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Characterization of a sample population of dental hygiene patients with and without periodontal disease as determined through fatty acid and fruit and vegetable intake

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Characterization of a sample population of dental hygiene patients with and without periodontal disease as determined through fatty acid and fruit and vegetable intake

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By

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Abstract

This study was designed to compare the fatty acid and fruit and vegetable intake of people with and without periodontal disease. Periodontal disease affects approximately 15 percent of the developed world population. Participants filled out a food frequency questionnaire (FFQ) for both fruit and vegetable and fat intake. A sample of whole blood (EDTA) was collected to determine the fatty acid profile of participants' red blood cell (RBC) membrane. This analytical procedure determines the average fatty acid intake of the participant over the previous 90 to 120 days. Participants were classified as case (n=10, patients diagnosed with periodontal disease) or control group (n=10, patients without periodontal disease). Our hypothesis was that the case group would have higher fat intake and lower fruit and vegetable intake, with lower levels of omega-3 fatty acids and higher levels of omega-6 fatty acids in the RBC membrane. FFQ results indicated that case participants consumed significantly higher amounts of total fat (109.02 vs. 94.46g/d; p= .05), saturated fat (35.102 vs. 28.242g/d; p= .033) and their diets had a higher percentage of total dietary fat (38.73% vs. 34.99%; p=.044). FFQ for fruit and vegetable intake showed no significance between the case and control groups (4.41 vs. 4.56 servings/d; p=.871). For the most part, there were no significant differences between the percent composition of the individual fatty acids isolated from the RBC membrane or in ratios of omega-3 to omega-6 fatty acid groups as determined by RBC fatty acid analysis. Results indicated a numerically lower intake of omega-3 fatty acids in the case than in the control group. Likewise, there was a numerically higher intake of omega-6 fatty acids in the case group. These numbers demonstrate a trend towards the hypothesis being correct and patients with periodontal disease consuming more omega-6 fatty acids and less omega-3 fatty acids.

Literature Review

General Overview

Periodontal disease is an inflammatory disease caused by gram-negative bacteria that colonize the gingival crevice leading to soft-tissue destruction with an abnormal inflammatory immune response.[1] The initiation of periodontal disease is prompted by a shift in the oral bacteria colonization from gram-positive to gram-negative species.[2] It is thought that many microorganisms play a role in the formation of periodontal disease.[1] Periodontitis leads to severe inflammation, destruction of gingival tissue, formation of deep pockets between the gingiva and tooth loss of alveolar bone and associated periodontal ligament and exfoliation of teeth.[2] Periodontal disease consists of periods of extremely heightened symptoms interspersed with periods of remission.[3] Dental caries and periodontal disease are the most prevalent chronic infectious oral conditions in humans. They affect about 10 to 15 percent of the developed world population and is the major cause of tooth loss in adults.[4] Tooth loss often acts as a consequence of chronic periodontal disease and a preventive measure for future infectious exposure. This indicates that edentulism occurring earlier in life might confer some protection against atherosclerotic development by minimizing lifetime oral infectious exposure.[5]

Evidence indicates associations between periodontitis and type-2 diabetes, cardiovascular and cerebrovascular disease. Patients with type-2 diabetes are 2.8 times more likely to have periodontal disease than patients without type-2 diabetes.[4] People with type 2 diabetes who are obese have about ninety-three percent higher risk than patients without type 2 diabetes.

Pathogenic similarities also exist between periodontitis and rheumatoid arthritis.[6] Several observational studies showed that there was a higher prevalence of periodontitis in patients with rheumatoid arthritis compared to the general population. However, a study performed by Cagri Esen et al. showed there was no difference between the group with periodontitis and the group with rheumatoid arthritis and periodontitis.[7]

It has been demonstrated that saturated fatty acids produce an inflammatory response through activation of the toll-like receptor signaling pathway.[8] Therefore an intake of saturated fatty acids leads to a greater tendency for periodontal disease. In the study conducted by Iwasaki et al. smoking was found to be a positive effect modifier for periodontal disease. Cigarette smoking causes periodontal disease to progress more quickly than periodontal disease progresses in nonsmokers.[8] Smoking appears to negate the association between an intake of saturated fatty acids and periodontal disease among smokers.[8] Intake of saturated fatty acids activate the nuclear factor-kappa B pathway and then further stimulates the production of inflammatory mediators in macrophages through the activation of TLR-4. This inflammatory pathway can lead to the development of periodontal disease.[8] Cells involved in the inflammatory cascade typically possess a high concentration of omega-6 polyunsaturated fatty acids (PUFA) in their cell membrane. When these immune cells are activated, synthesis of omega-6 eicasanoids, lipid-derived inflammatory mediators, occur, leading to the formation of proinflammatory mediators prostaglandin E_2 ($PGE_{2)}$ and leukotriene B_4 (LTB_4) via the cycloogenase and lipooxygenase pathways respectively.[3] Both PGE_2 and LTB_4 increase the production of IL-6 and enhance vascular permeability and vasodilation. Therefore, those whose cells contain a high proportion of omega-6 PUFA will be subjected to an elevation in both the strength and duration of the

inflammatory stage, resulting in more extensive tissue degradation.[3] Plasma C-reactive protein concentration has been inversely related to omega-3 PUFA, indicating a reduction in the inflammatory process due to higher plasma concentration of the omega-3 PUFAs.[3] Human studies concerning supplementation with omega-6 supplementation and induced gingivitis indicated a trend towards an anti-inflammatory effect.[3] This research, conducted by Dalai et al., indicated that an increase in n-3 PUFA would cause a decrease in the degree of periodontal bone loss.[3] There was also a similar negative correlation between n-3 PUFA concentration in red blood cell membrane and tooth loss. The ratio of n-6 to n-3 PUFAs was found to be a major factor in periodontal disease status.[3] It was suggested that dietary supplementation with n-3 PUFA would help to prevent and treat periodontal disease.[3]

