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The Association Between Physical Activity And Serum Immunoglobulin G (IGG) Antibodies Against Periodontal Bacteria

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THE ASSOCIATION BETWEEN PHYSICAL ACTIVITY AND SERUM IMMUNOGLOBULIN G (IGG) ANTIBODIES AGAINST PERIODONTAL BACTERIA

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ABSTRACT

The purpose of this study is to add more research and evidence to the mechanisms surrounding periodontal disease, periodontal microorganisms, and physical activity. The main objective of this study is to evaluate and explore the association between physical activity and serum IgG antibodies, which are grouped into four distinct clusters, formed from species specific 19 periodontal antibody titers. This cross sectional study divided physical activity into three categories: adequately physically active, inadequately physically active, and inactive based on MET scores. Certain models were adjusted for confounders including age, sex, race/ethnicity, income-to-poverty ratio, years of formal education, smoking, alcohol intake, BMI, waist circumference, diet, number of missing teeth, diabetes status, and frequency of visits to the dentist. The 5,611 participants came from NHANES III. Our findings show that in unadjusted and minimally adjusted models, physical activity is significantly, positively associated with the antibodies in the Orange-Blue cluster (a cluster associated with healthy periodontal states). However, after more complex adjustment, physical is not significantly associated with antibodies to oral microorganisms. These findings indicate that physical activity may improve the periodontal microbiota. However, the mechanisms surrounding periodontal microorganisms, physical activity, and periodontal disease are very complex and require further serious and complex research.

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CHAPTER I: Introduction

1.1 Statement of Problem

Periodontal disease occurs when inflammation in the gums causes attachment loss and loss of supporting alveolar bone. According to the Centers for Disease Control and Prevention CDC, approximately half of American adults aged 30 and older have periodontal disease, equivalent to about 64.7 million American adults with mild, moderate, or severe periodontitis. In adults aged 65 and older, the prevalence is even higher at 70.1 percent. And these percentages are exacerbated in the populations of men, those with lower incomes, less education, and smokers (CDC). This is a cause for concern because not only can periodontal disease cause bad breath and tooth loss, but it has been associated with systemic, and sometimes life threatening diseases such as cardiovascular disease, coronary heart disease and stroke, metabolic endotoxemia hyperglycemia, bacterial pneumonia, nonalcoholic liver diseases, rheumatoid arthritis, respiratory diseases, osteoporosis diabetes, obesity, preterm, and low birth weight (Li et al., 2000; Seymour et al., 2007; Merchant et al., 2014; Nagpal et al., 2014). However, the mechanisms that connect periodontitis and these serious diseases are still not completely understood.

It is also well-known that oral microorganisms are the primary cause of periodontal disease, though research is now emerging that the environment, behavior, and genetics may also contribute to the disease process (Brogden et al., 2002). Up until the

1970s, studies tended to focus on the overall mass of microorganisms as the cause of periodontal disease. However, recent research has challenged this way of thinking and has begun to look at specific bacterial species in their roles in the initiation and progression of periodontal disease. For example, Brogden et al. found that different strains of the same species might demonstrate different pathogenicity (Brogden et al., 2002). At the same time, certain microorganisms are frequently found together in diseased and healthy individuals (Lima et al., 2015). Socransky and colleagues grouped microorganisms associated with periodontal disease using cluster analysis. Based on these data, they grouped these microorganisms into five color coded categories (or complexes). Socransky's red complex is most strongly associated with periodontal problems (Socransky et al., 1998). It is generally preceded by colonization of the orange complex species (Guthmiller et al., 2002).

Physical activity is found to be inversely associated with periodontal disease in cross-sectional and prospective studies (Al-Zahrani et al. 2005, Bawadi *et al.* 2011, Merchant *et al.* 2003, Samnieng *et al.* 2013). Physical activity is hypothesized to impact periodontal disease through its effect on glucose metabolism. Physical activity improves glucose metabolism and reduces insulin resistance and hyperglycemia. Hyperglycemia has been associated with increased systemic inflammation; it is also related to deposition of advanced glycation end products in the periodontal tissues causing inflammation. Systemic and localized inflammation as a consequence of hyperglycemia is hypothesized to increase periodontal damage (Nagpal et al. 2015). However, the effect of physical activity on the microorganisms associated with periodontal disease is understudied.

The National Health and Nutrition Examination Survey III (NHANES III) is a large, nationally representative dataset, which contains information on antibodies against microorganisms related with periodontal disease, physical activity, periodontal disease and other factors related to periodontal disease and physical activity. The antibodies against periodontal 19 microorganisms were analyzed from blood serum samples. The data have been grouped into 4 clusters that are related with periodontal health.

1.2 Purpose

The purpose of this thesis is to examine the relationship between physical activity and antibodies against clusters of microorganisms related to periodontal disease by analyzing a cross-sectional dataset from the National Health and Nutrition Examination Survey (NHANES) III.

Research Questions:

Research Question 1: Is physical activity inversely associated with the Orange-Red cluster of antibodies?

Hypothesis: An increase in physical activity is associated with a decrease of the Orange-Red antibody cluster scores.

Research Question 2: Is physical activity positively associated with the Orange-Blue cluster of antibodies?

Hypothesis: An increase in physical activity is associated with an increase of Orange-Blue antibody cluster scores.

1.3 Significance of Research

This study attempted to identify the degree to which physical activity is associated with groups of antibodies against periodontal microorganisms that have been found to be associated with hyperglycemia and other health problems. This association has not been adequately evaluated before. The findings of this study adds to the understanding of the mechanisms through which physical activity and periodontal disease may be related.

1.4 Study Outline

Chapter I explains the problem of periodontal disease, its relationship to microorganisms, and experts' lack of knowledge on the mechanisms surrounding this relationship. It also states the purpose and objectives of this thesis and what this research contributes to the current research in this field by studying the relationship between physical activity and periodontal microorganisms. Chapter II consists of an explanation of Socransky's microbial complexes. It also gives a systematic literature review covering mechanistic studies and epidemiological studies on the relationship between physical activity and periodontal disease, obesity and periodontal disease, periodontal disease and microorganisms, and Periodontal Microorganisms and Hyperglycemia. Finally it gives an introduction to the small amount of research that is being done on the association between physical activity and microorganisms. Chapter III explains the data and Methods for research including the statistical analysis. Chapter IV presents a detailed report of the results of the research conducted. And Chapter V provides a summary and conclusion of the research.

CHAPTER II: Literature Review

2.1 Overview

Epidemiologists and other researchers have established an inverse association between physical activity and periodontal disease. They have also firmly established a positive association between microorganisms and periodontal disease. This study further investigates the complex relationship between physical activity and microorganisms.

2.2 Socransky's microbial complexes

Sigmund Socransky was a pioneer in the study of periodontal microbiology. He classified five complexes of bacterial species based on their role in periodontal disease. He labeled these complexes with the colors blue, yellow, purple, green, red, and orange. For example, the three species in the red complex, P. gingivalis, T. forsythia, and T. denticola, are associated with bleeding on probing. The red complex is also most strongly associated with increased pocket depth. The orange complex, consisting of P. intermedia, P. nigrescens, P. micros, F. nuc. vincentii, F. nuc. nucleatum, F. nuc. polymorphum, F. periodonticum, C. gracilis, C. rectus, E. nodatum, C. showae, and S. constellatus, is similar to the red and is also associated with increased pocket depth. These microorganisms often precede the colonization of the species in the red complex. The other species showed no association with pocket depth. Species in the yellow complex, S. mitis, S. oralis, S. sanguis, S. gordonii, and S. intermedius, and purple complex, V.

parvula and A. odontolyticus, are generally associated with healthy periodontal states. The organisms in the blue complex, which consisted of different Acctinomyces species, are found in both healthy and unhealthy periodontal states. And the green complex organisms, E. corrodens, C. gingivalis, C. sputigena, and C. ochracea, are weakly associated with periodontal disease (Haffajee et al., 2008).

2.3 Potential mechanisms between physical activity and periodontal disease

The pathophysiological mechanisms between physical activity and periodontal disease are under-studied. However, some authors have speculated on possibilities. A number of studies (Bawadi *et al.l*, Al-Zahrani *et al.*, and Merchant *et al.*) suggest insulin resistance as a possible mechanism. While this relationship has not yet been fully clarified, Bawadi *et al.* suggest that it is possible that the risk for periodontal disease increases with adiposity and insulin resistance caused by physical inactivity. However, the relationship between insulin resistance and periodontal disease is widely disputed in the scientific community (Watanabe K *et al.* 2014).

Al-Zahrani *et al.* also suggest that increasing insulin sensitivity could be a possible mechanism by which physical activity protects against periodontal disease and they cite many studies showing an association between physical activity and insulin sensitivity. They state that increasing insulin sensitivity leads to prevention of the progression of type II diabetes which is a known risk factor for periodontitis. They elaborate by pointing out that contracting muscles from physical exercise may have a synergistic effect with insulin to enhance glucose uptake into the cells. This could be related to increased blood flow and glucose transport to the muscles. Merchant *et al.* also expound on this point, stating that physical activity may increase translocation of GLUT4 glucose transporters into cells and increases GLUT4 expression. This may explain why even a small amount of physical activity can have a beneficial effect on glucose homeostatic for hours afterwards.

Therefore, physical activity can act as an alternative to maintaining glucose homeostasis. Physical activity is associated with a decreased risk of non-insulin dependent diabetes mellitus (NIDDM). And diabetes is associated with an increased prevalence and severity of periodontitis, higher blood glucose, and therefore, advanced glycation end products (AGE). These bind to receptors (RAGE) in the periodontium. This stimulates an inflammatory response. There is evidence that blocking RAGE can decrease the inflammatory response and decrease alveolar bone destruction (Lalla et al., 1998).

Another possibility is that physical activity reduces inflammation which has been shown to have a significant effect on the development of periodontitis (Al-Zahrani, 2005; Sanders, 2009). Al-Zahrani *et al.* cite studies showing the association between physical activity and plasma levels of inflammatory markers. They speculate that this could be related to the advantageous effect of physical activity on improving blood flow and efficiency of oxygen exchange. In a case control study, Sanders *et al.* studied the association between physical activity and inflammatory mediators and if this association different between people with periodontitis verses those without. Excessive inflammation is a part of the pathogenesis of periodontal disease. Sanders *et al.* did not find an association between physical activity and periodontitis. However, among the people with periodontal disease, physically active participants had lower measurements of inflammatory mediators. They concluded that physical activity may protect against an excessive inflammatory response among those with periodontitis.

Yet another possible mechanism is obesity (Al-Zahrani, 2005). Physical activity may protect against obesity by increasing the kilocalories used compared to the amount consumed. Obesity is associated with increased prevalence of periodontitis. Finally, physical activity could reduce stress which also has been associated with a higher prevalence of periodontitis (Al-Zahrani, 2005). Figure 2.1 shows a diagram of some of these hypothesized mechanisms.

