# Dietary Intakes and Periodontal Outcomes After Sanative Therapy

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Submitted in partial fulfillment of the requirements for the degree of
Master of Science in applied Health Sciences
(Health Sciences)

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# Abstract

Diet has an important role in the maintenance of oral health, but the relationship between diet and clinical outcomes following sanative therapy (ST) has not been investigated. Due to their antioxidant and anti-inflammatory properties, we hypothesized that periodontal patients with higher intakes of vitamin C, vitamin D, eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) would have greater reductions in probing depth (PD) after ST. Patients completed the Block food frequency questionnaire, a supplement use questionnaire and had their serum 25-hydroxyvitamin D measured. There were no significant associations between intakes of vitamin C, vitamin D, EPA, DHA and PD. There were, however, negative associations between intakes of linoleic acid, $\alpha$-linolenic acid or total vegetable intake and PD, as well as a positive association between the total omega-6/omega-3 ratio and PD ($p < 0.05$). Therefore, dietary intakes of essential fatty acids and vegetables may be important modulators of periodontal outcomes following ST.

# Acknowledgments

I would first like to thank my supervisor Dr. Wendy Ward for her years of invaluable mentorship. Wendy, you have always helped me strive for excellence and you have always had my best interest at heart. I couldn't have asked for a better supervisor. In fact, you are probably the only person who could get me to leave my comfy place in the city. The effort and dedication you have shown to me and to all of your students, whether it is helping us in the lab, editing our manuscripts, or writing us reference letters, is truly inspirational. You are an amazing role model and friend.

I would like to thank Dr. Peter Fritz for providing me with this wonderful research opportunity. Without his continual support and generosity, this research would not be possible. This research would also not be possible without the amazing hard-working team of staff at Dr. Fritz's clinic. I want to sincerely thank Michele Bowman for her continual dedication in keeping the study organized and tracking down patients to get those last food frequency questionnaires back, which was no easy task. I'd like to thank the four very talented hygienists, Cathy Hamm, Donna Lavoie, Linda Dakin and Teresa Shouldice-Fleming for their work on the front lines of the project. I want to thank the team of nurses, Margie Waldeck, Jeff Shapland and Mike Lavigne for their meticulous work collecting samples and data. I would also like to thank the rest of the clinic staff, Jordanna, Assunta, Candice, Patti, Ruth, Kelsey, and Jesse who are always happy to provide a helping hand throughout my study. I have greatly enjoyed the time I've spent at the clinic over the last two years and I will miss everyone.

I would like to extend my gratitude to my committee members who have taken the time to help make this thesis possible. Thank you Dr. Philip Sullivan for helping us

navigate the world of statistics and thank you Dr. Paul LeBlanc for your much appreciated scientific insight. Furthermore, I would like to sincerely thank my external examiner Professor Donna Hennyey for her commitment, attention to detail and for helping me highlight the real-world applicability of this research.

Lastly, I would like to show my appreciation to my friends and family who have supported me throughout my master's program. My peers in the Ward lab, Laura Castelli, Paula Miotto, Amanda Longo, Leslie Nash and Bryan Johnston have been invaluable colleagues who have not only taught me a lot but who have made this journey that much more enjoyable. I also want to give a special thank you to Bryan Johnston who was always willing to fill in for me and keep my study going when I had to be away. Last but not least, I would like to show my gratitude to my parents, Dave and Donna. They have always encouraged me to pursue my dreams and they have done everything in their power to help me succeed.

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| | |
|---|---|
| $1,25(OH)_2D$ | 1,25-dihydroxyvitamin D |
| 25(OH)D | 25-hydroxyvitamin D |
| AA | Arachidonic acid |
| ACCDQ | Anti-Cancer Council of Victoria Dietary Questionnaire |
| ALA | alpha-linolenic acid |
| ANCOVA | Analysis of covariance |
| BMI | Body mass index |
| BOP | Bleeding on probing |
| CAL | Clinical attachment loss |
| COPD | Chronic obstructive pulmonary disease |
| COX | Cyclooxygenase |
| CRP | C-reactive protein |
| DHA | Docosahexanoic acid |
| EPA | Eicosapentanoic acid |
| FFQ | Food frequency questionnaire |
| FGF | Fibroblast growth factor |
| GLA | Gamma-linoleic acid |
| GR | Gingival recession |
| HbA1c | Hemoglobin A1c |
| HRT | Hormone replacement therapy |
| IL | Interleukin |
| INF-λ | Interferon gamma |