Water-Soluble Vitamins and Periodontal Disease

Thiamin is required for metabolism of carbohydrates and branched-chain amino acids and a deficiency causes two main conditions: beriberi and Wernicke-Korsakoff syndrome.[6] Thiamin does not have oral manifestations. A deficiency in thiamin will not cause damage to the oral cavity.[6]

Riboflavin and niacin are involved in oxidation-reduction reactions.[8] A deficiency of riboflavin causes people to be at risk for developing edema of the pharyngeal and oral mucous membranes, angular cheilitis, stomatitis and glossitis.[6] A deficiency of niacin is called pellagra and manifests itself as bright red glossitis, burning mouth, erythema of the gingiva and dental caries.[6]

Vitamin B_6 is involved in carbohydrate, fat and protein metabolism. A deficiency of Vitamin B_6 causes glossitis, cheilitis and erythema of the gingiva in the oral cavity.[6]

Folic Acid aids in DNA synthesis and is involved in forming blood cells in the bone marrow.[6] A deficiency in folic acid leads to megaloblastic anemia, which results in large red blood cells. Oral manifestations of this deficiency include: burning of the tongue and oral mucosa, a red and swollen tongue and angular cheilitis.[6] Deficiency of folate is common in older adults and the prevalence of deficiency increases with age, because of the decrease rate of absorption by the gastrointestinal tract which comes with age.[9] Research has found that folate has a protective effect against various multisystem age-related disorders such as dementia, depression, myocardial infarction and stroke. Folate deficiency is associated with an increase in oxidative stress.[9] The increase in oxidative stress caused by the folate deficiency may increase the risk of periodontal disease. Low serum folate levels were associated with periodontal disease in older adults.[9]

Vitamin B_{12} is also required for DNA synthesis. A deficiency of vitamin B_{12} is called pernicious anemia and it manifests orally as a red, atrophic, beefy, burning tongue.[6] A deficiency in folic acid, vitamin B_{12} and iron is associated with recurrent aphthous stomatitis.[6]

Vitamin C is an antioxidant that is a cofactor in hydroxyproline and hydroxylysine formation.[6] A deficiency of vitamin C is called scurvy. Scurvy is a hemorrhagic disease, which presents with muscle weakness, lethargy, increased susceptibility to bone fractures, poor wound healing and painful and swollen joints.[10] Scurvy presents orally with hemorrhagic gingivitis with

enlarged blue or red gingiva, gingival bleeding, swollen gingiva and infections.[6] Scurvy also affects dentition, presenting with loose teeth, interdental infarcts and tooth loss.[6] With low intakes of vitamin C, the risk of periodontal disease rises by twenty percent.[6] Vitamin C is important in reducing the risk of periodontal disease because of the vitamin's antioxidant capacity and because vitamin C is a cofactor needed for collagen synthesis. Collagen is a major component of the periodontium. For collagen maturation to occur, adequate vitamin C must be available in the body to permit hydroxylation of lysine and proline.[11]

Antioxidants and Periodontal Disease

The equilibrium among free radicals and antioxidants in tissues as a necessary precondition for periodontal health is found to be disturbed in periodontitis.[11] This occurs as a result of excessive amounts of free radicals.[11] Increase in antioxidant intake causes a decrease in the progression of periodontal disease.[11] There is a significant inverse relationship between the number of teeth infected with periodontal disease with the daily intake levels of vitamin C, vitamin E, α-carotene and β-carotene, all strong antioxidants.[3] Vitamin C shows the strongest inverse association with periodontal disease.[6,10] Vitamin E has been shown to have a mitigating effect on periodontal inflammation and collagen breakdown.[9] Antioxidants decrease oxidative stress in periodontal tissue, which could be the explanation for the findings stated previously.[11]

Oxidative stress leads to an up-regulation of pro-inflammatory pathways implicated in the pathogenesis of periodontal disease.[4] Antioxidants counteract the harmful effects of free radicals created by the oxidative stress, to protect the tissue's integrity. An imbalance between

the levels of free radicals and antioxidants is suggested as a role in the onset of the inflammatory process that leads to periodontal disease.[4] Therefore periodontal disease has been associated with a reduction in total antioxidant status and an increase in oxidative damage within the oral cavity.[10] The decrease in the levels of these important antioxidants can be considered an important mechanism by which toxic effects of free radicals can initiate oral disease, such as periodontal disease.[12]

Dietary Intake and Periodontal Disease

Tooth loss changes food preferences and increases nutritional deficiencies in older people. There is unequivocal evidence of a relationship between dental and nutritional status as reported by the National Diet and Nutrition Survey.[15] The number of periodontal disease events during a six year study, by A. Yoshihara et al., significantly decreased with increasing amounts of dark green and yellow vegetables. However, other vegetables and fruits have not been found to have a relationship with periodontal disease. Periodontal disease events increased with an increase in intake of cereals, nuts and seeds, sugar and sweeteners and confectioneries in the same six year study[1]. A. Zare Javid et al. found there was no significant difference in periodontal status between a control group and a group with a dietary intervention, which was designed to promote an increased consumption of fruit, vegetables and whole grains between the first meeting and the six month follow-up.[17] A high-fiber low-fat diet has been reported to improve systemic inflammation by promoting body weight reduction. The improvement of periodontal markers found in the high-fiber low-fat diet might be through the reduction in systemic inflammation, via weight loss.[18]

Adiposity and Periodontal Disease

People without teeth are more likely to be underweight, because people without teeth have difficulty eating certain foods.[15] This can result in certain nutrient deficiencies. A patient having more than twenty teeth increases the likelihood of having an acceptable BMI (20-25) and being nutrient sufficient.[15]

Periodontal disease, as well as many other oral infectious diseases, cause an impairment of chewing ability and a tendency for exchanging hard and nutritious foods with soft ones, containing carbohydrates and unsaturated fatty acids. This can result in obesity.[19] Obese subjects' oral cavities are in poorer condition than normal weight subjects.[19] These poorer conditions are characterized as a higher number of lost teeth, an increased number of caries and an intensity of periodontal diseases.[19] Obesity increases the chance of periodontal diseases by as much as 77 percent. However, high BMI levels as a youth does not indicate a higher risk for developing periodontal disease later in life.[19]