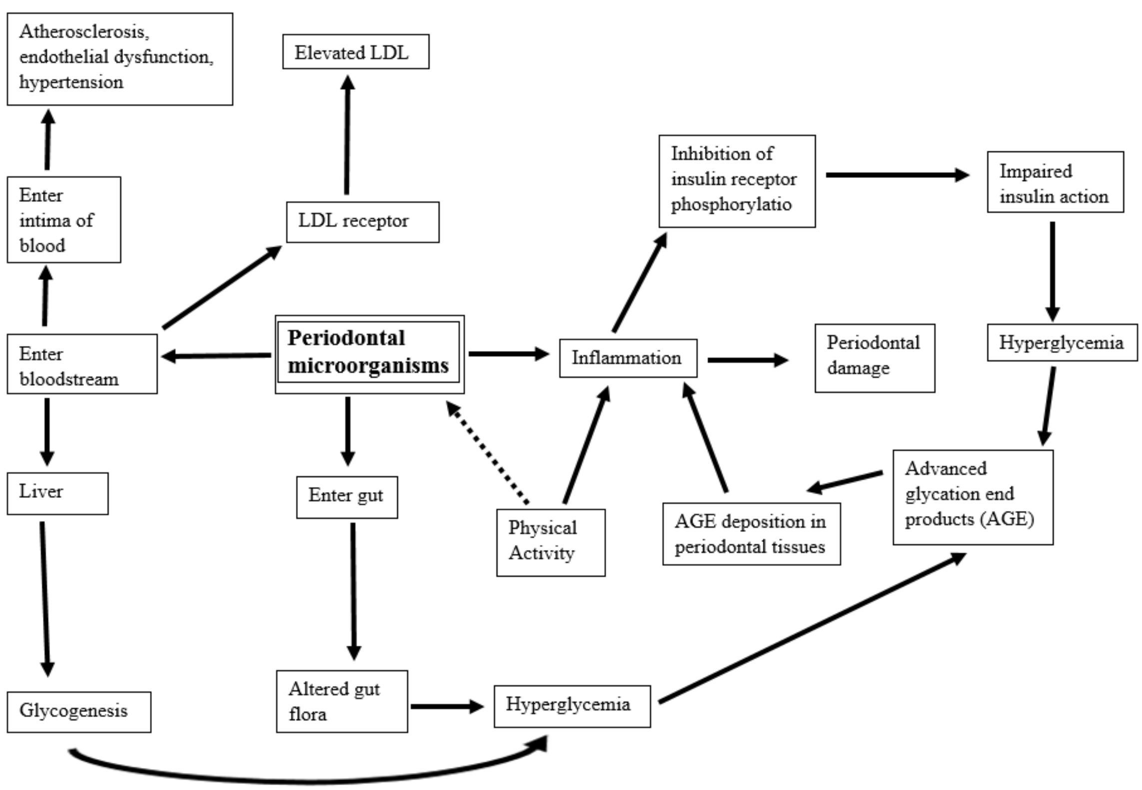

Figure 2.1: Diagram of Hypothesized Mechanisms

Periodontal→LDL: (Kim et al., 2006; Penumarthy et al., 2013; Katz et al., 2002)
Periodontits→inflamation→glucose/lipid metabolism←→insulin resistance, obesity, diabetes→hyperglycemia→AGEs deposition in tissues→hyperinflammation (Nagpal, 2014)
Physical Acitivty→Inflammation: (Al-Zahrani, 2005; Sanders, 2009)

2.4 Physical Activity and Periodontal Disease

A number of studies have found a negative association between physical activity and periodontal disease (Bawadi *et al.* 2011, Merchant *et al.* 2003, Al-Zahrani *et al.* Ag. 2005, Al-Zahrani et al, Oct. 2005; Samnieng *et al.* 2013). Bawadi *et al.* studied 340 18-70 year-olds from the outpatient clinics in the medical center of Jordan University of Science and Technology. They divided the people into high, moderate, and low levels of physical activity. For each person, they recorded the number of missing teeth, the plaque index, gingival index (a scale from one to four, used by dental professionals to visually analyze the extent of periodontal disease), and the clinical attachment loss (CAL). They found that along with a poor diet, lack of physical activity increases one's odds of having periodontal disease. More specifically, the researchers found that, compared to those who were moderately or rarely physically active, those who were highly physically active had statistically significantly lower average gingival index, CAL, and percentage of cites with CAL greater than three millimeters. After adjusting for gender, age, marital status, years of education, BMI, diabetes mellitus, plaque index, and number of missing teeth, individuals who rarely engaged in physical activity were more likely to have periodontal disease than those who were highly physically active with an odds ratio of 3.8.

A cross-sectional study conducted by Al-Zahrini *et al.* examined the association between periodontal disease and three health-enhancing behaviors: maintaining a normal weight, engaging in physical activity, and eating a high-quality diet. They found that after controlling for confounders, people who engaged in all three healthy habits were 40% less likely to have periodontitis than those who engaged in none of these behaviors. In a similar study, the same authors examined the relationship between periodontal disease and physical activity exclusively. They studied 2,521 individuals from the third National Health and Nutrition Examination Study (NHANES III) who were 18 or older, had periodontal examinations, and had reported similar physical activity/inactivity for 10 years or longer. Periodontal status was analyzed by probing depth and attachment loss at the mid-facial and mesio-facial aspects of each tooth. Periodontitis was defined as both an attachment loss of 3mm or more and a probing depth of 4mm or more. For physical activity, the researchers used the metabolic equivalent intensity rating (MET). They divided the participants into three groups: active (those who reported to meet the recommended five or more episodes of moderate physical activity per week or three or more episodes of vigorous intense activity per week), partially active (those who reported some activity, but less than required of the physically active), and inactive. They found the prevalence of periodontitis decreased as the amount of physical activity increased: 25.2% among inactive participants, 16.9% among partially active participants, and 13% among the participants who met the recommended amount of exercise.

Merchant *et al.* also studied the association between physical activity and risk of periodontal disease. In a prospective cohort study, the researchers studied 39,461 participants form the Health Professionals Follow-up study (HPFS). The participants were male, US based health professionals who were 40-75 years old at baseline. They were free of periodontitis, and other potentially confounding diseases at baseline. Periodontitis and physical activity were measured by validated questionnaires. Measurements of physical activity were based on MET's. Periodontitis was defined as the first report from participants gave of a professional diagnosis of periodontitis with bone loss. In a subsample of participants, they estimated periodontal bone loss in millimeters from bitewing radiographs using a periodontal probe, viewing box, and magnifying loupes. They then calculated the mean bone loss for each individual. The researchers found a 3% decrease in risk of periodontal bone loss for every 10-MET increase in average physical activity after adjusting for a number of confounders. In the categorical analysis, those in the highest quintile of physical activity had a 13% lower risk of periodontal disease than those in the lowest quintile. They also notably only found an association between physical activity and periodontitis in younger men, but not older men.

2.5 Periodontal disease and Microorganisms

The study of microorganisms and their effect on periodontal health is field of study that has made great strides recently. Over 600 oral bacterial species have been identified. There are approximately 630 different taxa in the oral cavity, though few seem to be important in relation to periodontitis (Kazor *et al.*, 2003). One of the earlier studies to organize oral microbes identified using culture was by Listgarten *et al.* in 1975. Subsequently, the use of DNA-probes to asses sub gingival plaque sped up the understanding of co-occurrence of oral bacteria. More recent technological advances have allowed for DNA-DNA hybridization, which led to Socransky’s color-coded complexes. The technology is continuing to improve at a fast pace.

The relationship between microorganisms and periodontal disease has been firmly established. Periodontitis is a disease driven by bacteria (Socransky *et al.*, 1991). However, researchers are still trying to decipher which oral bacteria or group of oral bacteria are responsible for these diseases. Three bacteria, *Aggregatibacter* (formerly

Actinobacillus), *actinomycetemcomitans, Tannerella forsythia* (formerly *Bacteroides forsythus*), and *Porphyromonas gingivalis* have been officially designated as etiological agents of periodontitis in a consensus report in 1996 (American Academy of Periodontology). A study done by Benachinmardi *et al.* found that anaerobic bacteria cause chronic periodontitis along with aerobes and microaerophilic organisms. Plaque bacteria are known to cause common diseases such as periodontitis (Nibali *et al.*, 2009).

2.6 Obesity/BMI and Periodontal Disease

It is well known that physical activity is associated with certain serious systemic diseases and poor health outcomes such as obesity. (CDC, 2015) There is also strong evidence emerging that periodontal disease is associated with many of the same outcomes. (Keller et al., 2015; Bascones-Martinez et al., 2011; Meyer et al., 2008) Therefore, it is useful to understand the research and mechanisms between periodontal disease and these outcomes in order to better be able to understand the association between physical activity and periodontal disease. There is strong evidence for a positive association between BMI and periodontal disease. In 2011, Suvan et al. systematically reviewed 33 articles. Their results supported the association between BMI and periodontal disease. In a meta-analysis of 19 of the papers, the odds ratio for the association between overweight/obese and periodontitis was 2.13 (CI: 1.40, 3.26). Another meta-analysis by Chaffee and Weston in 2010 found an odds ratio of periodontal disease of 1.35 (1.23, 1.47) comparing obese and non-obese individuals. Their meta-analysis suggested about a one-third increase in the prevalence odds of obesity for people with periodontal disease as well as a slight linear increase in the odds of periodontal disease with increasing BMI. This was based on a review of 70 studies representing 50 independent populations. The researchers found these associations to be stronger in non-smokers, women, and younger people.

A problem with most of the studies done on the association between obesity and periodontal disease is that they are cross-sectional. In these types of studies, it is impossible to determine a temporal sequence of events: weather periodontal disease causes weight gain or obesity is a risk factor for periodontal disease (Chaffee et al., 2010).

There are a few biologically plausible explanations for this association. First, clinical evidence has shown that obese individuals may have an increased local inflammatory response. This increased inflammatory state could predispose obese people to periodontal tissue destruction (Suvan, 2011). Clinical evidence also suggests that obesity could lead to an altered periodontal microflora because of the increase in proinflammatory cytokines (Suvan, 2011).

2.7 Periodontal Health and Hyperglycemia

Hyperglycemia is another health outcome that may be associated with both physical activity and periodontal microorganisms (Sigal et al., 2006; Zanusa et al., 2009), and therefore it is important to understand the mechanisms surrounding its relationship to periodontal health. Little research is available on the association between hyperglycemia and periodontal microorganisms. The little research there is shows mixed results. In 2014, Merchant et al. specifically studied the association between periodontal microorganisms and hyperglycemia. After analyzing data from 7848 adults from the NHANES III study, they found that higher orange-red cluster scores were associated with increased hyperglycemia whereas higher blue and orange cluster scores were associated with decreased hyperglycemia independent of known risk factors.

In 2012, Botero et al. studied individuals with (n=65) and without (n=81) diabetes. They found that among the people with diabetes, those that also had periodontitis presented higher glycemia and glycated hemoglobin when compared to people with gingivitis. Those with diabetes and hyperglycemia were more likely to develop periodontitis.

In a study done in 2015, Lima et al. found that pregnant women with good periodontal health did not have Socransky Red Complex, and those with gingivitis and periodontitis had a significantly higher probability of having this Red Complex. However, they did not find any association between hyperglycemia and periodontitis or with microorganisms. They concluded that Socransky Red Complex was not related to high blood pressure or high glucose levels. They found that glycemia was associated with CAL and number of teeth present.

2.8 Current Research on Physical Activity and Microorganisms

There is a small amount of research accumulating in regards to physical activity and microorganisms. For example, there is much current research on the gut and its association with physical activity. Physical activity is associated with diversity and composition of gut microorganisms (Clark *et al.* 2014; Queipo-Ortuño 2013; Matsumoto 2008). Studies have found that those who exercised less had less microbiota diversity, which has been linked to many conditions such as autism, GI disease, and obesity (Clark et al. 2014; Kang et al., 2013; Ott et al., 2004; Chang et al., 2008). Queipo-Ortuño et al. found that mice who exercised freely had increased numbers of Lactobacillus, Bifidobacterium, and B. coccoides_E. rectale when compared to mice with restricted exercise. Matsumoto et al. found that rats who had access to running wheels had significantly different cecum microbiota composition than rats without access to running wheels. While there is still little research on the association between physical activity and oral microorganisms, the oral and gut microbiome are inked and closely associated.

