level (CAL), plaque index (PLI; scored 0–3), and bleeding on probing (BOP; scored 0–3).^{35,36} A modified Gingival Index was used to assess the marked inflammatory changes that occur following ligature placement and plaque accumulation. To discriminate between changes in gingival color and swelling the following scoring was developed: 0 = no redness or swelling; 1 = mild redness/no swelling; 2 = moderate redness/no swelling; 3 = severe redness/mild swelling; 4 = severe redness/moderate swelling; 5 = severe redness/severe swelling. Where gingival swelling and visual inflammation were not compatible with the scale, the degree of swelling was always used and scored as a more objective measure of inflammation. Clinical evaluations were made at baseline (pre-ligature) and one month (21–35 days), two months (49–62 days), and three months (84–91 days) post-ligature placement. Following baseline clinical measures, 2–0 silk ligatures were placed and tied subgingivally at the cemento-enamel junction of each study tooth to facilitate the subgingival accumulation of bacterial plaque. Clinical measures were repeated under the same conditions at one month, two months, and three months following ligature placement.

Statistical Analysis

A mean was calculated for each index at each time for each animal. These means were used as the response variables in the analyses of the study. Repeated measures analysis of variance was used to evaluate each index over time (visit) and to evaluate diet effects. Two models were used to evaluate the data. First a **limited model** was used with only the effects of diet and discrete visit times (months 1, 2, and 3). A more **complete model** was then used to evaluate the effects of discrete visit times (months 1, 2, and 3), diet, gender, age, baseline measure of index being evaluated, and interactions of: Visit $\times$ Diet, and Diet $\times$ Gender. The purpose of using two models was to investigate the robustness of diet effects to both covariates and interactions. Ligatures used to induce periodontitis were lost on some animals at some teeth before the end of the 3 month period of time. A number of strategies were used to determine if the lost ligatures had an effect on any diet effect. An intent to treat analysis was done to investigate this possibility. Under the principal of intent to treat the last observation was brought forward. The analyses described above were done both with and without bringing the last observations forward. To further ensure that lost sutures did not affect the result, the number of teeth without lost ligatures at each time point was used as a covariate in the intent to treat analysis. The significant effects were robust to the different modeling and the intent to treat effects in that all four models had almost identical results. Significance levels for the intent to treat models will be reported in the results section (Table 2).

RESULTS

Baseline Clinical Measures

Animals were enrolled at a rate of approximately 1.5 control animals (n=32) for each experimental animal (N=23). There were no significant differences between the diet groups for either age or gender (Table 1). Baseline clinical values were also well balanced between the two groups (Table 3) with no significant differences found between the diet groups. However, in an examination of gender effects (Table 4), females had significantly higher BOP than males at baseline ($p < 0.0001$) and CR group females had significantly more baseline gingival inflammation than male and female control animals ($p < .05$).

Changes in Clinical Measures During the Development of Periodontal Inflammation and Disease

There were large changes in all clinical indices over the first month of ligature induced inflammation regardless of the diet group (Table 3; $p < 0.0001$). Plaque levels and BOP increased more than 50%, pocket depth and attachment level measurements, both increased by approximately 1mm. Females developed significantly more plaque ($p < 0.02$; Table 2) than males after ligatures were placed. However, these values were used as covariates in the models and did not have an effect on any of the clinical differences between groups when animals were grouped by diet; nor were there any significant interactions between gender and diet groups over time (Table 2). Gingival inflammation, measured by a modified gingival index, increased dramatically from mean levels of 0.67 and 0.70 for the CR and control groups, respectively, to 3.29 and 3.95 at one month. After one month, probing pocket depth levels, plaque levels and calculus levels remained constant. BOP levels increased significantly in both groups from 1 month to 2 months ($p < 0.0048$) and then decreased at 3 months to levels similar to levels found at 1 month. Attachment level measurements continued to increase in both groups from 1 month to 3 months, but the increase was only statistically significant in the control group ($p < 0.01$) (Figure 1). The estimate of the rate of attachment loss in the control group was 0.0062 mm. per day, a rate which translates to approximately 2.3 mm per year.