According to the National Health and Nutritional Examination Survey approximately 71% of adults over the age of sixty are overweight and 30% are obese.[20] A BMI of over 30 kg/m^2 is significantly associated with an increased likelihood in developing periodontal disease. However, BMI becomes a less valid way of measuring adiposity after 60 years of age because body composition changes.[20] Munoz-Torres et al. examined body composition to determine if central adiposity was a good indicator of risk for periodontal disease.[20] The results suggest that central adiposity is associated with periodontal disease.[20] The evidence is not overly significant,

which was calculated using *a priori* stratified analysis. Obesity may lead to periodontal disease by inducing a state of low-grade systemic inflammation and a pro-inflammatory environment.[20] This was conducive for a hyper-inflammatory response in the gingival tissue because of the presence of periodontal bacteria.[20]

Chronic Disease and Periodontal Disease

It has been long established that periodontal disease is associated with an increased risk in cardiovascular disease.[21] Recent findings suggest another link between periodontal disease and cerebrovascular disease. T. Wu et al. found an increased risk between total cerebrovascular disease and nonhemorrhagic stroke and periodontitis.[21] The study found that periodontal bone loss was associated with an increased risk for stroke. This was statistically significant as measured by a multivariate analysis conducted using the Cox proportional hazard model.[21] People with cerebral infarction have poorer periodontal status, as measured by gingival bleeding on probing, subgingival calculus, suppuration in the gingival pocket, third degree caries lesions and vertical bone pockets, in comparison with control subjects.[21] There are a few potential pathophysiologic links between periodontal disease and cerebrovascular disease. The bacteria from the periodontal disease could induce an abundant production of proinflammatory cytokines, cause inflammatory cell proliferation into large arteries and stimulate hepatic synthesis of clotting factors and thus contribute to atherogenesis and thromembolic events.[21] There are also several periodontal pathogens that can induce platelet aggregation and may thus be thrombogenic when entering the systemic circulation as in periodontitis.[21]

Periodontal disease has traditionally been viewed as a consequence of diabetes. When compared with healthy adults, adults with periodontal disease are twice as likely also present with type 2 diabetes.[5] Chronic inflammation in response to periodontal bacteria might be the link between periodontal disease and type 2 diabetes.[22] Systemic inflammation is a major indicator for type 2 diabetes. Patients with periodontal disease have consistently shown to have elevated levels of systemic inflammation as measured by an elevated serum C-reactive protein levels.[22] R. Demmer et al. reported the odds of being diagnosed with diabetes was increased by fifty percent for people who already had periodontitis.[5]

Materials and Methods

Participant Selection

Twenty participants from the large case study were identified for use in this thesis. Ten patients were identified as part of the case group (had periodontal disease) while another 10 were part of the control group (did not have periodontal disease). Patients were selected for these groups based upon the periodontal disease markers (periodontal probing depth and bleeding index).

All participants involved in this study filled out two food frequency questionnaires (fruit and vegetable screener and brief fat screener developed by Block et al.), self-reported anthropometric data and had 5 mL of whole blood (EDTA tube) collected for analysis of the fatty acid profile of the red blood cell membrane and water soluble vitamins (plasma). Participants underwent this research experiment on a completely voluntary basis and this research project was approved by the ETSU Institutional Review Board (IRB). Informed consent

was obtained on each participant per IRB protocols. Participants were offered 20 dollars for their participation in the study.

Self-reported patient and anthropometric data

Patients were asked to provide information related to their anthropometric measurements and patient identification including current body weight, height, sex and age. Height and weight data were used to calculate the patients' body mass index (BMI).

Food Frequency Questionnaire

Each participant filled out two food frequency questionnaires (FFQ) upon admittance into this study. Block et al. designed these questionnaires that have been validated through research and licensing was received for intended survey use.[24] The FFQs took approximately 5 minutes to complete and was an inexpensive tool for researchers to assess a snapshot of each patient's dietary habits. The brief fat screener consisted of 15 items designed to assess dietary fat consumption by each patient. The 15 questions were directed toward meats and snacks that were consumed. The fruit and vegetable screener consisted of 10 questions designed to assess fruit and vegetable, fiber and micronutrient intake in much less time than traditional food records. The sex and age of each patient was used as a potential covariate in order to create prediction equations using the food screener scores that were obtained. The equations (developed by Block et al.) were used to compute point estimates of selected nutrients such as: total fat, saturated fat, percent fat, fruit/vegetables servings, vitamin C, magnesium and potassium.[24]

Prediction equations for Block Dietary Fat Screener:

Total fat (gm) = 32.7 + [2.4 * (Meat/snack score)] + (11.2 * Sex)

Saturated fat (gm) = 9.4 + [0.88 * (Meat/snack score)] – (3.5 * Sex)

Percent fat (%) = 19.8 + [0.6 * (Meat/snack score)] + (2.3 * Sex)

Prediction equations for Block Fruit/Vegetable/Fiber Screener:

Fruit/Vegetable servings = -0.23 + [0.37 * (Fruit/vegetable score)] – (0.55 * Sex)

Vitamin C (mg) = 56.5 + [6.6 * (Fruit/Veg/Beans score)] – (26.7 * Sex) – (0.45 * Age)

Magnesium (mg) = 272 + [11.6 * (Fruit/Veg/Beans score)] – (92.3 * Sex) – (1.7 * Age)

Potassium (mg) = 2348 + [114.8 * (Fruit/Veg/Beans score)] – (759 * Sex) – (13.8 * Age)

Dietary fiber (gm) = 12.6 [0.77 * (Fruit/Veg/Beans score)] – (5.12 * Sex) – (0.16 * Age)

Laboratory Methods

Red blood cell (RBC) fatty acid profile

Blood samples were collected from trial participants in 5-mL EDTA vacutainers. Samples were transported to the Nutrition and Dietetics Research Laboratory at the ETSU Valleybrook campus. Blood samples were processed within 24 hours. Each sample was centrifuged at room temperature for 10 minutes at 4000 RPM. The resulting supernatant, composed of plasma, was decanted using a Pasteur pipette and transferred to a 3 mL amber glass vial. Vials were stored at -30º C until further analysis for antioxidant potential of the plasma (FRAP) and water soluble vitamins assays. Equal parts normal saline (0.9% NaCl) was added to the remaining sample, vortexed briefly to disrupt the RBC pellet and centrifuged for 5 minutes at 4000 RPM. The clear supernatant layer was removed and discarded. This process was repeated two more times with the clear supernatant being removed and discarded each time.