CHAPTER III: Methods

3.1 NHANES III

This thesis uses a cross-sectional study design, analyzing data from the National Health and Nutrition Examination Survey (NHANES) III, a nationally representative sample of the non-institutionalized U.S population collected through multistage probability cluster sampling. It is conducted by the National Center for Health Statistics (NCHS) which is part of the Center for Disease Control and Prevention (CDC). The NHANES program began in the 1960s. Data collection for NHANES III more specifically was conducted from 1988 to 1994. During these years, the NCHS conducted a complex, stratified, multistage sample survey from a representative sample of the US civilian, noninstitutionalized population. It includes about 33,994 individuals selected from households in 81 counties in states throughout the US. 31,311 of these individuals underwent a comprehensive physical examination. Black Americans and Mexican Americans were selected in large proportions in NHANES III as these minority groups can have different health status and characteristics. Each of these groups composed 30 percent of the sample. The participants were aged 2 months and older. Infants and young children (1-5 years-old) and older participants (60+ years-old) were sampled at a higher rate than previous NHANES projects. The NHANES III project also put special emphasis on environmental effects on health (National Center for Health Statistics).

The interview includes demographic, socioeconomic, dietary, and health-related questions. The examination component includes medical, dental, and physiological measurements as well as laboratory test results conducted by medical personnel. The data include information on a variety of health risks and behaviors, periodontal disease, clinical and antibody information related to periodontal disease, and physical activity.

An oral health examination was given to participants aged 2 years and older. In this examination, a dentist performed a number of oral-facial assessments. For participants 13 years and older, the dentists recorded information related to periodontal conditions such as pocket depth, loss of attachment, the presence of calculus and bleeding, and other related conditions. Blood samples were collected from sample participants to perform assays. The National Institute of Dental and Craniofacial Research tested surplus sera for antibodies to periodontal pathogens in participants 12 years old or more. The data were released in March 2002 (Hyman). Complete serum immunoglobulin (IgG) antibody data against 19 oral microorganisms are available for participants 40 years and older. In 2008, the Centers for Disease Control and Prevention released the analysis of these IgG antibody titer values for oral microorganisms from stored serum samples from the participants of NHANES III. The level of IgG antibody was determined using "checkerboard" immunoassay (CDC).

3.2 Study Population

The study population for this study consists of NHANES III participants aged 40 and over with complete serum IgG antibody data against 19 microorganisms. Records of these individuals are linked with relevant dental, medical, and socioeconomic, anthropometric, laboratory, and nutritional information.

These are available in NHANES III using the unique participant identification number. All data for this report are available at http://www.cdc.gov/nchs/nhanes/nh3data.htm.

3.3 Inclusion and exclusion criteria

A total of 33,994 people participated in NHANES III. Of these, 14,464 were at least 40 years old, 11,448 completed interviews, 9,379 were given a physical examination, 8,153 provided blood samples analyzed for IgG antibody titer levels against a panel of 19 periodontal bacteria. It was necessary to exclude those under 40 as younger participants did not provide blood samples. 3,295 of these participants had missing data on antibody clusters. Finally, this study excludes people who are edentulous in at least one arch (2,542). Of the remaining participants, there were none with missing data on physical activity. This leaves a final population size of 5,611. Figure 3.1 shows a flowchart demonstrating how I got the final analytical sample.

These exclusions limit the generalizability of the results. Since we excluded a number of participants, including 2010 participants missing data on physical activity, the results may not be generalizable to the entire NHANES population or the whole US population. However, while using this population damages generalizability, it is not likely to bias the relationship between physical activity and periodontal microorganisms.

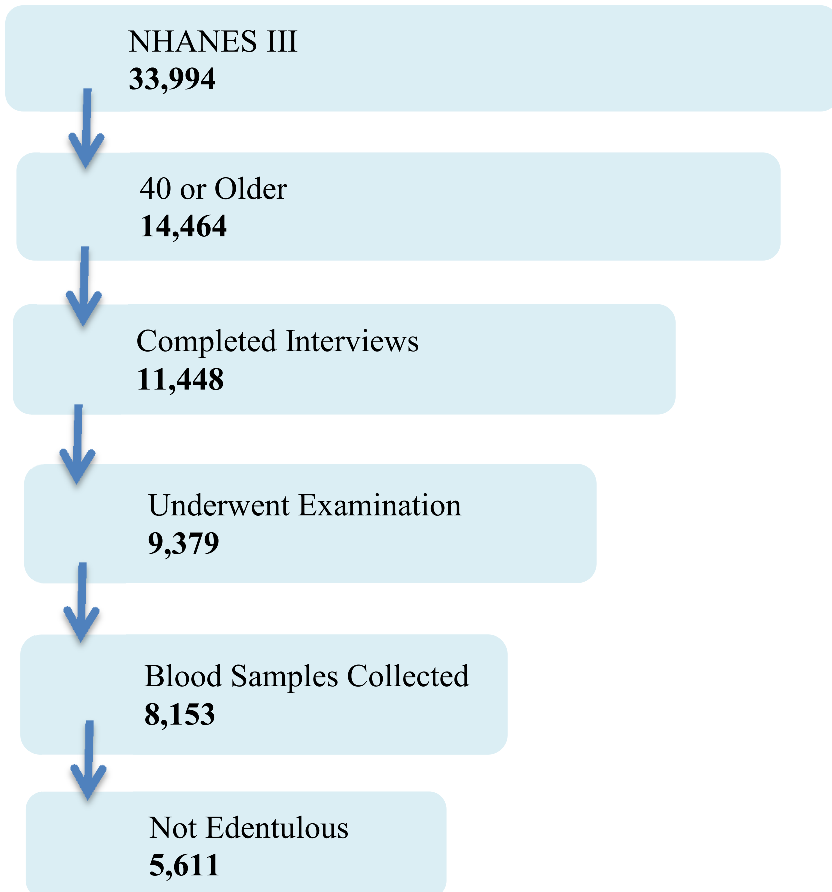

Figure 3.1: Inclusion/Exclusion Flowchart

3.4 IgG Assay for Periodontal Bacteria

Researchers from the Oral and Diagnostic Sciences Laboratory at Columbia University College of Dental Medicine used sera from NHANES III participants to test for IgG antibodies against 19 oral bacterial species using the "checkerboard" immunoassay technique. IgG antibodies are long-term antibodies and so indicate that the microorganisms either are or were present in the past. Therefore, this study is really looking at the association between serological *markers* and physical activity. These markers may be an imperfect surrogate for the bacteria that caused them. Therefore, to extend the results of this study to make inferences about the association between certain bacteria and physical activity is risky. This long-term antibody is appropriate for the cross-sectional study design. These results are in gravimetric units. Details can be found in the NHANES III documentation (Centers for Disease Control and Prevention, 2008), which contains details of this procedure. Explanation of the strains used to prepare whole cell antigenic extracts for the checkerboard immunoassay are provided in the Appendix of the documentation.

3.5 Cluster Formation and Names

Serum IgG antibodies titer values against periodontal microorganisms for each of the 19 oral bacterial species were log transformed and standardized by dividing them by the log-transformed population standard deviation. Standardized *z score*s of these IgG antibodies were used in cluster analysis to derive four mutually distinct groups of IgG antibodies against periodontal bacteria. Then, cluster scores were calculated for everyone in the population (Merchant et al., 2014). The names of the clusters were derived from Socransky's microbial color complexes. For example, the orange-red cluster, contains antibodies against four organisms, of which three are from Socransky's orange complex and the remaining one from the red complex. Therefore, it was named the orange-red cluster. Based on this approach, the other clusters were labeled red-green, yellow-orange, and orange-blue.

Detailed information on these clusters can be seen in Figure 2. The name of Socransky's microbial color complexes are shown in parenthesis.

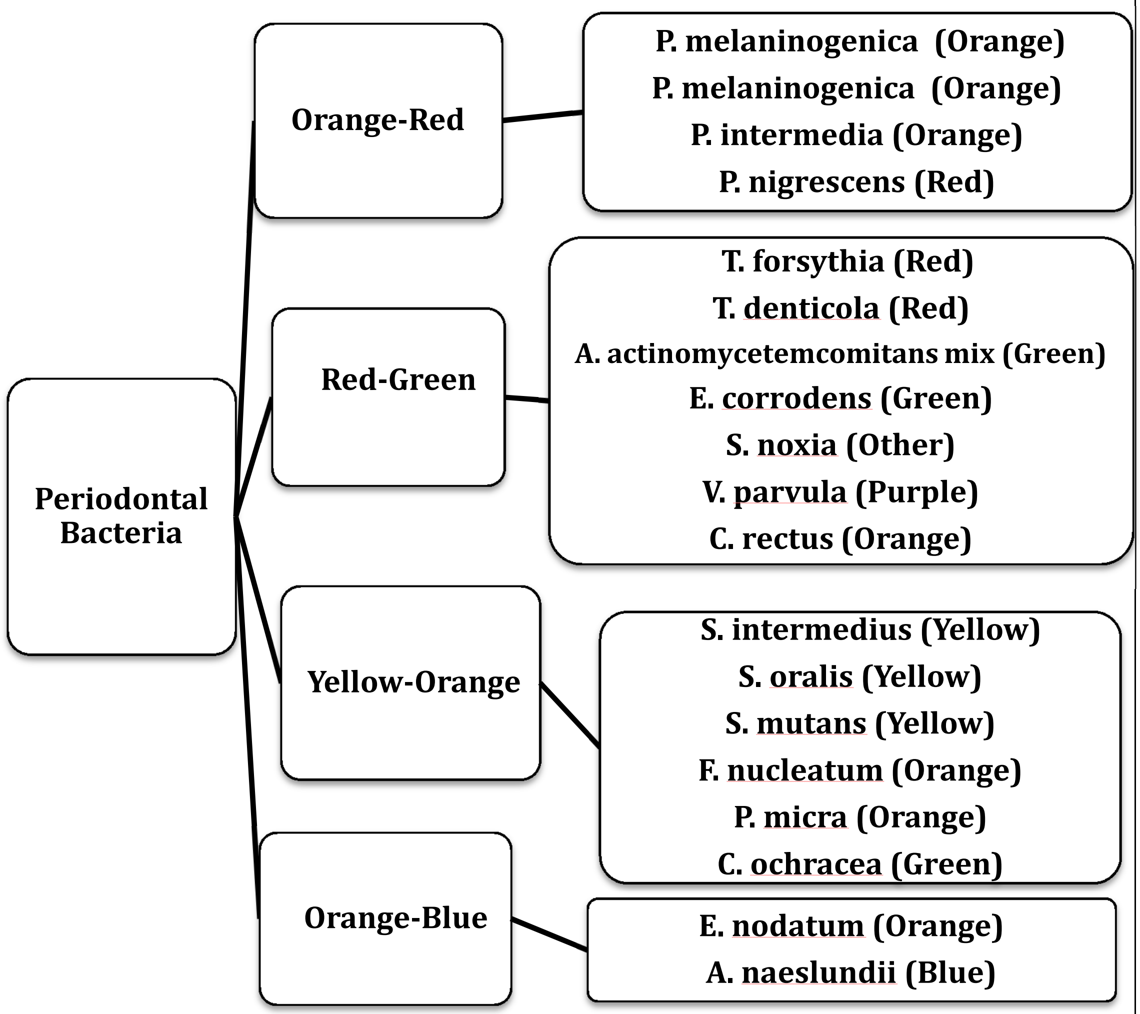

Figure 3.2: Naming of Clusters

From these clusters, everyone in the population was given a cluster score by summing the *z scores* for each antibody titer. For example, the orange-blue cluster contains the antibody titers *E. nodatum* and *A. naeslundii*. These antibody titers' *z scores* were summed in order to determine the score for that cluster. The names of the clusters are adapted from Socransky's color coding system for periodontal bacteria (Socransky and Haffajee, 2002, 2005).