Effects of a Reduced Calorie Diet on Clinical Measures

There was no difference between groups in plaque levels at any time point between animals grouped by diet. However, all of the other measures showed significantly less deterioration in the CR group (Tables 2,3,4). Inflammation was significantly reduced in animals on the CR diet. Levels of bleeding on probing (BOP) were significantly different between groups ($p < 0.0015$) on a consistent basis during the study with the CR group having lower levels. The modified GI, was also consistently and significantly ($p < 0.0001$) lower in the animals of the CR group. In a similar manner, pocket depth (PD) was consistently lower in the CR group ($P < 0.0016$) and remained constant over time. Attachment loss (AL) was also significantly less in the CR group ($p < 0.0038$). Attachment loss increased in CR and control groups from the 1 month visit to the 3 month visit. However, a significant ($p < 0.01$) increase in destruction from baseline was only observed in the control group (Figure 1).

Three male monkeys (2 CON; 1 CR) were exited from the study prior to completion of the month 3 evaluation due to medical conditions. Because all animals were part of an ongoing study of the effects of CR on aging, the specially prepared chow could not be softened as is usually done as part of the ligature model. As a result, ligature displacement occurred in a small number of teeth prior to evaluation at month 1 (3 %), month 2 (5.5 %), and month 3 (17.5 %) days, with a cumulative loss within the CON and CR groups of 14.6 % and 21.7 %, respectively. Male monkeys displaced a higher percentage of ligatures than female monkeys (24.7 % versus 10.7 %); however, CR males lost the majority of ligatures prior to the month 2 evaluation, in contrast to CON males, which lost the majority of ligatures after to the month 2 evaluation. Due to the loss of ligatures, an intent-to-treat analysis was performed. When a ligature was lost the last observation was brought forward for the missing observation. Table 5 shows the distribution of lost ligatures by time, gender and diet group. By month 3 the distribution of ligatures was balanced by group. Although a greater number of males lost ligatures, the number of animals who lost ligatures was similar in each group. Due to the low number of ligatures lost, and subsequent low statistical power, these findings were not subjected to hypothesis testing. The mean indices in the intent to treat analysis (Table 6) were virtually identical to those found in Table 3 where all available observations were used. Additionally, for both the limited model and the more complete model the analysis of all of the effects for all of the clinical indices were virtually identical (Table 2). Thus, the loss of ligatures did not affect the results.

DISCUSSION

Considerable attention has been given to the negative effects of obesity and type 2 diabetes on the progression of periodontal disease and their co-relationship with the secretion of pro-inflammatory cytokines such as TNF α , IL-1 and IL-6.¹⁻³ In contrast, the effects of a reduced calorie diet have not been explored in humans or other models although studies support the concept that maintaining normal weight combined with regular exercise reduces the prevalence of periodontal disease and associated pro-inflammatory markers.¹ Dietary caloric restriction has been shown, in multiple animal models, to decrease weight, impact physiologic and hormonal functions, increase lifespan, reduce age-related disease, and reduce the release and effects of pro-inflammatory mediators, therefore minimizing local and systemic signs of inflammation.⁴⁻³⁰ In the current study, a cohort of nonhuman primates that have been part of a National Institute on Aging intramural study on the effects of long term caloric restriction for 13-17 years, were subjected to an inflammatory challenge utilizing the ligature model of periodontitis induction over a 3 month period. Male and female rhesus monkeys were represented in both CON and CR groups. At baseline, male and female monkeys were found to have comparable clinical scores on all clinical measures, with the exception of GI and BOP. Females exhibited a modest but significantly higher mean BOP than males in both the CON and CR groups and a higher mean GI than males in the CR group. During the first month of ligature placement, clinical measures of plaque and gingival inflammation increased in both the CON and CR groups. While the plaque scores remained constant after the first month there was a difference in the inflammatory response to plaque accumulation observed in the CR and CON monkeys. Monkeys in the CR group exhibited significantly less gingival inflammation and bleeding on probing (lower mean GI and BOP) than animals in the CON group at all post-ligature intervals. These findings suggest that long-term CR dampened the inflammatory response of ligature-induced inflammation in this non-human primate model.