Isolation of RBC and lipid extraction

Kang and Wang developed the procedure for lipid extraction and methylation of RBC. The procedure was modified in the Nutrition and Dietetics Research Laboratory.[24] The final RBC pellet, developed from the procedure above, was put back into suspension in 25 µL of saline solution (0.9% NaCl) and vortexed. One- hundred µL of the RBC were extracted and transferred to clear, 150 mm screw-top test tubes with Teflon lined caps. Duplicate assays were developed for each sample to reduce experimental variance. Two mL of hexane (Fisher Scientific) and 2 mL of boron triflouride- methanol solution ($BF_{3;}$ Sigma- Aldrich) were added to the red blood cells and vortexed to ensure homogenous mixing. Vortexed tubes were placed in a heating block for one hour at 100º C to create an environment suitable for fatty acid methylation. This process created fatty acid methyl esters (FAME). The solution was allowed to cool to room temperature. Deionized distilled water (1.5 mL) (DDW) was added, the test tubes were vortexed briefly and centrifuged for one minute at 4000 RPM. The hexane layer (top layer) was removed using a Pasteur pipette and added to a new, 150 mm screw-top test tube, dried under nitrogen in a heated water bath at 50º C. Once the hexane layer had evaporated, FAME were put back in suspension with 275 µL of hexane and transferred to a 1.5 mL amber glass autosampler vial. These vials were equipped with a low volume insert of 300 µL (Fisher Scientific) and an internal standard (5 µL, C-17 fatty acid; Nu-Check Prep, Inc.), which were added to validate FAME time signature for peak identification. Samples were stored at -30º C until analysis by gas chromatography.[24]

Analysis of fatty acids

Flame ionization gas chromatography (GC, Shimadzu) was used for analyzing FAME using a capillary column (Phenomenex, Zebron ZB-WAX). Helium was used as a carrier gas with a flow rate of 30 mL/min and a temperature program of a constant temperature ramp (2º C/min) between hold temperatures were the conditions of the column necessary for the analysis. The temperature was held for 5 minutes at an initial temperature of 160º C; then held for 8 minutes, 10 minutes, 15 minutes, 15 minutes and 20 minutes at increasing increments of 10º C per hold time for the initial temperature. The total run time for each sample in the GC was 100 minutes. Additional instrument conditions included: autosampler injection volume of 1 µL; flame ionization detector (FID) temperature of 255º C; injector port temperature of 250º C; hydrogen flow rate of 40 mL/min; and flow rate of 400 mL/min1q1'q. Individual peaks were identified using known standards and individual fatty acids were measured as a percent of total area under the peak.

FRAP Assay

Plasma was evaluated for antioxidant activity capacity using a spectrophotometric technique (ferric reducing ability of plasma (FRAP) assay). The procedure was described and modified by Benzie and Strain. FRAP assay was used to determine the total antioxidant capacity of each patient's plasma. The FRAP reagent was made fresh before each series of measurements to maintain validity. The reagent contained the following materials: 200 mL of acetate buffer (300 mM, pH 3.6), 20 mL TPTZ solution (0.062g 2, 4, 6- tripyridyl-s-triazine in 20 mL 4 mM HCl), 20 mL $FeCl_3$ solution (0.2748g $FeCl_3$- $6H_2O$ in 50 mL DDW) and 24 mL DDW. Samples were run in triplicate with 20 µL of serum added to 60 µL of DDW and 1000 µL of FRAP

reagent. The solution was briefly vortexed and incubated for 4 minutes at room temperature. The absorbance was determined against a blank vial consisting of 1000 µL of FRAP reagent and 80 µL of DDW. The standard curve was determined using a series of standards for diluting 1 mM ferrous sulphate solution. The concentration of the series of dilutions included: 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM. FRAP values were determined by UV-visible spectrophotometer run at 592nm.

Statistical Analysis

The data for FRAP, RBC fatty acid profile, RBC fatty acid ratios, FFQ surveys and self-reported anthropometric data was analyzed by using general linear models and correlates (bivariates) program in SPSS version 22. Case status was identified as the independent variable (case vs. control patients) while data from laboratory analysis and surveys were used as dependent variables. Differences between the values in the general linear models were considered significant if the P-value was ≤ 0.05. For the correlation coefficients, the Pearson correlation was evaluated and considered statistically significant if the P-value was ≤ 0.05.

Results

Participants

Once enrolled in the trial, all twenty participants filled out a questionnaire related to anthropometric measures, two food frequency questionnaires (FFQ, fruit and vegetable intake and fat intake), consented to have a blood draw and consented to have periodontal measures completed by a dental hygienist. Two groups were formed as defined by whether the patient had periodontal disease or did not have periodontal disease. The case group had 10 participants who were diagnosed with periodontal disease. The control group had 10 participants who were not diagnosed with periodontal disease. The mean age of the participants was 58.1 ± 11.02 years for

the case group and 56.1 ± 13.42 years for the control group as show in *Table 1*.The sex of the participants was also a factor when choosing participants. There was no significant difference in the age and sex of the participants between the case and control group ($p>0.05$).

The BMI of each participant was calculated by using the self-reported anthropometric measurements of each participant. The average BMI of the control group was 30.65 ± 6.01 and 28.68 ± 4.11 for the case group. Based on the BMI value, the case group was considered to be overweight (Overweight: 25.0-29.9). However, the control group was considered to be obese with an average BMI over 30 (Obese: >30.0).