According to Socransky's color scheme, organisms in the red and orange clusters have been linked with periodontal disease. Yellow and purple cluster organisms are associated with a good periodontal health. The blue cluster organisms are found in both diseased and healthy periodontal states. The remaining green cluster organisms are slightly associated with periodontal disease (Socransky and Haffajee, 2002, 2005).

It is worth mentioning that several studies have found that partial mouth examinations, and NHANES III specifically, underestimate periodontal disease (Papapanau et al., 2012; Susin et al., 2005; Kingman et al., 2002; Kingman et al., 2008; Beck et al., 2006). This is an advantage of using antibodies as a surrogate for periodontal disease.

3.6 Outcome variable

The main outcomes of interest are the sum of the z scores of the 4 clusters of antibodies described previously: Orange-Red, Red-Green, Yellow-Orange, and Orange-Blue. A number of studies have found that even after successful therapy to periodontal disease, serum IgG antibodies to periodontal bacteria persist in the serum. (Papapanou et al. 2004; Darby et al. 2001; Alfakry et al. 2011). Therefore, IgG antibodies measure past and present periodontal infection.

3.7 Predictor variable

The main exposure of interest is physical activity. The NHANES III survey included detailed questions on the participants' leisure time physical activity. Participants were asked if in the past month they had walked a mile or more without stopping, jog or ran, rode a bicycle or exercise bike, swam, did aerobics or aerobic dancing, did other types of dancing, did calisthenics or exercises, gardened or did yard work, or lifted weights (National Center for Health Statistics). For all positive responses, the participants were then asked how often they performed these physical activities in the past month. The participants were also asked to provide information on up to four other exercise, sports, or physically active hobbies not previously mentioned. The NHANES III survey did not collect information on the duration the participant spent on these activities which limits the precision of the estimates.

The National Center for Health Statistics (NCHS) assigned a five-digit code and a metabolic equivalent (MET) intensity level for each activity (CDC, 1996). One MET is defined as the amount of energy expenditure used at a resting metabolic rate such as sitting or watching television. The list of codes and their corresponding intensity ratings can be found in Appendix 2 of the CDC's Household Adult Data File Documentation (CDC, 1996). This five digit code corresponding to the MET's of certain activities was compiled in a compendium by Ainsworth et al. and updated in 2011. For example, the activity of running/jogging has a MET worth of 8.0.

There are many different methods of analyzing physical activity in NHANES III. A few researchers simply use a continuous variable to analyze the data (Cho et al., 2003; Clark et al., 2003). Others categorized physical activity. After calculating the leisure time physical activity (LPA), Park et al. categorized physical activity into a dichotomous variable: those with an LPA of under 3.5 and those over 3.5. But others created three categories (Sun et al., 2007; Simon et al., 2000).

A number of researchers ran analysis using the frequency of physical activities, disregarding the intensity ratings (Abramson et al., 2002; Parekh et al., 2012; Loucks et al., 2007; Schmitz et al., 2011). According to Abramson et al. (2002) of the participants 40 and over 34.8% engaged in physical activity 0 to 3 times in the month, 31.5% engaged in physical activity 4 to 21 times in the month, and 33.7% 22 or more times. However, most studies used the same method explained in this paper: the sum of intensity ratings multiplied by the times per month of each activity (Parekh et al., 2012; Cho et al., 2003; Clark et al., 2003; Park et al., 2003; King et al., 2001; Liangpunsakul et al., 2010; Schmitz et al., 2011). Loucks et al. (2007) dichotomized physical activity into some or none. Romieu et al., (2004) simply used hours of TV watching as a surrogate for physical activity in children.

For this study, the complex method of categorization of physical activity used is the same as that used by Beddhu et al. (2009). This method takes into consideration both the frequency and the intensity of the activities. Based on the recommendations of the CDC and the American College of Sports Medicine as described by Pate et al. (1995), Beddhu et al. categorized participants as sufficiently active if they got moderate intensity physical activity (METs ranging from 3 to 6) at least five times per week or vigorous intensity physical activity (METs greater than 6) at least three times per week. Participants were categorized as inactive if they did not report any physical activities. And the participants who were active, but did not meet the recommendations were categorized as insufficiently active. Thus three categories of physical activity were created: sufficiently active, insufficiently active, and inactive. This approach of considering both frequency and intensity of the activities was also used by many other researchers (Parekh et al., 2012; King et al., 2001; Liangpunsakul et al., 2010; Schmitz et al., 2011; Bassett et al., 2002).

3.8 Exposure and Outcome

As mentioned, physical activity was only measured based on the previous month. IgG antibodies on the other hand measure past and present periodontal infection. Therefore, conclusions about temporality are troublesome and some may question the usefulness of analyzing an association between these two measurements. But there are a number of reasons the results are still useful. As periodontal disease is a chronic infection, the antibodies reflect a measure of lifetime periodontal health. IgG antibodies measure both past and present disease (Dye et al., 2009; Sandholm et al., 1987; Patil et al., 2011; Eggert et al., 1987). In terms of physical activity, asking people to recall their physical activity from their whole lives are likely to produce incorrect results. Researchers have found that studies that limit the reporting interval to a relatively short time (less than three months) have higher reliability due to the limitations of human memory (Shephard 2003; Shephard 1986). Moreover, numerous studies have found that physical activity as measured from reports from the past month is correlated with long-term, past physical activity (Chasan-Taber et al., 1999; Chasan-Taber et al., 2002; Wolf et al., 1994). Also, studies have shown that physical activity, assessed over the previous month, is related to periodontal disease using NHANES III data (Al-Zahrani et al., 2005). Therefore it is reasonable to analyze the relationship between physical activity and periodontal microorganisms. Finally, as this is a cross-sectional analysis, it is not possible to assess temporality.

3.9 Covariates

Covariates include age, sex, race/ethnicity, income-to-poverty ratio, years of formal education, smoking, alcohol intake (current, past, never), BMI, central adiposity (waist circumference), diet (daily intake of calories; grams of carbohydrate, protein, fat, and fiber), number of missing teeth, diabetes status (yes/no), and frequency of visits to the dentist.

The age of each participant was obtained using the birth date as reported on the Screener Questionnaire. Age was be analyzed as a continuous variable

The Family questionnaire asked about the sex of the Family Reference Person and of each sample person.

Race/ethnicity was obtained from both the Family Questionnaires and the Screener Questionnaires. Race/ethnicity is separated into four categories: non-Hispanic white, non-Hispanic black, Mexican American and others. Non-Hispanic white served as the reference.

The poverty income ratio or poverty index is based on the family income and the family size using tables published by the Census Bureau each year. It was computed as the midpoint income category of the family (obtained in the Family Questionnaire) divided by the poverty threshold, the age of the family reference person, and the calendar year of the interview. This data is useful when comparing data over time because it is relatively standardized for factors such as inflation. Many programs such as the USDA food assistance program (WIC), Food Stamp Program, School Lunch and Breakfast Programs use certain eligibility cut points. The cut points chosen in this analysis are based off these federal assistance program eligibility criteria. This variable was divided into three categories: less than or equal to 1.3, greater than 1.3 to 3.5, and greater than 3.5 (CDC, 1996).

For education, participants were asked the highest grade/year of regular school that they completed. I divided this variable into three categories: those who did not complete high school, those who completed high school, and those who completed at least one year of education after high school. Those who did not complete high school are the reference level.

NHANES smoking data were collected using these two questions: “Have you smoked at least 100 cigarettes during your entire life?” and “Do you smoke cigarettes now?” If the participant answered “No” to the both questions s/he was categorized as never smoked. If the participant answered yes to the first question and no to the second, then the participant was categorized as a former smoker. And if the participant answered yes to both questions then they were categorized as a current smoker.

For alcohol, participants were asked if they had had at least 12 drinks of any kind of alcoholic beverage in their entire life. They were then asked if they had had at least 12 drinks of any kind of alcoholic beverage in the last 12 months. Based on their answers to these questions, the participants were grouped into three categories: non-drinkers, former drinkers, and current drinkers.

Central adiposity was measured by absolute waist circumference. While waist circumference and BMI are interrelated, waist circumference provides prediction of risks beyond that of BMI (National Institutes of Health, 1998; Sahakyan, 2015). Abdominal obesity is defined by the National Institutes of Health as a waist circumference greater than 102 cm (40 inches) in men or 88 cm (35 inches) in women (National Institutes of Health, 1998). Participants were grouped into either a normal waist circumference group or an elevated waist circumference group.

The weight and height for each participant were measured in the exam. These were used to calculate the BMI. It was calculated from height and weight using the following formula: weight(kg)/(standing height(cm)/100)^2. BMI was be analyzed as a continuous variable. The linear association between BMI and periodontal disease has been thoroughly studied (Al-Zahrani et al., 2005; Borges-Yáñez et al., 2006; Bouchard et al., 2006; Ekuni et al., 2008; Furuichi et al., 2003; Marugame et al., 2003; Shimazaki et al. 2010; Xiao et al., 2009).

Data for the participants' diet were collected in the mobile examination center and home examinations. Respondents were asked to recall all the food and beverages, except plain water, they consumed during the previous 24 hours. The total nutrients intakes were included in this dataset for respondents whose dietary recalls were coded complete and reliable. US Department of Agriculture's food composition databases were used to assign nutrient values to these dietary recalls (measured in grams). From this information, we can calculate the total grams of dietary fibers, total carbohydrate, protein, and total fat consumption for each participant during the previous 24-hour time period.

All teeth were examined (excluding third molars) in two randomly selected quadrants, one from upper and one from lower, at two fixed sites per tooth (mesio-buccal and mid-buccal). Data were collected on each tooth, including if it was missing. The total number of missing teeth was added up and recorded for each participant. This was put into four categories: 0, 1-5, 6-10, and more than 10 missing teeth.

In the adult questionnaire, participants were asked how often they went to the dentist and how many days ago they had last seen a dentist or dental hygienist. Based on their answers to these two questions, participants were divided into three categories. If the respondent had seen a dentist in the last year, or if they answered that they went to a dentist once a year, they were put into the “yearly” category. If the respondent had been to the dentist in the last two years, or if they reported that they went to the dentist every two year or less often than every two years, then they were put into the “every two years or less” category. If the respondent had not been to the dentist in the last two years or said they went to the dentist “whenever needed” and reported they did not follow a schedule, they were put into the “no schedule” category.

People were classified as having diabetes if they had ever been told by a doctor that they had diabetes (and it wasn’t while they were pregnant), or if they were taking insulin or diabetes pills such as oral agents or oral hypoglycemic agents to lower their blood sugar. Women who only had gestational diabetes were not classified as diabetic.

Possible confounders were based off a literature review and by looking at the distribution of each variable across levels of physical activity. Regression analysis was used to analyze if the variable is associated with the exposure (physical activity) and the outcome (cluster z scores).

3.10 Possible effect modifiers

Possible effect modifiers were identified through previous studies (Sanders et al., 2014; Dickie de Castilhos et al., 2012). They include sex, age, smoking, diabetes status and central adiposity (waist circumference). I tested for interaction by showing the results stratified by these variables.