The lower inflammatory response observed in the CR group compared to the CON group is consistent with other studies that have demonstrated reductions in inflammatory mediators, such as TNF α and IL-6, in response to dietary restriction.¹⁸ Consistent with the effects of CR on periodontal inflammation, the development of periodontal pocketing and progression of clinical attachment loss was significantly greater in the CON group than the CR group and was found to advance at a significantly slower rate in animals in the CR group compared to animals in the CON group. In addition to the anti-inflammatory effects of CR on cytokine production, CR has been found to decrease the production of reactive oxygen species and oxidative damage of mtDNA.³⁷ CR has also been shown to improve metabolic parameters, such as blood glucose levels and insulin sensitivity, that are triggered by inflammatory peptides, including TNF α .¹¹ Hence, it appears that CR affects multiple immunologic and physiologic mechanisms that may explain the anti-inflammatory effects and reduction of disease noted in the periodontally challenged CR group.

Epidemiologic studies provide strong evidence that men are at greater risk for developing advanced periodontal disease than women. In the present study, male and female monkeys in the CON group were found to develop significantly greater increases in periodontal pocketing and clinical attachment loss than animals on the dietary CR regimen with no differential effect of gender. In this experimental disease model, CR effected reductions in the development of gingival inflammation and periodontal breakdown equally in both male and female primates. These findings are consistent with the hypothesis that CR modulates the development of periodontal breakdown by damping the inflammatory response.

Numerous studies now link excess body weight to inflammatory diseases, such as periodontal disease.¹ Obesity is associated with more severe periodontal disease, independent of glucose tolerance. The adipocyte is now recognized to contribute substantially to circulating levels of

inflammatory cytokines, including TNF α , IL-1, and IL-6. Our future studies, designed to investigate the effects of CR on the changes in inflammatory mediators in serum and gingival crevicular fluid during the initiation of active periodontitis using the ligature model, will provide some insight into the local and systemic effects of CR on pro-inflammatory mediators that may affect the progression of periodontal inflammation and disease. In addition, we are currently investigating whether CR had any qualitative impact on the development of the plaque biofilm that could potentially impact the development of inflammation and disease. These data will be presented in future papers.

In conclusion, the results of this controlled study provide the first experimental evidence that long term dietary CR dampens clinical inflammation and reduces the degree of periodontal breakdown secondary to acute microbial challenge. These clinical findings are consistent with, and extend, a growing body of evidence indicating that the modulating effects of dietary CR include an anti-inflammatory component.

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Figure 1.
Changes in Clinical Attachment Loss in Millimeters From Baseline (Mean $\pm$ SE)

Table 1

Baseline Group Characteristics

Diet	Gender	N	Age*
Control	Female	16	16.1 ±0.8
	Male	16	18.5 ±0.3
CR	Female	11	15.0 ±0.8
	Male	12	18.3 ±0.3

* mean ± standard error

Table 2

Summary of Statistical Significances Using Limited and Complete Models

Response	Limited Model		Complete Model	
	Adjusted R ²	Significant effects	Adjusted R ²	Significant Effects
PLI	.701	N. S.	.684	Gender p=.02, Baseline PLI, p=.0006
GI	.716	Diet p < .0001	.709	Diet p < .0001, Age, p=.004
BOP	.618	Diet p=.0024, Visit p=.0025	.606	Diet p=.0015, Visit p=.0048 [#]
AL	.660	Diet p =.003, Visit p=.0003	.650	Diet = .0038, Visit = .0007 [*]
PD	.739	Diet p=.0045	.728	Diet p=.0016

Hypothesis testing: Intent to treat responses were used. Both models were repeated measures analysis of variance. Effects in the limited model were diet and visit (Month 1, 2, 3). Effects in the complete model were visit (Month 1, 2, 3), diet, gender, age, baseline measure of index being evaluated. Interactions of Visit × Diet and Diet × Gender.

[#] Month 2 was different (Tukey's test, P < 0.05) from month 1 and 3

^{*} Linear effect from month 1 to month 3 was stronger (p=.0001) than using discrete time points (visits) (p=.0007)

Table 3

Clinical measures over time

Visit	Diet	N	PLI		PD		AL		BOP		Modified G.I.	
			Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
Baseline	Control	32	1.11	0.12	2.19	0.06	0.22	0.06	1.35	0.13	0.70	0.10
	CR	23	1.01	0.11	2.15	0.09	0.16	0.06	1.29	0.15	0.67	0.16
Month 1	Control	32	1.45	0.11	3.54	0.12	1.28	0.08	2.49	0.19	3.95	0.12
	CR	23	1.44	0.13	3.16	0.12	1.06	0.09	2.00	0.22	3.29	0.19
Month 2	Control	32	1.50	0.08	3.63	0.10	1.45	0.09	3.07	0.18	3.90	0.12
	CR	23	1.50	0.12	3.22	0.11	1.09	0.11	2.21	0.23	3.36	0.14
Month 3	Control	30	1.36	0.09	3.48	0.11	1.68	0.09	2.46	0.18	4.02	0.09
	CR	22	1.42	0.12	3.10	0.10	1.27	0.07	1.84	0.19	3.20	0.11