Table 1: Self-reported anthropometric data of participants reported as mean ± standard deviation (std) and classified as case or control with the corresponding level of significance.

Variable	**Control mean ± std**	**Case mean ± std**	***P*-value**
Age (years)	56.1 ± 13.42	58.1 ± 11.02	0.734
Sex	5 females 5 males	7 females 3 males	-
BMI (kg/m^2)	30.65 ± 6.01	28.68 ± 4.11	0.446

FRAP Assay

A total of 20 FRAP assays (10 per group) were conducted and included in the analysis of this study as shown in *Table 2*. The average concentration of FRAP values for the control group was 0.5217 ± 0.15 µmol/L and 0.5427 ± 0.07 µmol/L for the case group. The difference of the FRAP values was not considered statistically significant ($p= .700$).

Table 2: Analysis from FRAP Assays reported as mean ± std and classified as case or control with the corresponding level of significance.

	Control mean ± std	**Case mean ± std**	***P*- value**
FRAP Assay (µmol/L)	0.5217 ± 0.15	0.5427 ± 0.07	0.700

Responses to Food Frequency Questionnaire

Descriptive statistics pertaining to the food frequency questionnaires are reported in *Table 3.* The data was derived from prediction equations that were used with the answers reported in the FFQs. Prediction equations were developed (by Block et al.) and have been validated.[23] The brief fat screener and the fruit and vegetable screener are the two FFQs used in this study. The results of the FFQs revealed that case participants consumed significantly higher amounts of total fat (g), saturated fat (g), unsaturated fat (g) and percent fat. The average total fat consumed by the control group was 94.46 ±16.42 g and the case group consumed an average of 109.02 ± 14.55 g of total fat ($p< 0.05$). The average saturated fat consumed for the control group was 28.24 ± 7.06 g and 35.10 ± 6.17 for the case group, which was also significant ($p=0.033$). The average unsaturated fat consumption for the control group was 66.22 ± 11.34 g and 73.92 ± 10.25 g for the case group. However, these values were not considered significant but they were approaching significance ($p= 0.129$). The average percent fat reported by the FFQs of the control group was 34.99 ± 4.10 % and 38.73 ± 3.62 % for the case group; these values were statistically different ($p= 0.044$).

Prediction equations were also developed for saturated fat (g) and unsaturated fat (g) as a percent of total fat (g) and the ratio between the two. The average saturated fat as a percent of total fat for the control group was 29.71 ± 4.62 % and 32.16 ± 3.42 % ($p> 0.195$).The average unsaturated fat as a percent of total fat for the control group was 70.28 ± 4.62 % and 67.84 ± 3.42 % for the case participants ($p=.195$). Finally, the ratio of saturated to unsaturated fat as a percent of the total fat reported by the control group was 0.43 ± 0.09 and 0.48 ± 0.07 for the case group ($p=.200$).

The responses for the fruit and vegetable questionnaire revealed that control participants consumed a numerically higher amount of servings of fruits and vegetables per day than the case participants. The servings of fruits and vegetables reported for the control group was 4.56 ± 1.94 servings per day and 4.41 ±2.09 servings per day; these values were not statistically significant (p= 0.871). These results were consistent with there being no statistically significant difference between the case and control for any of the consumed nutrients calculated by the fruit and vegetable FFQ and the FRAP assay as noted by *Table 3* and *Table 2.* The average vitamin C (mg) reported for the control group was 140.01 ± 46.066 mg and 147.97 ± 48.41 mg for the case. These values are not statistically different (p= 0.711). The average amount of magnesium (mg) consumed for the control group was 345.08 ± 80.78 mg and 366.79 ± 88.15 mg for the case group; these values were not significant (p= 0.573). The average potassium (mg) consumed, according to the FFQ, for the control group was 3318.12 ± 785.30 mg and 3506.62 ± 853.40 mg. These values were also not considered statistically different (p=0.614). Finally, the dietary fiber (g) was considered not statistically different as well (p=0.652). The dietary fiber consumed by the control group was 15.33 ± 5.36 g and 16.48 ± 5.77 g for the case group.

Table 3: Data from Food Frequency Questionnaires with the corresponding value of significance for each variable.

Variable	**Control mean ± std**	**Case mean ± std**	***P*-value**
Total fat (g/day)	94.46 ± 16.42	109.02 ± 14.55	0.05
Saturated fat (g/day)	28.24 ± 7.06	35.10 ± 6.17	0.033
Unsaturated fat (g/day)	66.22 ± 11.34	73.92 ± 10.25	0.129
Percent dietary fat (%)	34.99 ± 4.10	38.73 ± 3.62	0.044
Saturated fat (% of total fat)	29.71 ± 4.62	32.16 ± 3.42	0.195
Unsaturated fat (% of total fat)	70.28 ± 4.62	67.84 ± 3.42	0.195
Saturated: Unsaturated calculated ratio	0.43 ± 0.09	0.48 ± 0.07	0.200
Fruit/Vegetable (servings/day)	4.56 ± 1.94	4.41 ± 2.09	0.871
Vitamin C (mg/day)	140.01 ± 46.06	147.97 ± 48.41	0.711

Magnesium (mg/day)	345.08 ± 80.78	366.79 ± 88.15	0.573
Potassium (mg/day)	3318.12 ± 785.3	3506.62 ± 853.40	0.614
Dietary Fiber (g/day)	15.33 ± 5.36	16.48 ± 5.77	0.652

Analysis of RBC Fatty Acid Composition

Fatty acid composition of red blood cells for case and control patients is reported in *Table 4* and *Table 5.* These tables display the results of individual fatty acid profiles as well as calculated ratios of average saturated fatty acids, average unsaturated fatty acids, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). This research study hypothesized that the case group would consume more total fat, have lower levels of omega-3 fatty acids and higher levels of omega-6 fatty acids. Results from the analysis of the red blood cell membrane fatty acid profile do support this theory. The average omega-3 fatty acids found in the red blood cell membrane by the control group was 9.59 ± 9.54 % and 9.18 ± 8.22 % (p= 0.919) for the case group. The average omega-6 fatty acids found in the RBC membrane by the control group was 22.22 ± 4.76 % and 23.52 ± 8.64 % for the case group (p= 0.683). The average ratio of omega-6 fatty acid to omega-3 fatty acid found in the RBC membrane of the control group is 7.79 ± 10.46 and 4.27 ± 3.09 for the case group (p= 0.322). These are not statistically significant but the values do represent a trend toward lower omega-3 fatty acid consumption and higher omega-6 fatty acid consumption for the case group. The patient population included in this study is only a small portion (20 patients) of the complete study population (132 patients) and final results still need to be evaluated for significance.