For age, the CDC has separate recommendations for people who are 65 and older (CDC, Physical Activity Basics). Therefore when analyzing for effect modification, age was be separated into two categories: those under 65 and those 65 and older.

3.11 Statistical Analysis

Statistical analyses and data management were performed using SAS 9.4. The Center for Disease Control and Prevention provides sample weights, cluster, and strata variables to account for the complex sampling design. These were used in all analyses. A statistical significant threshold level of 0.05 was be used.

Descriptive statistics were obtained using SAS procedures for complex surveys such as proc surveymeans and proc surveyfreq. The linear regression models were run using proc surveyreg with log-transformed z score clusters as the outcome and the three levels of physical activity as the predictor while controlling for the other three clusters. The first model only adjusted for the other three clusters. The second model adjusts for age and sex. The third model makes further adjustments for race/ethnicity, income-to-poverty ratio, years of formal education, smoking status, alcohol intake, central adiposity, missing teeth, body mass index, dentist visits, and fiber intake. And the fourth model makes even further adjustments for diet—fiber, protein, fat, and carbohydrate consumption. The results were back transformed to natural units by exponentiation of the log-transformed estimates.

CHAPTER IV: Results

4.1 Sample Characteristics

After the exclusions previously described in Chapter III, a total of 5,611 participants were analyzed. The characteristics of this sample population are presented in the column labeled “Total” in Table 4.1. Of this sample the average age was about 55. About 47% were male and 53% female. Around 80% of the participants were white, 9% black, 4% Mexican American, and 7% were other races/ethnicities. In terms of the poverty index, 12% fell into the category of “lower,” 39% into the category of “middle,” and 49% into the category of “higher.” About 22% of the participants did not graduate from high school, 31% had a high school degree, and 46% received at least 1 year of education after high school. About 47% of the participants had never smoked, 33% were former smokers, and 20% smoked. In terms of alcohol consumption, 14% did not drink, 35% were former drinkers, and 20% drank alcohol. The overall average BMI was 27 kg/m^2. The majority (53%) of the participants had healthy waist circumferences while the minority (47%) had elevated waist circumferences. In terms of diet, in the 24 hours prior to the interview, the participants ate an aver of 18 grams of dietary fibers, 251 grams of carbohydrates, 78 grams of protein, and 78 grams of fat. About 42% of participants had no missing teeth, 45% had one to five missing teeth, 10% had six to ten missing teeth, and 3% had more than ten missing teeth. And in terms of annual visits to the dentist, 18%

followed no schedule, 78% went to the dentist at least once a year, and 4% went to the dentist every two years or less.

Table 4.3 compares the study population to those who were eliminated based on missing data on physical education or antibody clusters or were excluded because they were edentulous. There are no major differences between this population and the study population. Those who were excluded were slightly different in a few ways: more lower and middle income, less educated, more likely to smoke or have smoked, less likely to drink, and were less likely to go to the dentist at least once a year. These differences were small and probably do not affect the results drastically.

4.2 Physical Activity among the Study Sample

As previously described, leisure-time physical activity was divided into three categories: those who were sufficiently active, i.e. they met the recommendations of the CDC and the American College of Sports Medicine (Pate et al, 1995); those who were insufficiently active, i.e. they did *not* meet the recommendations of the CDC and the American College of Sports Medicine; and those who reported no physical activity. Of the study population, about a third (33%) were sufficiently active, over a half (53%) were insufficiently active, and a smaller portion (13%) were inactive. This can also be seen in the first row of Table 4.1.

4.3 Covariates across Levels of Physical Activity among the Study Sample

All of the covariates were significantly associated with physical activity. For continuous covariates, this means that the mean "score" of the participants in one category of physical activity is significantly different from one or both of the other mean "scores" of the participants in the other categories of physical activity. The participants who were insufficiently active had the lowest mean age (53). Inactive and Sufficiently Active participants had similar mean ages: 57 and 56 respectively. Those who were inactive had the highest average BMI at 28 kg/m^2. Insufficiently active participants had only a slightly lower average BMI at 27 kg/m^2. And those who were sufficiently active had the lowest average BMI at 26 kg/m^2. Those who were insufficiently active tended to eat more dietary fibers than those who were inactive; but those who were sufficiently active tended to eat even more than both of the other groups. The same trend was seen in carbohydrates with inactive participants eating the least, insufficiently active eating more, and sufficiently active eating the most on average. Participants who were insufficiently active at the most proteins and fats on average; sufficiently active ate the second most; and inactive ate the least of both proteins and fats on average.

For categorical variables, a significant p-value means that the covariate is significantly related with the level of physical activity. For example, women were more likely to be inactive, while men were more likely to be sufficiently active. Whites were more likely to be insufficiently active than inactive. And African-Americans, Mexican-Americans, and other races/ethnicities were more likely to be inactive. People with high income-to-poverty ratios were more likely to be either sufficiently or insufficiently active than inactive. However people with lower income-to-poverty ratios were more likely to be inactive than to report any amount of activity. The participants who did not graduate from high school were more likely to be inactive, whereas those who had at least one year of education after high school were less likely to be inactive and instead more likely to be sufficiently active. Former smokers seem to tend toward sufficiently active while current smokers tend towards inactive. Both non-drinkers and former drinkers of alcohol were most likely to be inactive. However, current alcohol drinkers tended not to be inactive. Participants with elevated waist circumferences did not tend to be sufficiently active. They were more likely to be insufficiently active, and even more likely to be inactive. The participants who were missing 6 or more teeth tended to be inactive rather than active. Finally, those who had no scheduled visits to the dentist tended to be inactive whereas those who went to the dentist at least once a year tended to be active as opposed to inactive. These results are also shown in Table 4.1.\

4.4 Mean Cluster Scores across Levels of Physical Activity

Table 4.2 shows the distribution of cluster scores across levels of physical activity. All clusters other than Orange-Blue do not seem to be associated with levels of physical activity. However, getting physical activity does seem to be associated with higher Orange-Blue cluster scores, which contains antibodies to bacteria associated with a healthy periodontal state.

4.5 Association between Covariates and Cluster Scores

Table 4.3 shows the association between covariates and cluster scores. The bold numbers represent a significant association. For continuous variables, the table shows the estimated regression coefficients. The number represents the estimated mean change in cluster score for every unit change in the covariate. For categorical variables, the number represents the mean cluster core in that category of the covariate. A significant p-value (<0.05) indicates that the mean cluster score in one category of the covariate is significantly different form the mean cluster score in at least one other category of the covariate.

Table 4. 1: Characteristics of Study Population

	Study Population (N=5,611)	**Inactive** (N=1,195)	**Insufficiently Active** (N=2,732) (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (N=1,684) (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)	**p-value***
Total	100	13.7	53.1	33.1	
Age (years) *mean (SE)*	54.6 (0.45)	57.1 (0.52)	52.9 (0.49)	56.3 (0.65)	<.0001
Sex *%*					<.0001
Male	46.6	32.2	48.4	49.6	
Female	53.4	67.8	51.6	50.4	
Race/Ethnicity *%*					<.0001
White	79.6	62.8	83.9	79.5	
Blacks	9.0	17.0	7.7	7.8	
Mexican-American	4.2	7.9	3.8	3.2	
Others	7.2	12.3	4.5	9.4	
Income-to-Poverty Ratio *%*					<.0001
Lower, ≤1.3	12.1	26.9	8.8	11.3	
Middle, ≤3.5	39.0	46.4	38.0	37.5	
Higher, >3.5	49.0	26.6	53.1	51.2	
Years of Formal Education (years completed) *%*					<.0001
<High school	21.6	42.5	18.5	17.9	
Graduated high school	31.5	33.5	33.0	28.2	
≥1 year after high school	47.0	24.0	48.5	54.0	
Smoker *%*					0.0028
Never	46.8	48.5	45.9	47.6	

Former Current	33.4 19.8	25.9 25.6	33.5 20.5	36.1 16.3	
Alcohol Intake *%* Non-drinker Former Current	13.7 35.3 51.1	25.4 43.8 30.8	10.4 33.4 56.2	14.0 34.7 51.3	<.0001
BMI (kg/m^2) *mean (SE)*	27.3 (0.15)	28.31 (0.42)	27.5 (0.20)	26.5 (0.16)	<.0001
Central Adiposity$^+$ *%* Normal Elevated	53.1 46.9	40.8 59.2	52.1 47.8	59.4 40.6	<.0001
Diet(gm/day) *mean (SE)* Dietary fibers Carbohydrates Proteins Total fats	18.1 (0.45) 250.8 (3.81) 78.2 (1.15) 78.2 (1.48)	15.40 (0.40) 231.7 (6.25) 70.2 (1.74) 70.6 (2.13)	17.9 (0.30) 250.7 (4.48) 80.2 (1.40) 81.5 (1.69)	19.7 (0.34) 256.0 (5.36) 78.1 (1.54) 75.4 (2.62)	<.0001 <.0001 <.0001 <.0001
Missing Teeth *%* 0 1-5 6-10 >10	41.7 45.5 9.7 3.2	30.9 47.3 15.1 6.7	43.4 45.7 8.8 2.2	43.5 44.3 8.8 3.4	<.0001
Visits to the Dentist *%* No schedule At least once/year Every 2 years or less	17.9 78.4 3.7	29.0 68.1 3.0	16.5 79.4 4.1	15.4 81.2 3.4	<.0001

Values with parentheses indicate mean (*SE*). Stand-alone values indicate percentages.
*The p-value for continuous variables represents the results of an F-test. A significant value indicates that at least one mean of a covariate in a certain level of PA is significantly different from at least one other mean of that covariate in a different level of PA.
*The p-value for categorical variables is the result of a Rao-Scott Chi-Square Test. It indicates that the covariate is significantly related to physical activity.
$^+$ Normal ≤ 102 cm (40 inches) in men or 88 cm (35 inches) in women.

Table 4. 2: Cluster Scores by Levels of Physical Activity

	Study Population (N=5,611)	**Inactive** (N=1,195)	**Insufficiently Active** (N=2,732) (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (N=1,684) (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)	**p-value***
Orange-Red	-8.1 (10.8)	0.7 (19.5)	-15.1 (15.0)	-2.2 (10.6)	0.65
Red-Green	22.3 (35.0)	37.6 (40.6)	14.4 (36.4)	27.7 (41.0)	0.77
Yellow-Orange	20.6 (21.9)	23.1 (30.2)	21.7 (25.0)	18.0 (23.5)	0.97
Orange-Blue	25.3 (5.8)	3.4 (8.9)	32.9 (7.0)	24.6 (6.9)	**0.01**

Values with parentheses indicate mean (standard error).
*The p-value represents the results of an F-test. A significant value indicates that at least one mean of a cluster score in a certain level of PA is significantly different from at least one other mean of that cluster score in a different level of PA.

The table seems to indicate that there is a strong association between antibody cluster scores and both race and smoking status as all the clusters are significantly associated with these covariates. The Orange-Blue cluster scores seem to be more associated with the covariates, with over half of the covariates significantly associated with Orange-Blue. Orange-Red was also associated with half of the covariates. Alcohol and BMI do not appear to be associated with any of the cluster scores and within the diet categories, only carbohydrate consumption was significantly associated with the Orange-Blue cluster.

4.6 Linear Regression Analysis of the Association between PA and Cluster Scores

Table 4.5 provides the results of the linear regression analysis conducted with physical activity and cluster z scores. Most of the associations are insignificant. However, some significant associations were found between physical activity and Orange-Blue cluster z scores in models one and two.