| | |
|---|---|
| IGF | Insulin-like growth factor |
| IU | International unit |
| Kcal | Kilocalorie |
| L | Liter |
| LA | Linoleic acid |
| LOX | Lipooxygenase |
| μg | Microgram |
| μL | Microliter |
| μmol | Micromole |
| mg | Milligrams |
| mL | Milliliter |
| MLR | Multiple linear regression |
| mm | Millimeter |
| MMP | Matrix metalloproteinase |
| NBP | Nosocomial bacterial pneumonia |
| ng | Nanogram |
| NHANES | National health and nutrition examination survey |
| Nmol | Nano mole |
| OHIP | Oral health impact profile |
| OPG | Osteoprotegerin |
| OR | Odds ratio |
| PD | Probing depth |
| PDGF | Platelet derived growth factor |

| | |
|---|---|
| PG | Prostaglandin |
| PMN | Polymorphonuclear cell |
| PUFA | Polyunsaturated fatty acid |
| RA | Rheumatoid arthritis |
| RANKL | Receptor activator of nuclear factor kappa-B ligand |
| RDA | Recommended dietary allowance |
| ROS | Reactive oxygen species |
| SD | Standard deviation |
| SEM | Standard error of the mean |
| ST | Sanative therapy |
| SUPP | Supplements |
| SVCT | Sodium dependent vitamin C transporter |
| TGF | Transforming growth factor |
| TNF-α | Tumor necrosis factor-alpha |
| LT | Leukotriene |
| TX | Thromboxane |

# Chapter One

# LITERATURE REVIEW

## 1.1 Periodontal Disease

Periodontal disease is a condition characterized by inflammation of the tissues that surround and support the teeth. It is caused by bacteria on the surface of the teeth and below the gum line, as well as an over active immune response to that bacteria. In periodontal disease, inflammation results in loss of connective tissue supporting the teeth and loss of alveolar bone from the jaw. Unlike gingivitis, which is a reversible condition, periodontitis is chronic and often non-reversible. If left untreated, patients with periodontal disease will lose their teeth.

According to the 2007-2009 Canadian Health Measures Survey, 16% of Canadian adults (20 – 79 years of age) have moderate periodontal disease while 4% of Canadian adults of severe periodontal disease [1]. The prevalence is also higher in specific populations. For example, 24% of older Canadian adults (60 – 79 years of age) have signs of moderate periodontal disease and 22% of current smokers have signs of moderate periodontal disease.

### 1.1.1 Anatomy and Definitions

#### 1.1.1.1 Anatomy of the Periodontium

The periodontium consists of both hard and soft tissues. The hard tissues of the periodontium include the alveolar bone and cementum while the soft tissues include the periodontal ligament and gingivae [2] (**Figure 1.1**). Alveolar bone is a specialized type of bone that sits over the jaw and forms sockets in which the teeth sit. Alveolar bone is composed of an outer periostium, an underlying layer of compact bone, and the inside is composed of cancellous bone. Alveolar bone is laid down by osteoblasts, maintained by

osteocytes, and broken down by osteoclasts to allow for remodeling. The cementum is a hard tissue covering of the root of a tooth. It is primarily composed of calcium hydroxyapatite crystals embedded in fibrous tissues. The cementum is formed by cementoblasts and maintained by cementocytes. The periodontal ligament forms a connective tissue attachment between the cementum and the alveolar bone. It consists of directional collagen bundles running between the alveolar bone and cementum and between adjacent teeth and gingivae. Periodontal ligament attaches to bone and cementum through specialized fibres called Sharpey's fibres. The periodontal ligament acts as a shock absorber during normal activities such as chewing. It is also highly vascularized and rich in sensory nerve fibres. The periodontal ligament and fibrous connective tissues are formed by fibroblasts and by the epithelial cells of the gingivae. The gingivae (or gums) are a special connective tissue covering that attaches to teeth at their necks. It is composed of a surface keratinized epithelium, which forms part of the oral mucosa. There are three types of gingivae; attached gingivae that lays on top of alveolar bone, marginal gingivae that forms the gingival margin (does not sit on top of bone) and junctional tissue that forms the anatomical junction between the teeth and the oral epithelium. In healthy individuals, the junctional tissue attaches at the cemento-enamel junction. The top of the gingivae is known as the gingival margin and the pit-like depression between the gingival margin and the junctional epithelium forms the gingival crevice.