Table 4: Analysis of Individual Fatty acid profiles of RBC membranes for cases and controls. Fatty acids are expressed by average % area under the peak and reported as mean ± std.

Fatty Acid, omega type, carbon: double bonds	**Control mean ± std**	**Case mean ± std**	***P*-value**
Myristic, 14:0	1.19 ± 0.93	1.06 ± 0.72	0.724
Palmitic, 16:0	21.43 ± 5.52	20.63 ± 3.47	0.702
Palmitoleic ,16:1	1.98 ± 1.52	1.42 ± 1.13	0.357
Stearic, 18:0	11.53 ± 8.42	12.20 ± 7.73	0.854
Oleic (n-9), 18:1	12.74 ± 3.83	11.20 ± 4.48	0.418
Linoleic (n-6), 18:2	11.62 ± 5.95	11.12 ± 1.51	0.800
Linoleic (n-6), 18:2 IT	3.94 ± 5.04	3.96 ± 4.52	0.992
Arachidic, 20:0	1.13 ± 2.93	1.98 ±5.05	0.648
Arachidonic (n-6), 20:4	11.58 ± 7.06	12.38 ± 6.13	0.790
EPA (n-3), 20:5	4.53 ± 7.21	4.01 ± 8.58	0.885
Behenic, 22:0	.90 ± .91	.98 ± .52	0.799
Adrenic (n-6), 22:4	2.33 ± 2.21	3.81 ± 3.49	0.275
Lignoceric, 24:0	1.29 ± 1.46	1.83 ± 1.09	0.367
DPA (n-3), 22:5	2.15 ± 1.25	2.23 ± 0.98	0.873
DHA (n-3), 22:6	2.16 ± 1.29	2.44 ± 1.03	0.593

Table 5: Analysis of Fatty Acid Ratios. Fatty acids are expressed by average % area under the peak and reported as mean ± std.

Fatty Acid Types & Ratios (% area under chromatogram curve)	**Control mean ± std**	**Case mean ± std**	***P*- value**
Average Omega-6	22.22 ± 4.76	23.52 ± 8.64	0.683
Average Omega-3	9.59 ± 9.54	9.18 ± 8.22	0.919
Omega-6: Omega-3 Ratio	7.79 ± 10.46	4.28 ± 3.09	0.322
MUFA	17.62 ± 5.67	15.62 ± 6.14	0.466
PUFA	43.71 ± 8.18	44.22 ± 6.62	0.879
Total Saturated FA	38.67 ± 6.78	40.13 ± 7.83	0.662
Total Unsaturated FA	61.32 ± 6.78	59.87 ± 7.83	0.662
Saturated: Unsaturated Ratio	0.65 ± 0.17	0.69 ± 0.21	0.587

Periodontal Measures

Descriptive statistics pertaining to the periodontal measure for each patient can be found in *Table 6.* This table displays the results for bleeding index and periodontal probing depth in the two groups. The differences between control and case groups are highly significant with a p-value of < 0.0001 for each periodontal measure. The average bleeding index for control group was 0.10 ± 0.316 % and 34.20 ± 19.19 % for the case group. The average periodontal probing depth for control group was 2.00 ± 0.00 mm and 5.40 ± 0.843 mm for the case group.

Table 6: Analysis of Periodontal measures with the corresponding value of significance for each variable.

Periodontal Measures	**Control mean ± std**	**Case mean ± std**	***P*- value**
Bleeding Index (%)	0.10 ± 0.316	34.20 ± 19.19	0.0001
Probing Depth (mm)	2.00 ± 0.00	5.40 ± 0.843	0.0001

Correlation Coefficients

The correlation coefficients between dependent variables are listed in *Tables 7, 8 and 9.* Correlation coefficients detect the relationship between two dependent variables.

	BI	N6N3	Age	PD	TF	MUFA
BI Pearson Correlation	1	.161	.045	.691	-.084	-.364
Significance		.255	.426	.000	.362	.057
N6N3 Pearson Correlation	.161	1	-.144	.121	.596	-.101
Significance	.255		.278	.311	.004	.340
Age Pearson Correlation	.045	-.144	1	-.037	.026	-.273
Significance	.426	.278		.438	.457	.122
PD Pearson Correlation	.691	.121	-.037	1	-.061	-.328
Significance	.000	.311	.438		.399	.079
TF Pearson Correlation	-.084	.596	.026	-.061	1	.154
Significance	.362	.004	.457	.399		.258

MUFA Pearson Correlation	-.364	-.101	-.273	-.328	.154	1
Significance	.057	.340	.122	.079	.258	
PUFA Pearson Correlation	.185	-.296	-.006	.249	-.694	-.584
Significance	.218	.110	.491	.145	.000	.003
TOTUS Pearson Correlation	-.030	-.439	-.200	.074	-.746	-.011
Significance	.449	.030	.199	.378	.000	.482
TOTSAT Pearson Correlation	.030	.439	.200	-.074	.746	.011
Significance	.449	.030	.199	.378	.000	.482
SATUNSAT Pearson Correlation	.074	.387	.295	-.021	.646	-.134
Significance	.378	.051	.104	.465	.001	.286
N3 Pearson Correlation	-.080	-.917	-.003	.041	-.734	-.119
Significance	.369	.000	.496	.432	.000	.309
N6 Pearson Correlation	.238	.461	-.069	.206	-.190	-.491
Significance	.156	.024	.386	.192	.211	.014
FRAP Pearson Correlation	.237	-.005	.284	.070	-.184	-.099
Significance	.157	.492	.112	.384	.219	.339
FV Pearson Correlation	-.278	.184	.285	-.060	.225	-.188
Significance	.118	.226	.112	.401	.170	.213
Vit C Pearson Correlation	-.082	.275	.156	.078	.434	-.101
Significance	.365	.127	.256	.372	.028	.336