Table 4. 3: Association between Covariates and Cluster Scores

	Orange-Red	Red-Green	Yellow-Orange	Orange-Blue
Age (years) *Estimated Regression Coefficients* p-value	**1.3** **0.04**	0.8 0.49	-0.2 0.80	**-1.6** **0.00**
Sex Male Female p-value	 **23.0** **-35.1** **0.00**	 **43.6** **2.8** **0.04**	 27.8 14.4 0.46	 27.0 25.4 0.80
Race/Ethnicity White Blacks Mexican-American Others	 **-33.4** **105.8** **44.7** **104.5** **0.00**	 **5.1** **87.5** **67.6** **155.4** **0.01**	 **11.6** **59.7** **44.7** **110.1** **0.00**	 **27.7** **11.4** **32.9** **13.3** **0.02**
Income-to-Poverty Ratio Lower, ≤1.3 Middle, ≤3.5 Higher, >3.5 p-value	 2.1 -5.9 -13.2 0.83	 -20.9 21.2 41.6 0.61	 -34.1 16.7 45.1 0.25	 **-22.1** **16.1** **44.5** **0.00**
Years of Formal Education (years completed) <High school Graduated high school ≥1 year after high school p-value	 10.2 -18.3 -10.3 0.44	 9.8 8.5 43.5 0.47	 -9.1 8.9 48.3 0.1	 **-7.9** **21.3** **42.1** **0.00**
Smoker Never Former Current p-value	 **8.5** **3.5** **-67.7** **0.03**	 **75.4** **14.2** **-68.0** **0.00**	 **23.5** **-44.0** **54.9** **0.04**	 **41.0** **24.9** **-8.7** **0.00**
Alcohol Intake Non-drinker Former Current p-value	 14.1 2.7 -22.9 0.24	 79.9 41.9 0.0 0.22	 59.9 35.9 6.7 0.37	 23.6 25.1 26.6 0.96
BMI (kg/m^2) *Estimated Regression Coefficients* p-value	0.73 0.10	0.3 0.89	2.0 0.73	0.6 0.23
Central Adiposity⁺ Normal Elevated	 -8.2 -6.6	 25.1 23.6	 25.1 20.3	 30.3 20.3

p-value	**0.01**	0.95	**0.04**	0.11
Diet(gm/day) *Estimated Regression Coefficients* Dietary fibers (p-value) Carbohydrates (p-value) Proteins (p-value) Total fats (p-value)	0.6 (0.33) 0.0 (0.5) -0.2 (0.20) -0.1 (0.22)	1.2 (0.3) 0.1 (0.23) -0.3 (0.33) -0.1 (0.6)	0.88 (0.38) 0.1 (0.32) -0.3 (0.24) -0.1 (0.58)	0.5 (0.17) **0.1** **(0.01)** 0.1 (0.47) 0.05 (0.45)
Missing Teeth 0 1-5 6-10 >10 p-value	**-29.5** **1.4** **22.0** **22.0** **0.03**	32.1 18.7 10.2 53.9 0.22	28.5 22.4 0.3 42.2 0.74	**45.7** **17.7** **-8.0** **-35.6** **0.00**
Visits to the Dentist No schedule At least once/year Every 2 years or less p-value	-3.1 -13.5 49.9 0.10	9.2 26.9 49.2 0.87	-8.4 29.8 40.9 0.51	**6.6** **28.5** **50.7** **0.04**
***bold** indicates a significant p-value *For continuous variables, the table shows the estimated regression coefficients. The number represents the estimated mean change in cluster score for every unit change in the covariate. *For categorical variables, the number represents the mean cluster core in that category of the covariate.				

Table 4. 4: Comparison between Study Population and Excluded Participants

	Study Population (N=5,611)	**Excluded from Study***(N = 3,295)
Age (years) *mean (SE)*	54.6 (0.45)	59.4 (0.54)
Sex *%* Male Female	 46.6 53.5	 52.5 47.4
Race/Ethnicity *%* White Blacks Mexican American Others	 79.6 9.0 4.2 7.2	 78.2 12.9 3.7 5.2
Income-to-Poverty Ratio *%* Lower, ≤1.3	 12.1 39.0	 19.0 42.36

Middle, ≤3.5 Higher, >3.5	49.0	38.4
Years of Formal Education (years completed) *%* <High school Graduated high school ≥1 year after high school	21.6 31.5 47.0	34.7 30.2 35.0
Smoker *%* Never Former Current	46.8 33.4 19.8	37.8 38.0 24.2
Alcohol Intake *%* Non-drinker Former Current	13.7 35.3 51.1	15.7 41.5 42.9
BMI (kg/m^2) *mean (SE)*	27.3 (0.15)	26.8 (0.19)
Central Adiposity[+] *%* Normal Elevated	53.1 46.9	52.1 47.9
Diet(gm/day) *mean (SE)* Dietary fibers Carbohydrates Proteins Total fats	18.1 (0.45) 250.8 (3.81) 78.2 (1.15) 78.2 (1.48)	16.8 (0.39) 225.7 (3.71) 71.9 (1.35) 73.9 (1.55)
Missing Teeth *%* 0 1-5 6-10 >10	41.7 45.5 9.7 3.2	61.5 24.6 8.9 5.0
Visits to the Dentist *%* No schedule At least once/year Every 2 years or less	17.9 78.4 3.7	32.6 64.5 2.8

Values with parentheses indicate mean (*SE*). Stand-alone values indicate percentages.
*Excluded includes the participants who were missing data on physical activity or antibody cluster information or were edentulous.

Model 1 only adjusted for clusters other than the one being directly measured as the outcome. Model 2 makes further adjustments for age and sex. On average, both sufficiently and insufficiently active participants had significantly higher *z* scores of the Orange-Blue clusters. This table only compares sufficiently active and insufficiently active to the reference level, inactive. But it does not compare between each other. A test was run to analyze the difference between these two levels, and no significant difference was found between insufficiently and sufficiently active. The first two models adjust for much fewer confounders. Therefore, these significant results in models 1 and 2 may be due to confounding. No statistically significant results were found in models 3 or 4.

Table 4.6 shows the results of models 3 and 4 stratified by gender. Again, no significant results were found in model 3 or 4. Table 4.7 shows the results of model 3 and 4 stratified by age. Again, no significant results were found in either model. Table 4.8 shows the cluster *z* scores stratified by adiposity status (normal or high) in models 3 and 4. There were no significant results when the participants were stratified according to adiposity status.

Table 4.9 shows the results stratified by diabetes status. There were only 556 (about 10%) participants categorized as diabetic. There were no significant results found in either Model 3 or Model 4 for either of the categories of diabetes. Table 4.10 shows the results stratified by smoking status (non-smokers, former smokers, and current smokers) in models 3 and 4. Again, none of the results were significant for any of the smoking categories.

Table 4. 5: Association between Physical Activity and Cluster Scores

	Inactive (no physical activity)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)
Orange-Red			
Model 1	Reference level	-14.80 (-43.33, 13.72)	-1.09 (-27.45, 25.27)
Model 2		-18.31 (-46.81, 10.18)	-9.91 (-36.12, 16.29)
Model 3		-0.76 (-30.36, 28.84)	2.31 (-26.23, 30.81)
Model 4		-2.25 (-32.70, 28.18)	0.04 (-28.84, 28.91)
Red-Green			
Model 1	Reference level	-25.16 (-58.33, 8.00)	-7.82 (-41.50, 25.86)
Model 2		-25.54 (-58.17, 7.09)	-11.96 (-46.18, 22.25)
Model 3		-10.77 (-40.03, 18.50)	1.73 (-31.23, 35.70)
Model 4		-10.77 (-40.39, 18.85)	1.72 (-32.07, 35.52)
Yellow-Orange			
Model 1	Reference level	15.29 (-11.95, 42.53)	0.0 (-26.03, 26.03)
Model 2		17.42 (-10.49, 45.34)	5.36 (-21.70, 32.32)
Model 3		1.72 (-26.17, 29.61)	-11.88 (-39.63, 15.88)
Model 4		2.16 (-26.16, 30.48)	-11.59 (-39.71, 16.96)
Orange-Blue			
Model 1	Reference level	**31.30 (14.12, 48.47)**	**22.17 (6.38, 37.96)**
Model 2		**26.17 (8.38, 43.96)**	**22.46 (5.93, 39.00)**
Model 3		7.61 (-8.38, 22.31)	6.97 (-8.38, 22.31)
Model 4		8.54 (-7.28, 24.37)	7.34 (-8.10, 22.78)

*__Bold__ indicates a significant p-value (<0.05).
*Model 1 adjusts for the other three clusters.
Model 2 further adjusts for age and sex.
Model 3 further adjusts for race/ethnicity, income-to-poverty ratio, years of formal education, smoking status, alcohol intake, central adiposity, missing teeth, body mass index, dentist visits, diabetes, and fiber intake.
Model 4 further adjusts for diet—fiber, protein, fat, and carbohydrate consumption.

4.7 Stratified Results by Possible Effect Modifiers

Tables 4.6 through 4.10 show the results stratified by possible effect modifiers. These tables only show the results of models 3 and 4. Model 1 and 2 are not shown as they were under adjusted, not taking into account important confounders.

Table 4. 6: Association between Physical Activity and Cluster Scores Stratified by Sex

		MALES (2,675)		FEMALES (2,936)	
	Inactive (no physical activity)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)
Orange-Red	Reference level				
Model 3		-8.2 (-45.7, 29.3)	8.2 (-32.5, 49.0)	6.8 (-34.1, 47.8)	-6.04 (-45.2, 33.1)
Model 4		-10.5 (-49.6, 28.5)	5.0 (-37.2, 47.0)	5.6 (-35.3, 46.6)	-6.9 (-46.4, 32.7)
Red-Green	Reference level				
Model 3		-2.8 (-55.9, 50.2)	19.8 (-31.1, 70.7)	-19.1 (-48.2, 10.0)	-12.4 (-46.8, 22.1)
Model 4		-1.9 (-54.5, 50.7)	20.4 (-30.9, 71.7)	-20.2 (-50.2, 9.7)	-12.5 (-48.4, 23.4)
Yellow-Orange	Reference level				
Model 3		1.3 (-42.6, 45.3)	-22.0 (-57.1, 13.0)	3.6 (-27.9, 35.2)	0.8 (-34.7, 36.3)
Model 4		0.8 (-43.0, 44.7)	-22.5 (-57.6, 12.6)	5.3 (-26.9, 37.5)	1.7 (-34.9, 38.4)
Orange-Blue	Reference level				
Model 3		-1.3 (-25.2, 22.6)	9.1 (-33.3, 15.1)	11.1 (-10.2, 32.3)	17.4 (-4.8, 39.6)
Model 4		0.2 (-24.8, 25.2)	-8.0 (-31.7, 15.8)	11.7 (-10.0, 33.4)	17.4 (-4.9, 39.8)

*__Bold__ indicates a significant p-value (<0.05).
*Model 2 adjusts for other clusters, age, and sex.
Model 3 further adjusts for race/ethnicity, income-to-poverty ratio, years of formal education, smoking status, alcohol intake, central adiposity, missing teeth, body mass index, dentist visits, diabetes, and fiber intake.
Model 4 further adjusts for diet—fiber, protein, fat, and carbohydrate consumption.