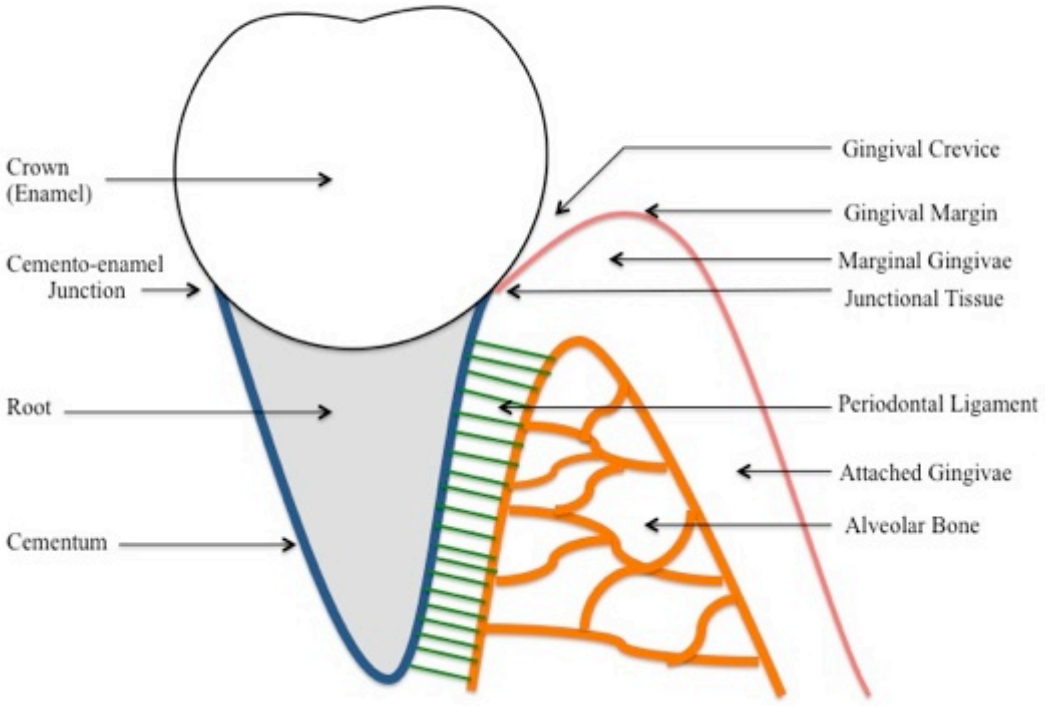


**Figure 1.1** Anatomy of the periodontium.

### 1.1.1.2 Clinical Measures

Periodontal status is assessed through a number of clinical markers. Probing depth (PD) is a measure of the distance from the gingival margin to the bottom of the gingival crevice. A probing depth of 3 millimeters (mm) or less is normal, while a probing depth of 4 mm or more indicates periodontal disease. PD is routinely used to evaluate disease severity, disease progression and response to treatment [3]. Another common measure is gingival recession (GR), which is a measure of the exposure of the root surface. GR is measured as the distance between the gingival margin and the cemento-enamel junction.