	PUFA	TOTUS	TOTSAT	SATUNSAT	N3
BI Pearson Correlation	.185	-.030	.030	.074	-.080
Significance	.218	.449	.449	.378	.369
N6N3 Pearson Correlation	-.296	-.439	.439	.387	-.917
Significance	.110	.030	.030	.051	.000
Age Pearson Correlation	.-.006	-.200	.200	.295	-.003
Significance	.491	.199	.199	.104	.496
PD Pearson Correlation	.249	.074	-.074	-.021	.041
Significance	.145	.378	.378	.465	.432
TF Pearson Correlation	-.694	-.746	.746	.646	-.734
Significance	.000	.000	.000	.001	.000
MUFA Pearson Correlation	-.584	-.011	.011	-.134	-.119
Significance	.003	.482	.482	.286	.309
PUFA Pearson Correlation	1	.818	-.818	-.718	.589
Significance		.000	.000	.000	.003
TOTUS Pearson Correlation	.818	1	-1.00	-.979	.641
Significance	.000		.000	.000	.001
TOTSAT Pearson Correlation	-.818	-1.000	1	.979	-.641

Significance	.000	.000		.000	.001
SATUNSAT Pearson Correlation	-.718	-1.00	1	.979	.641
Significance	.000	.000		.000	.001
N3 Pearson Correlation	.589	.641	-.641	-.577	1
Significance	.003	.001	.001	.004	
N6 Pearson Correlation	.602	.393	-.393	-.354	-.229
Significance	.002	.043	.043	.063	.166
FRAP Pearson Correlation	.123	.081	-.081	-.055	-.018
Significance	.303	.367	.367	.408	.470
FV Pearson Correlation	-.097	-.253	.253	.289	-.165
Significance	.342	.141	.141	.109	.244
Vit C Pearson Correlation	-.217	-.339	.339	.341	-.331
Significance	.179	.072	.072	.070	.077

	N6	FRAP	FV	Vit C
BI Pearson Correlation	.238	.237	-.278	-.082
Significance	.156	.157	.118	.365
N6N3 Pearson Correlation	.461	-.005	.184	.275
Significance	.024	.492	.226	.127
Age Pearson Correlation	-.069	.284	.285	.156
Significance	.386	.112	.112	.256
PD Pearson Correlation	.206	.070	-.060	.078
Significance	.192	.384	.401	.372
TF Pearson Correlation	-.190	-.184	.225	.434
Significance	.211	.219	.170	.028
MUFA Pearson Correlation	-.491	-.099	-.188	-.101
Significance	.014	.339	.213	.336
PUFA Pearson Correlation	.602	.123	-.097	-.217
Significance	.002	.303	.342	.179
TOTUS Pearson Correlation	.393	.081	-.253	-.339
Significance	.043	.367	.141	.072
TOTSAT Pearson Correlation	-.393	-.081	.253	.339
Significance	.043	.367	.141	.072
SATUNSAT Pearson Correlation	-.354	-.055	.289	.341
Significance	.063	.408	.109	.070
N3 Pearson Correlation	-.229	-.018	-.165	-.331
Significance	.166	.470	.244	.077
N6 Pearson Correlation	1	.231	.110	.128
Significance		.163	.322	.295

FRAP Pearson Correlation	.231	1	-.241	-.242
Significance	.163		.153	.152
FV Pearson Correlation	.110	-.241	1	.859
Significance	.322	.153		.000
Vit C Pearson Correlation	.128	-.242	.859	1
Significance	.295	.152	.000	

Discussion

Participants

The aim of this study was to explore the potential relationships between dietary intake and periodontal disease. In order to accurately form a comparison between dietary intake and periodontal disease, case participants consisted of patients over the age of 25 years old who had been diagnosed with periodontal disease. Control patients were people over the age of 25 years old who did not exhibit periodontal disease. The subject of this thesis includes 20 patients; 10 patients were identified for the case group and 10 for the control group. The mean age for the participants in the case group was 58.1 ± 11.02 years of age and 56.1 ± 13.42 for the control group as shown in *Table 1.* There was no significant difference in age between the case group and the control group (p=0.734) and as a result was not included as a covariate in the statistical model.

The average BMI for the control group was 30.65 ± 6.01 and 28.68 ± 4.11 for the control group, as shown in *Table 1*. Based on the BMI value, the control group was obese (BMI> 30) and the case group was considered overweight (BMI> 26 and <30). There was no statistical difference between BMI of each group (p= 0.446). Research finds that obesity increases the chance of periodontal disease by as much as 77%.[20] Propić et al. studied a correlation between obesity and general condition of the oral cavity in a group of men and women of ages 31-60

years old. That study demonstrated that obese (BMI> 30) subjects' oral cavities were in poorer condition, meaning the obese patients had a higher number of lost teeth, an increased number of dental caries and an increase in the intensity of periodontium diseases.[20] With both of the groups being at least overweight, the factor in periodontal disease formation must be found somewhere else.