Table 4. 7: Association between Physical Activity and Cluster Scores by Age

		<65 years-old (3,846)		≥65 years-old (1,765)	
	Inactive (N=1,195) (no physical activity)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)
Orange-Red	Reference level				
Model 3		-1.2 (-36.0, 33.6)	0.2 (-37.1, 37.5)	-8.1 (-67.3, 51.0)	12.9 (-48.1, 74.0)
Model 4		-3.8 (-39.5, 31.9)	-3.0 (-40.2, 34.3)	-7.5 (-66.4, 51.3)	14.9 (-47.1, 76.9)
Red-Green	Reference level				
Model 3		-4.2 (-38.6, 30.2)	8.6 (-31.5, 48.7)	-31.7 (-68.3. 28.3)	-16.5 (-61.3, 28.3)
Model 4		-4.4 (-39.4, 30.7)	8.4 (-32.9, 49.7)	-30.8 (-67.2, 5.5)	-14.8 (-59.7, 30.2)
Yellow-Orange	Reference level				
Model 3		-5.8 (-37.6, 26.1)	-20.3 (-54.5, 13.9)	26.4 (-8.7, 61.5)	12.0 (-28.4, 52.3)
Model 4		-4.9 (-37.3, 27.3)	-19.4 (-54.4, 15.5)	26.0 (-8.5, 60.4)	10.3 (-30.4, 51.0)
Orange-Blue	Reference level				
Model 3		6.8 (-13.6, 27.2)	4.8 (-16.5, 26.0)	6.9 (-13.3, 27.1)	5.3 (-15.6, 26.3)
Model 4		8.1 (-12.9, 29.2)	5.5 (-15.8, 26.9)	1.5 (-31.0, 34.0)	5.3 (-25.0, 35.6)

***Bold** indicates a significant p-value (<0.05).
*Model 2 adjusts for the other clusters, age, and sex.
Model 3 further adjusts for race/ethnicity, income-to-poverty ratio, years of formal education, smoking status, alcohol intake, central adiposity, missing teeth, body mass index, dentist visits, diabetes, and fiber intake.
Model 4 further adjusts for diet—fiber, protein, fat, and carbohydrate consumption.

Table 4. 8: Association between Physical Activity and Cluster Scores by Central Adiposity

		NORMAL ADIPOSITY (2,558)		ELEVATED ADIPOSITY (2,840)	
	Inactive (no physical activity)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (N=1,684) (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)
Orange-Red	Reference level				
Model 3		16.1 (-22.5, 54.8)	32.5 (-7.8, 72.8)	-11.8 (-45.2, 21.6)	-24.7 (-59.7, 10.1)
Model 4		15.6 (-22.9, 54.1)	31.0 (-8.8, 70.9)	-15.0 (-49.8, 19.9)	-28.0 (-63.3, 7.3)
Red-Green	Reference level				
Model 3		-22.3 (-60.3, 15.7)	-3.4 (-37.3, 30.5)	-4.8 (-39.9, 30.3)	2.7 (-49.8, 55.1)
Model 4		-23.0 (-60.8, 14.8)	-34.2 (-38.4, 30.0)	-3.9 (-40.1, 32.2)	4.0 (-49.1, 32.2)
Yellow-Orange	Reference level				
Model 3		4.2 (-30.1, 38.3)	-20.5 (-50.1, 9.0)	1.8 (-32.4, 36.2)	0.1 (-41.3, 41.4)
Model 4		4.7 (-29.3, 38.8)	-20.2 (-49.9, 9.5)	2.7 (-32.5, 37.8)	-0.8 (-41.1, 42.6)
Orange-Blue	Reference level				
Model 3		-1.9 (-25.0, 21.1)	3.4 (-23.2, 30.0)	13.7 (-6.7, 34.1)	2.5 (-21.7, 26.8)
Model 4		-1.1 (-24.2, 22.1)	4.6 (-21.8, 31.0)	14.0 (-6.9, 34.8)	0.9 (-23.7, 25.4)

*__Bold__ indicates a significant p-value (<0.05).
*Model 3 further adjusts for race/ethnicity, income-to-poverty ratio, years of formal education, smoking status, alcohol intake, central adiposity, missing teeth, body mass index, dentist visits, diabetes status, and fiber intake.
Model 4 further adjusts for diet—fiber, protein, fat, and carbohydrate consumption.
*Normal Adiposity ≤ 102 cm (40 inches) in men or 88 cm (35 inches) in women.

Table 4. 9: Association between Physical Activity and Cluster Scores in No Diabetes vs Diabetes

		NO DIABETES (5,049)		DIABETES (556)	
	Inactive (no physical activity)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)
Orange-Red	Reference level				
Model 3		-1.4 (-33.0, 30.2)	1.8 (-28.7, 32.3)	-29.5 (-89.2, 36.3)	-16.6 (-85.8, 52.6)
Model 4		-3.0 (-35.3, 29.3)	-0.4 (-31.2, 30.4)	-21.4 (-86.1, 43.2)	-14.6 (-85.4, 56.3)
Red-Green	Reference level				
Model 3		-11.5 (-40.7, 17.8)	1.9 (-31.8, 35.5)	-20.7 (-82.9, 41.5)	-26.7 (-78.0, 24.6)
Model 4		-11.5 (-41.1, 18.2)	1.9 (-32.4, 36.3)	-19.3, (-84.1, 45.5)	-26.9 (-78.2, 24.4)
Yellow-Orange	Reference level				
Model 3		2.4 (-26.7, 31.5)	-12.5 (-41.0, 15.9)	13.6 (-36.9, 64.0)	22.0 (-18.9, 62.8)
Model 4		2.8 (-26.6, 32.3)	-12.1 (-41.0, 16.9)	11.2 (-41.2, 63.7)	21.2 (-19.1, 61.6)
Orange-Blue	Reference level				
Model 3		7.2 (-10.7, 25.1)	6.6 (-12.3, 25.6)	12.2 (-27.2, 77.6)	12.2 (-50.4, 74.7)
Model 4		8.3 (-10.3, 26.9)	7.1 (-12.0, 26.2)	25.2 (-24.6, 75.0)	12.7 (-48.3, 73.8)

*__Bold__ indicates a significant p-value (<0.05).
*Model 3 further adjusts for race/ethnicity, income-to-poverty ratio, years of formal education, smoking status, alcohol intake, central adiposity, missing teeth, body mass index, dentist visits, diabetes status, and fiber intake.
Model 4 further adjusts for diet—fiber, protein, fat, and carbohydrate consumption.
*Diabetes = ever been told by a doctor they had diabetes (not while pregnant), or taking insulin or diabetes pills

Table 4. 10: Association between Physical Activity and Cluster Scores by Smoking Status

		NEVER-SMOKERS (2,726)		FORMER SMOKERS (1,734)		CURRENT SMOKERS (1,151)	
	Inactive (no physical activity)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)
Orange-Red Model 3	Reference Level	-24.0 (-63.7, 15.8)	-34.1 (-73.4, 5.2)	14.5 (-22.1, 89.7)	33.1 (--22.9, 89.2)	25.9 (-58.4)	54.0 (-16.9, 124.8)
Model 4		-23.5 (-63.2, 15.6)	-37.3 (-76.9, 2.2)	14.8 (-36.5, 66.2)	30.8 (-36.5, 66.2)	18.3 (-66.4, 103.1)	53.2 (-16.4, 103.1)
Red-Green Model 3	Reference Level	-23.6 (-68.5, 21.4)	-7.6 (-47.9, 32.6)	-24.4 (-72.4, 23.6)	-8.4 (-68.3, 51.5)	22.4 (-28.6, 73.4)	26.5 (-40.8, 93.8)
Model 4		-23.2 (-67.9, 21.6)	-8.6 (-49.4, 32.2)	-24.7 (-72.3, 23.0)	-7.0 (-65.9, 52.0)	19.2 (-33.1, 71.5)	26.3 (-43.6, 96.3)
Yellow-Orange Model 3	Reference Level	18.3 (-25.6, 62.2)	5.3 (-37.3, 48.0)	16.0 (-20.5, 52.6)	4.7 (-41.5, 51.1)	-19.5 (-67.6, 28.6)	-45.4 (-94.3, 3.5)
Model 4		18.4 (-26.0, 62.8)	6.8 (-37.3, 50.8)	15.6 (-20.7, 51.8)	4.6 (-40.3, 49.4)	-15.6 (-64.2, 33.0)	-45.7 (-96.4, 5.0)
Orange-Blue Model 3	Reference Level	15.8 (-10.3, 41.9)	14.8 (-6.9, 36.6)	16.3 (-14.2, 46.8)	2.3 (-33.3, 37.8)	-24.7 (-52.9, 3.4)	-6.4 (-41.7, 29.0)
Model 4		15.9 (-10.4, 42.3)	14.7 (-7.9, 37.3)	18.0 (-11.6, 47.6)	3.3 (-30.8, 37.5)	-25.3 (-54.9, 4.3)	-4.0 (-41.4, 33.4)

***Bold** indicates a significant p-value (<0.05).
Model 3 further adjusts for race/ethnicity, income-to-poverty ratio, years of formal education, smoking status, alcohol intake, central adiposity, missing teeth, body mass index, dentist visits, diabetes status, and fiber intake.
Model 4 further adjusts for diet—fiber, protein, fat, and carbohydrate consumption.

4.8 Further Analysis

Looking back at the main analysis as shown in Table 4.5, physical activity is significantly associated with higher Orange-Blue cluster scores in models 1 and 2. But while the significance goes away in more adjusted models, there may be a general trend of lower Orange-Red cluster scores and higher Orange-Blue cluster scores with physical activity. It is possible that the insignificance of the results is due to an issue of collinearity. Model 5 adjusts for the other clusters, age, sex, race, smoking status, and BMI. BMI is removed in model 6. The results of this model are shown in Table 4.11. The results of Model 5 are still significant. In Model 6, for sufficiently active, the significance is barely lost. And the significance is maintained for insufficiently active. This is evidence that the insignificant results in models 3 and 4 may be due to collinearity.

Table 4. 11: Association between Physical Activity and Cluster Scores

	Inactive (no physical activity)	**Insufficiently Active** (engages in physical activity, but less than sufficiently active)	**Sufficiently Active** (3-6 METs at least 5 times/week or >6 METs at least 3 times/week)
Orange-Red	Reference level		
Model 5		-15.5 (-49.9, 18.8)	-7.4 (-46.4, 31.7)
Model 6		-6.0 (-32.8, 20.7)	-4.0 (-30.6, 22.5)
Red-Green	Reference level		
Model 5		-15.5 (-49.9, 18.8)	-7.4 (-46.4, 31.7)
Model 6		-14.89 (-48.9, 19.1)	-5.9 (-43.1, 31.4)
Yellow-Orange	Reference level		
Model 5		5.9 (-23.1, 35.0)	-2.6 (-32.8, 27.6)
Model 6		5.8 (-23.2, 34.8)	-3.0 (-32.4, 26.5)
Orange-Blue	Reference level		
Model 5		**17.0 (0.4, 33.6)**	**16.5 (0.2, 32.9)**
Model 6		**16.7 (0.0, 33.4)**	15.8 (-0.6, 32.2)

*Bold indicates a significant p-value (<0.05).
*Model 5 adjusts for other clusters, age, sex, race, smoking status, and BMI.
*Model 6 adjusts for other clusters, age, sex, race, and smoking status

CHAPTER V: Discussion

The main finding of this study is that physical activity may be slightly associated with antibodies related to periodontal microorganisms. There was an inverse association between physical activity and the orange-blue cluster, which contains antibodies against microorganisms that are associated with reduced risk of periodontal damage, in unadjusted models and models adjusting only for sex and age. However this association attenuated after further adjustment for potential confounders.