Clinical attachment loss (CAL) is the distance between the cemento-enamel junction and the bottom of the gingival crevice. When the gingival margin is below the cemento-enamel junction, CAL is the sum of PD and GR. Measurements of PD, CAL and GR are demonstrated in **Figure 1.2.** CAL is accepted as the gold standard for periodontitis, however, it is considered a better measure of past periodontal disease activity, whereas PD is a better measure of present periodontal disease activity [4]. This is because when periodontitis is treated, inflammation resolves and PD is returned to normal but GR may still be present. For these reasons, CAL and PD are both used in epidemiology and clinical trials, however, since CAL is cumbersome and time consuming to measure, PD alone is usually used to measure disease activity in a clinical setting. Other common measures include bleeding on probing and plaque score. Bleeding on probing (BOP) is the number of sites that bleed when probed and is a measure of acute inflammation and oral hygiene care. Plaque score is the number of teeth with visible plaque and is a useful measure because it reflects a patient's hygiene practices. PD, CAL and BOP are measured at six sites around each tooth; three sites on the front surface (buccal, mesial buccal, and distal buccal) and three sites on the back surface (lingual, mesial lingual, distal lingual).

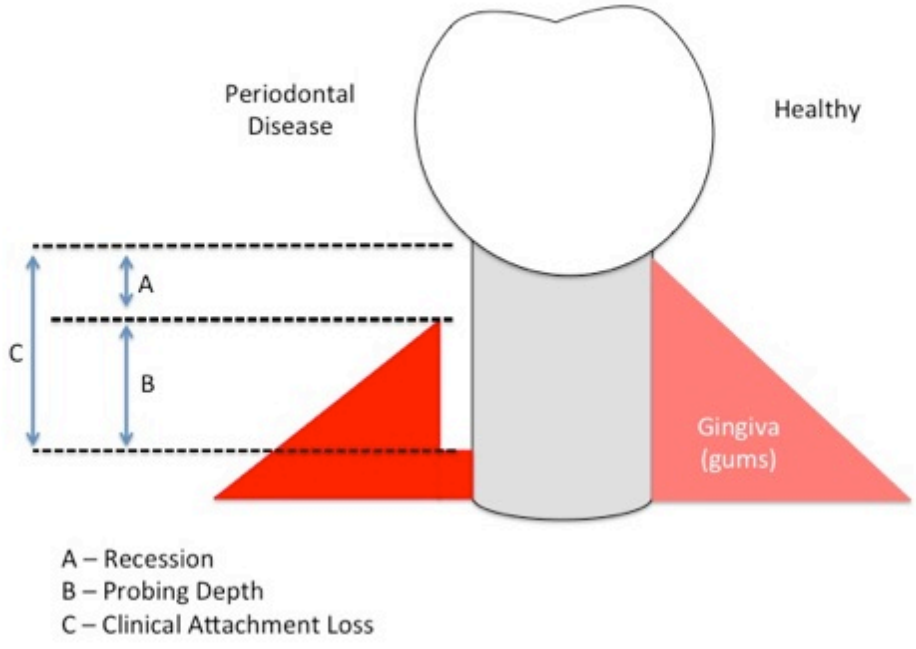


**Figure 1.2** Measurement of recession, probing depth and clinical attachment loss.

### 1.1.1.3 Definition of Periodontal Disease

The current classification system for periodontal disease was established in 1999 by the American Academy of Periodontology [5]. They define chronic periodontitis as ‘‘an infectious disease resulting in inflammation within the supporting structures of teeth, progressive attachment and bone loss. It is characterized by pocket formation and/or gingival recession. It is recognized as the most frequently occurring form of periodontitis. Its onset may be at any age but is most commonly detected in adults. The prevalence and severity of the disease increases with age. It may affect a variable number of teeth and has variable rates of progression.’’ Chronic periodontitis can be classified as either localized or generalized. Localized periodontitis is when ≤ 30% of sites are affected and

generalized periodontitis is when > 30% of sites are affected. Periodontitis can be further classified based on severity. Moderate periodontitis is defined as having ≥ 2 interproximal sites (mesial or distal) with CAL ≥ 4 mm (not on same tooth) or ≥ 2 interproximal sites with PD ≥ 5 mm (not on same tooth). Severe periodontitis is defined as having ≥ 2 interproximal sites with CAL ≥ 6 mm (not on same tooth) and ≥ 1 interproximal site with PD ≥ 5 mm.