FRAP Assay

Ferric reducing antioxidant power (FRAP) assays were conducted on each participant and included in the analysis of this study. Results from the FRAP assay are listed in *Table 2*. The ferric reducing ability of the plasma in the case group was found to be numerically higher antioxidant capacity (p=0.700) as compared to the control group. The average concentration of FRAP values for the case group was 0.5427 ± 0.07 µmol/L and 0.5217 ± 0.15 µmol/L for the control group. Oxidative stress was defined as a disturbance between the production of highly reactive molecular species and antioxidant defense systems, leading to potential damage. Chronic or excess production of reactive oxygen species (ROS) within the periodontal pocket is a possible cause of the periodontal tissue damage. Antioxidants counteract the harmful effects of free radicals (ROS).[25] Therefore, periodontal disease has been associated with a reduction in antioxidant activity leading to the increase in oxidative stress.[10] The decrease in the levels of these important antioxidants can be considered an important mechanism by which toxic effects of free radicals can initiate periodontal disease.[13] Diets rich in vegetables and Vitamin C (a potent antioxidant) appear to be associated positively with better periodontal disease health and negatively with periodontal disease progression.[25]

Food Frequency Questionnaire

Several variables within the food frequency questionnaire were evaluated and shown to have significant difference between control and case groups. These variables can be found in *Table 3.* Results of the FFQs revealed that case participants consumed significantly higher amounts of: total fat (g), saturated fat (g) and a higher dietary fat percent. The average total fat consumption reported by the control group was 94.46 ± 16.42 g/day and 109.02 ± 14.55 g/day for the case group. These values were significant (p=0.05). This showed a relationship between an increase in fat consumption and periodontal disease in the case group. Prediction equations were used to develop saturated fat and unsaturated fat as a percent of total fat consumed and the ratio between the two (Saturated: Unsaturated). The average saturated fat as a percent of total fat for the control group was 29.71 ± 4.62 % and 32.16 ± 3.42 % for the case group (p= 0.195). The average unsaturated fat as a percent of total fat for the control group was 70.28 ± 4.62 % and 67.84 ± 3.42 % for the control group (p=0.195). These results were consistent with the red blood cell membrane fatty acid, which can be found in *Table 5*. Saturated fatty acids are believed to raise total and low-density lipoprotein cholesterol.[8] This diet-induced hyperlipidemia induces downstream pro-inflammatory mediator production. Increased inflammation is recognized as one of the key underlying etiologic conditions for the development and progression of periodontal disease.[8] The percent of saturated fat was higher in the case group than the control indicating a possible association between saturated fatty acids and periodontal disease.

The fruit and vegetable FFQ revealed that control participants consumed slightly more servings of fruits and vegetables than the case group, which was 4.56 ± 1.94 servings/day for the control group and 4.41 ± 2.09 servings/day for the case group. The hypothesis was that the control group would consume more fruits and vegetables than the case. The control participants

did not consume statistically more fruits and vegetables than the case group. However, they did consume numerically more than the case group (p= 0.871). When the complete data set of 132 patients is analyzed a more accurate determination of the relationship between fruit and vegetable intake and periodontal disease can be assessed. The higher fruit and vegetable consumption is not reflected in the nutrient analysis. Vitamin C has a very strong inverse relationship with periodontal disease. Vitamin C is a potent antioxidant and has the ability to decrease oxidative stress in periodontal tissue.[12] However, the patients in the case group consumed more vitamin C (147.97 ± 48.41 mg/day) than the control group (140.01 ± 46.06 mg/day). This could be explained by the amount of food the case group consumes. The case group could consumed more fats in total than the control group; as indicated by total fat (gram) consumed and as a result consumed more total nutrients than the control group.

Analysis of RBC Fatty Acid Profile

Fatty acid composition of red blood cell membranes for case and control patients is found in *Table 4* and *Table 5.* Results of individual fatty acid profiles as well as calculated ratios of average saturated fatty acid (FA), average unsaturated FA, MUFAs and PUFAs are indicated in these tables. This thesis hypothesized that there would be lower consumption of omega-3 fatty acids and higher consumption of omega-6 fatty acids in the case group than in the control. The results from the analysis of the red blood cell membrane fatty acid profile did support this hypothesis. The average omega-6 fatty acids consumed by the control group was 22.22 ± 4.76 % and 23.52 ± 8.64 % by the case group (p= 0.683). The average omega-3 fatty acid consumption found in the red blood cell membrane of the control group was 9.59 ± 9.54 % and 9.18 ± 8.22 for the case group (p= 0.919). There is not significant statistical difference between the case and control group for consumption or omega-3 and omega-6 fatty acids. However, there is a

numerically lower percent of consumption of omega-3 fatty acids in the case group and a numerically higher percent of consumption of omega-6 fatty acids in the case group as well. Fatty acids are responsible for many functions of immune cells such as regulation of gene expression by either altering receptor activity, signaling or activation of transcription factors and the production of lipid mediators.[2] Therefore, alteration of the fatty acid composition in the cell membrane may result in changes to the type of lipid mediators synthesized and the subsequent inflammatory cascade. Omega-3 fatty acids are found to have anti-inflammatory effects. Increasing intake of omega-3 fatty acids should decrease the risk of developing or the progression of periodontal disease.[2] Analysis of the entire population (132 patients) for the large study will provide more evidence as to whether or not there is a relationship of omega-3 and 6 fatty acid intake on the incidence of periodontal disease.

The ratio of n-6 to n-3 PUFAs was found to be a major factor in periodontal disease status. The 2:1 or lower ratio of omega-6 to omega-3 fatty acids has been shown to suppress inflammation and therefore aid in preventing periodontal disease.[3] The ratio found in most western diets is 16:1, which increases inflammation and may be a cause of periodontal disease and many other chronic diseases.[3] The ratio was found in the control group to be 7.79 ± 10.46 and 4.28 ± 3.09 in the case group. This is not found to be statistically significant ($p=0.322$). However, it will be more apparent if there is a relationship between omega fatty acid ratio and periodontal disease when the entire population is analyzed.

Conclusion

Analysis of the complete data set (132 patients) will provide a more accurate representation of the interaction between nutrient intake and periodontal disease. In the summer

of 2015, water soluble vitamins in the plasma, as determined by HPLC, will be available to further analyze the effects of dietary intake on periodontal disease. This thesis consists of only preliminary data. When the complete data set is analyzed there should be more conclusive and significant results regarding each variable. This project has been designed to test the hypothesis that people who consume more fruits and vegetables and less fat will be less likely to develop periodontal disease. Data from all patients in the study may provide stronger statistical power to indicate whether the proposed hypothesis is correct; however, the initial results reported in this thesis do trend toward a validation in the hypothesis.

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