This is the only known study that examins the association between physical activity and antibodies to periodontal microorganisms. Table A.1 in the Appendix shows a summary of studies that have assessed the association between physical activity and periodontal health. Most studies found an inverse association between physical activity and periodontal health (Al-Zahrani et al., Aug. 2005; Al-Zahrani et al., Oct. 2005; Merchant et al, 2003; Bawadi et al., 2011; Samnieng et al., 2013). Only one study found no association (Sakke et al., 1995), which examined the association between physical activity and periodontal pocket depth.

Wakai et al., (1999) used a number of different measures for physical fitness and had mixed results. For example certain physical fitness traits were associated with periodontal health such as aerobic capacity, foot balance, and reaction time. However, others were not such as grip strength, sit-ups, and sit-and-reach tests. VO_2 at anaerobic threshold (ml/kg/min) was only associated in the model adjusted for age and sex at the α=0.1 level. However, in the fully adjusted model, it was not. VO_{2max} appeared to be associated with decreased risks, but there was no clear linear trend. And many of these associations did not exist in the fully adjusted model. The outcome used by Wakai et al. was the Community periodontal Index of Treatment Needs (CPITN) score. The CPITN procedure includes the evaluation of pockets, calculus, and gingival bleeding. 10 specified index teeth are examined. For those under 20, only six index teeth are specified.

A number of these studies do not look at physical activity alone, but look at a number of predictors including physical activity (Al-Zahrani et al., Aug 2005; Samnieng et al., 2012; Wakai et al., 1999). For example, Al-Zahrani et al. (Aug 2005) found that having any ***one*** of the healthy behaviors (including getting recommended PA) was associated with 16% reduction in prevalence of periodontitis.

Also, none of these studies use microorganisms or antibodies to microorganisms as the outcome. Instead they used an indicator of periodontal destruction. These include clinical attachment loss (Al-Zahrani et al., Aug. 2005; Al-Zahrani et al., Oct. 2005; Bawadi et al., 2011; Samnieng et al., 2013), probing depth (Al-Zahrani et al., Aug. 2005; Al-Zahrani et al., Oct. 2005; Bawadi et al., 2011; Samnieng et al., 2013; Sakki et al., 1995), bone loss (Merchant et al., 2003), plaque index and gingival index (Bawadi et al., 2011), and salivary flow rate (Samnieng et al., 2013).

In order to understand why these results seem to differ from much of the current research, it is important to understand the mechanisms surrounding physical activity, periodontal health, and antibodies to periodontal microorganisms. These studies propose a number of possible mechanisms by which physical activity can effect periodontal health. One is that increased physical activity improves insulin sensitivity and/or decreases the risk of type II diabetes which leads to decreased periodontal disease.

However many of the models adjusted for diabetes status and still find an association (Al-Zahrani et al., Oct. 2005; Merchant et al., 2003; Bawadi et al., 2011).

Another proposed mechanism is that physical activity reduces inflammation, which is an important factor in the pathogenesis of periodontitis (Al-Zahrani et al., Aug. 2005). For example, two studies of NHANES III data have found that physical activity reduces inflammatory markers such as C-reactive protein in the plasma (Abramson et al., 2002; Ford et al., 2002).

Al-Zahrani et al. (Aug. 2005) also points out that there is evidence that physical activity reduces prostaglandin (PGE2) synthesis (American Cancer Society). Therefore physical activity could reduce PGE2 synthesis in the gingival tissue and thus protect against periodontal damage (Al-Zahrani et al., Aug. 2005).

Finally, Wakai et al. (1999) propose that physical activity improves a person's immunological response which would protect against the pathogens of periodontal disease.

There are a number of explanations for how the results of this study relate to the existing literature. As previously stated, the majority of the existing research has found an association between physical activity and periodontal destruction and disease. However, this study did not find any significant associations between physical activity and antibodies to periodontal microorganisms.

Many of the outcomes of the existing research, such as periodontal destruction, are irreversible and permanent. Antibodies, on the other hand, could indicate the past or present periodontal state. High levels of antibodies against periodontal microorganisms may be protective against future infection. Therefore, physical activity could in some way improve this process of preventing future infection and protect against periodontal disease.

A related explanation is that physical activity could not actually protect against periodontal infection, but instead, protect against the *consequences* of periodontal disease such as periodontal destruction. For example, as mentioned, physical activity could protect against inflammation which could improve bone attachment loss and probing depth scores (Al-Zahrani et al., Aug. 2005) of a person who is already infected with periodontal disease. The common symptoms of periodontal disease include increased probing depth, increased clinical attachment loss, and bone resorption. So for people infected with periodontal disease, physical activity may help lessen or slow the severity of their symptoms.

Al-Zahrani et al. (Oct. 2005) did a very similar study. They also used NHANES III data and used the same levels of physical activity as the predictor. The major differences were that that study used participants 18 and older (whereas this study used participants 40 and older) and the outcomes were bone attachment loss and probing depth. Al-Zahrani et al. found that engaging in the recommended levels of physical activity was significantly associated with lower periodontal prevalence (OR=0.58). The difference of findings could be due to the different outcomes, periodontal destruction vs periodontal antibodies as described above. Compared to this study, Al-Zahrani used a larger sample size, a younger population with a wider range of physical activity levels. The differences in findings could be due to the fact that this studies population size was smaller as the age-range was smaller. This could have caused more error in measuring physical activity in older subjects or variation in antibody levels. Also, older participants may have more chronic conditions, lower levels of physical activity, or a higher level of disease. Al-Zahrani on the other had used younger participants with a wider range of physical activity.

Sakki et al. (1995) did not find an association between physical activity and periodontal health (measured in pocket depth). Like this study, Sakki used an older population (55 year olds). Older populations may have lower levels of physical activity or physical activity may have a slightly different effect.

This study had a number of strengths. First, the participants were a large, representative, population-based sample. Measurements of potential confounders were carefully measured. Also, IgG antibodies to oral microorganisms are a unique measure of periodontal health. They are a measure of cumulative lifetime exposure to periodontal disease. And they may be less prone to measurement error than other predictors of periodontal health used in research (Papapanau et al., 2012; Susin et al., 2005; Kingman et al., 2002; Kingman et al., 2008; Beck et al., 2006). Also, clustering the antibody titers prevents an individual antibody titer form having too much influence. This makes the measurement more stable.

This study has a number of limitations. First, the generalizability of this study is limited as it only considered people who were 40 and older. However, there was not antibody data available for younger participants. Second, using antibodies as the outcome makes the results harder to interpret. But antibodies can also reveal information that was not possible to study otherwise and offer a new perspective, different from periodontal disease and destruction. Third, this is a cross-sectional study. Therefore, inferences on causality or temporality are risky.

In summary, there is slight evidence that being physically active is associated with periodontal antibodies against microorganisms among middle aged and older individuals in this cross-sectional investigation.

This is the first study to look at this specific association and adds another piece of evidence to the mechanisms surrounding periodontal disease, physical activity, and systemic diseases. Future research needs to evaluate these associations prospectively among a wider age range of individuals with more carefully measured variables.

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APPENDIX A: Literature Review Table

Table A.1 Studies examining the association between physical activity and periodontal health

Study	Predictor	Outcome	Confounders Considered	Population	Findings
Al-Zahrani et al. Increased physical activity reduces prevalence of periodontitis. Oct. 2005	PA categorized Vigorous = 3-6 METs at least 5 times/week or >6 METs at least 3 times/week Moderate = less than vigorous Sedentary = no PA (same as this study)	3mm or more bone attachment loss and 4mm or more probing depth.	age, race-ethnicity, gender, poverty index, education attainment, cigarette smoking, body mass index, healthy eating index, vitamin and mineral supplement use, time elapsed since last dental visit, percentage of sites with calculus and percentage of sites with gingival bleeding.	NHANES III 18 and older Only selected participants who had similar PA to NHANES II Excluded diabetics	Engaging in the recommended level of physical activity was significantly associated with lower periodontitis prevalence (OR=0.58, 95% CI, 0.35–0.96). Association was not strong among current smokers
Al-Zahrani et al. Periodontitis and three health-enhancing behaviors: maintaining normal weight, engaging in recommended level of exercise, and consuming a high-quality diet. Aug. 2005	3 behaviors including PA categorized Vigorous = 3-6 METs at least 5 times/week or >6 METs at least 3 times/week Moderate = less than vigorous Sedentary = no PA (same as this study)	3mm or more bone attachment loss and 4mm or more probing depth.	age, gender, race/ethnicity, cigarette smoking, other tobacco products, education, diabetes, poverty index, census region, acculturation, vitamin use, calculus, gingival bleeding, and time since last dental visit.	NHANES III 18 and older Excluded pregnant and nursing women Excluded “others” race/ethnicity Excluded BMI <18.5 N=12,110	Having any one of the healthy behaviors (including getting recommended PA) was associated with 16% reduction in prevalence of periodontitis.
Merchant AT,	Questionnair	bone loss and	Age, smoking,	US male	3% decreased risk

Pitiphat W, Rimm EB, Joshipura K. Increased physical activity decreases periodontitis risk in men. 2003	e: Calculated METs per week.	mean estimated radiographic bone loss.	alcohol, total calories, BMI, and diabetes	health professionals 40-75 years	for every 10-MET increase
Bawadi HA, et al. The association between periodontal disease, physical activity and healthy diet among adults in Jordan. 2011	7-d recall of physical activity 7 questions Classified into 3 categories based on a scoring protocol	Plaque index, gingival index, probing pocket depth, clinical attachment loss	gender, age, marital status, years of education, BMI, diabetes mellitus, plaque index and number of missing teeth	340 people 18-70 years old	Individuals who were highly physically active had a significantly lower average plaque index, average gingival index, average clinical attachment loss (CAL) and percentage of sites with CAL ≥ 3 mm compared to individuals with a low level of physical activity and individuals with a moderate level of physical activity.
Samnieng et al. The relationship between seven health practices and oral health status in community-dwelling elderly Thai. 2013	7 self-reported health practices PA-2 categories 'No' or 'Regular'	at least one site of 3 mm or deeper attachment loss and 4 mm or deeper pocket depth	age, gender, education, income, systemic disease, taking medicine, tooth brushing, dental flossing, dental health check-up, smoking, drinking, eating breakfast, sleeping time, eating between-meal snacks, maintaining weight and physical activity	612 people ≥60 years old Thai	Participants who had NO regular physical activity had a significantly higher prevalence of periodontal disease and lower salivary flow rate than their counterpart ($p < 0.05$).
Sakki et al. Association of	Lifestyle factors	Periodontal pockets	Tooth-brushing frequency,	1,012 55-yr-old citizens of	No association between

lifestyle with periodontal health. 1995	including physical activity PA was 2 categories: less than 15 min/day of walking/biking to work and only 1nce exercise in a week=low. Otherwise high	deeper than 3 mm,	time of last dental visit, frequency of dental visits, diet, alcohol, tobacco smoking, occupational status, family incomes, vocational education, sex	Oulu, Finland	periodontitis and physical activity and pocketing
Wakai K, et al. Associations of medical status and physical fitness with periodontal disease. J Clin Periodontol. 1999	Medical information/ health checkup including: Physical fitness test: including general endurance (maximum oxygen uptake and anaerobic threshold), muscle strength (grip strength), muscle endurance (sit-ups in 1 minute), flexibility, ability to balance, and agility.	Community periodontal index for treatment needs (CPITN) scoring system. This includes periodontal pockets, calculus, gingival bleeding, tooth mobility, inflammation	Sex, age, smoking habits, fasting plasma glucose, and simplified debris index	517 males and 113 females from Nagoya, Japan from 1992-1997	VO2max associated with decreased risk, but no clear linear trend. No significant association between grip strength, sit-ups, or flexibility. Balance associated with decreased risk.