### 1.1.2 Pathogenesis of Periodontal Disease

The initiation of periodontal disease begins with chronic bacterial exposure. Although pathogenic organisms are a prerequisite for periodontitis, it has been estimated that bacterial exposure only accounts for approximately 20% of disease expression [6]. Therefore, periodontal disease is largely driven by host factors, namely the host immune response. In response to bacteria and bacterial biofilms, immune cells such as neutrophils, macrophages, B cells and T cells are recruited into the periodontium [7]. This results in the release of pro-inflammatory cytokines, matrix metalloproteinases (MMPs), reactive oxygen species (ROS), and mediators for osteoclastic bone resorption.

Periodontitis ultimately results in both soft tissue (periodontal ligament and gingivae) and hard tissue (alveolar bone) destruction. Most of the soft tissue destruction is mediated by MMPs. MMPs are released by a variety of host cells such as polymorphonuclear leukocytes, macrophages, fibroblasts, bone, epithelial, and endothelial cells and they catalyze the breakdown of the cell membrane and extracellular matrix proteins such as collagen, gelatin, proteoglycans, fibronectin, laminin, and elastin [8]. Periodontal disease is characterized by an imbalance in MMP activity. For example,

there is increased secretion of MMP-8 (neutrophil-derived collagenase) and MMP-9 (neutrophil-derived gelatinase) by polymorphonuclear cells (PMNs), which effectively degrade the type 1 collagen found in periodontal ligament and gingivae [9]. Furthermore, transcription of MMP genes is up regulated by cytokines known to be important in periodontal disease progression, including interleukin (IL)-1β and tumor necrosis factor alpha (TNF-α) [10]. Hard tissue destruction is largely mediated by over activity of osteoclasts. Osteoclast differentiation and activity is directly up regulated by the immune response through mediators such as IL-1ß, TNF-α, IL-6 [11, 12]. In addition, reactive oxygen species (ROS) play an important role in the pathogenesis of periodontal disease. ROS are a diverse group of oxygen containing molecules with unpaired electrons, which makes them extremely reactive and capable of oxidizing and damaging biological molecules. Immune cells, especially neutrophils generate ROS in periodontal tissue as part of the normal response to bacterial pathogens [13]. ROS has been shown to cause lysis of gingival epithelial cells [14], activate osteoclasts [15], cause de-polymerization and residue modification of extracellular matrix proteins [16], modify type 1 collagen making it more susceptible to proteolysis [17] and up regulate MMP expression [18]. A simplified schematic of periodontal tissue destruction is depicted in **Figure 1.3**.

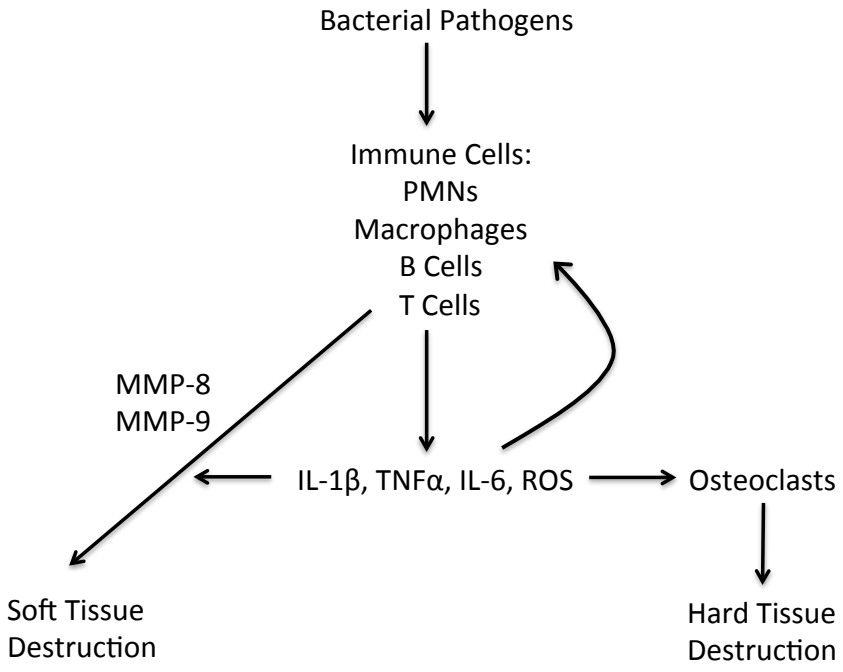


**Figure 1.3** Pathogenesis of periodontal disease.

### 1.1.3 Risk Factors for Periodontal Disease

The prevalence and severity of periodontal disease has been associated with both genetic and environmental risk factors [19].