Table 4

Clinical measures over time by gender

Visit	Gender	Diet	N	Mean	PLI Std Err	Mean	PD Std Err	Mean	AL Std Err	Mean	BOP Std Err	Mean	Modified GI Std Err
Baseline	Female	Control	16.00	0.96	0.16	2.02	0.07	0.20	0.09	1.61	0.19	0.67	0.16
	CR	Control	11.00	1.06	0.19	2.14	0.16	0.27	0.11	1.61	0.24	0.94	0.27
Month 1	Male	Control	16.00	1.27	0.18	2.36	0.08	0.24	0.07	1.08	0.14	0.74	0.13
	CR	Control	12.00	0.97	0.12	2.17	0.10	0.06	0.03	1.00	0.16	0.43	0.14
Month 2	Female	Control	16.00	1.68	0.16	3.50	0.15	1.31	0.12	2.96	0.19	3.94	0.13
	CR	Control	11.00	1.62	0.26	3.24	0.14	1.13	0.10	2.24	0.26	3.49	0.23
Month 3	Male	Control	16.00	1.22	0.14	3.58	0.18	1.24	0.10	2.02	0.29	3.97	0.20
	CR	Control	12.00	1.28	0.09	3.08	0.19	1.00	0.15	1.78	0.35	3.10	0.29
Month 3	Female	Control	16.00	1.54	0.12	3.54	0.16	1.59	0.12	3.08	0.24	4.06	0.15
	CR	Control	11.00	1.45	0.21	3.40	0.16	1.33	0.16	2.64	0.33	3.68	0.21
Month 3	Male	Control	16.00	1.46	0.10	3.72	0.11	1.31	0.14	3.05	0.28	3.74	0.17
	CR	Control	12.00	1.53	0.13	3.06	0.14	0.88	0.12	1.81	0.27	3.06	0.16
Month 3	Female	Control	16.00	1.45	0.08	3.31	0.15	1.59	0.12	2.67	0.27	4.11	0.10
	CR	Control	11.00	1.55	0.21	3.07	0.16	1.23	0.10	2.03	0.32	3.22	0.17
Month 3	Male	Control	14.00	1.25	0.17	3.68	0.16	1.77	0.13	2.22	0.22	3.92	0.15
	CR	Control	11.00	1.29	0.14	3.14	0.12	1.31	0.09	1.65	0.21	3.18	0.16

Table 5

Distribution of lost ligatures

Visit	Gender	Diet	Total missing sutures	In total number of animals
Month1	Female	Control	2	2
		CR	2	2
	Male	Control	0	0
Month2		CR	1	1
	Female	Control	3	3
		CR	4	4
	Male	Control	1	1
		CR	6	5
Month3	Female	Control	4	4
		CR	5	5
	Male	Control	10	6
		CR	10	6

Table 6
Clinical measures under the assumption of intent to treat (Control n=32, experimental n=23)

Visit	Diet	PLI		PD		AL		BOP		Modified GI	
		Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
Baseline	Control	1.11	0.12	2.19	0.06	0.22	0.06	1.35	0.13	0.70	0.10
	CR	1.01	0.11	2.15	0.09	0.16	0.06	1.29	0.15	0.67	0.16
Month1	Control	1.42	0.10	3.52	0.12	1.26	0.08	2.47	0.19	3.90	0.12
	CR	1.41	0.13	3.12	0.11	1.03	0.09	1.95	0.21	3.20	0.19
Month2	Control	1.49	0.08	3.59	0.10	1.41	0.09	2.98	0.17	3.83	0.12
	CR	1.46	0.12	3.20	0.12	1.41	0.11	2.20	0.21	3.22	0.15
Month3	Control	1.36	0.08	3.47	0.11	1.60	0.09	2.49	0.17	3.96	0.10
	CR	1.40	0.12	3.04	0.10	1.20	0.07	1.77	0.17	3.07	0.12