#### 1.1.3.1 Non-Modifiable Risk Factors (genetics, sex, age)

Familial aggregation studies have demonstrated a significant genetic component to periodontal disease and that genetics may account for up to 50% of disease expression in adults [20]. The identification of specific genetic risk factors for periodontal disease is one of the current challenges in periodontal research. Vijayalakshmi [21] has provided an

excellent summary of genetic polymorphisms identified to date. Examples include polymorphisms in genes for cytokines (IL-1, TNFα, IL-10), receptors (Fc gamma receptor, formyl peptide receptor 1), metabolism (vitamin D receptor, calcitonin receptor), antigen recognition (human leukocyte antigens) and innate immunity receptors (toll-like receptor 2 and 4, CD14, caspase recruitment domain-containing protein 15). These associations, however, are based on a limited number of studies and therefore the clinical implications of these findings are not yet known.

Age and sex have also been identified as risk factors for periodontal disease. There is a greater prevalence of periodontal disease with age; however, this appears to be mediated by oral hygiene status rather than by age itself [22]. In this study, oral hygiene status was defined as either good or poor depending on the need for professional removal of plaque and calculus (hardened plaque). The prevalence of periodontitis has also been shown to be higher in males compared to females; however, it is thought that this phenomenon is largely attributable to behavioral differences [23]. The greater prevalence seen in men may be due to lower frequency of brushing and flossing [24], greater tobacco use [25] and less frequent use of oral health care services [26].

#### 1.1.3.2 Modifiable Risk Factors (oral hygiene, smoking, stress, obesity, systemic diseases)

Numerous modifiable risk factors have been identified and include oral hygiene, smoking, stress, obesity, and the presence of systemic diseases. Systemic diseases will be reviewed in section 1.1.4.

Oral hygiene, especially plaque control, is an extremely important component of periodontal disease prevention and management [27]. This is true because the removal of plaque removes the etiological agent of periodontal disease. Interestingly though, a meta-analysis has concluded that there is no evidence that personal oral hygiene care prevents or controls chronic periodontitis [28]. However, this finding may be due to the use of measures such as brushing frequency and technique to assess oral hygiene, which do not take oral hygiene efficacy (the actual success of plaque removal) into account [19]. Surrogate measures of oral hygiene care such as plaque score are significantly related to measures of early periodontitis [29].

Smoking is undeniably a significant risk factor for periodontal disease. Epidemiological evidence from the National Health and Nutrition Examination Survey (NHANES) in the United States has suggested that approximately half of the periodontitis cases are attributed to smoking [30]. The mechanisms are not fully understood but smoking is associated with lower levels of anti-inflammatory and anti-resorptive factors such as IL-10 and osteoprotegerin (OPG) and higher levels of pro-inflammatory cytokines such as IL-6 and interferon gamma (INF-λ) in periodontal tissues [31]. It is also known that smokers have a lower proportion of blood vessels in periodontal tissue and therefore a reduced blood flow to the area [32]. When it comes to periodontal disease treatment, most clinical studies show that both surgical and non-surgical periodontal therapies are less effective in smokers [33]. Fortunately, there are several studies that report benefits to periodontal health and treatment with smoking cessation [34] although being a former smoker is still associated with some risk of periodontal disease [30].

Stress is currently considered a risk factor for periodontal disease [35]. The mechanisms have not been fully elucidated but the relationship is thought to arise through health-impairing behaviors (poor oral hygiene, smoking, alcohol consumption, and poor nutritional intake) and physiological factors such as higher glucocorticoid levels that may contribute to inflammation.

It has been estimated that after dental plaque and smoking, obesity is the next biggest risk factor for periodontal disease [36]. Most of the evidence for this association is epidemiological. A body mass index (BMI) of 25-29.9 confers a relative risk for periodontitis of 3.4 while a BMI greater than 30 confers a relative risk of 8.6 [37]. BMI is also significantly correlated with the severity of attachment loss with greater attachment loss when insulin resistance is present [38]. Additionally, it has been shown that the distribution of fat is an important consideration as waist-to-hip ratio may be a better predictor of periodontal disease than BMI [39]. Furthermore, physical activity has been associated with a lower prevalence of periodontitis [40]. The underlying mechanism is not yet known but it is suspected to be due to inflammatory cytokines and adipokines derived by adipose tissue [19].

### 1.1.4 Periodontal Disease and Systemic Conditions

Periodontal disease is associated with a number of other chronic diseases including diabetes, cardiovascular disease, osteoporosis, respiratory diseases and rheumatoid arthritis. Clinically, it is important for dental professionals to assess a patient's overall health in order to identify those at greater risk for periodontitis.

Similarly, the presence of systemic conditions may also have important implications for designing treatment plans for patients with periodontitis.

#### 1.1.4.1 Diabetes

A meta-analysis of 57 studies concluded that type 2 diabetes mellitus is a risk factor for periodontitis [41]. On average, patients with diabetes have 1 mm more clinical attachment loss compared to non-diabetics. The proposed mechanism is thought to be through high levels of advanced glycosylation end products in periodontal tissue [42]. These end products bind to immune cells and fibroblast resulting in a hypersensitive response to pathogens (increased cytokine expression and increased mediators of both hard and soft tissue destruction). Moreover, these end products inhibit collagen formation by fibroblasts and thereby impair wound healing.

Because inflammation promotes insulin resistance and dysregulation of glycemia [43], it is plausible that periodontal treatment can improve glycemic control in diabetics. Two meta-analyses have concluded that periodontal therapy can reduce hemoglobin A1c (HbA1c) levels significantly by 0.40% [44, 45]. This has significant clinical implications as a 0.20% reduction in HbA1c has been associated with a 10% decrease in mortality in the general population [46].

#### 1.1.4.2 Cardiovascular Disease

Cardiovascular diseases are one of the leading causes of death worldwide. Cardiovascular diseases describe a broad range of pathologies that include ischemic heart disease, ischemic stroke, and myocardial infarction. Based on the results from several

meta-analyses, the Canadian Dental Association has concluded that there is a significant relationship between periodontitis and cardiovascular disease independent of other risk factors [47]. This association may be explained by the fact that many of the systemic markers in periodontal disease [C-reactive protein (CRP), IL-1β, IL-6, IL-8, TNF-α] are also associated with cardiovascular disease [48]. Furthermore, periodontal disease has been associated with endothelial dysfunction, another risk factor cardiovascular disease [49]. There is strong evidence that periodontal treatment reduces c-reactive protein levels and improves endothelial function while there is a moderate amount of evidence suggesting periodontal therapy reduces IL-6 and lipid levels [50, 51]. It is likely that these changes translate into a reduced risk for cardiovascular diseases but the magnitude of these effects has not yet been calculated.

#### 1.1.4.3 Osteoporosis

Osteoporosis is a skeletal disorder characterized by low bone mass and microarchitecture deterioration, which predispose an individual to fragility fractures. Postmenopausal women with osteoporosis, especially, are at a significantly higher risk (odds ratio of 2.58) for periodontal disease [52]. We have also known for decades that there is an inverse relationship between bone mineral density and tooth loss [53-55]. The relationship between periodontitis and osteoporosis likely stems from the fact that they share many pathological features in common such as an increase in inflammation and an increase in bone resorption. In a recent study, it was suggested that the relation between these two diseases is at least partly mediated by low vitamin D, increased receptor

activator of nuclear factor kappa-B ligand (RANKL) and an increase in inflammatory cytokines [56].

#### 1.1.4.4 Respiratory Diseases

Periodontal disease has been associated with an increased risk for both nosocomial bacterial pneumonia (NBP) and chronic obstructive pulmonary disease (COPD) [57]. The incidence of NBP in people with periodontitis has been attributed to colonization of the respiratory tract by pathogens in dental plaque [58]. Fortunately, professional oral hygiene care has been shown to reduce the incidence of pneumonia in high-risk patients (e.g. elderly people in nursing homes) [59]. It is currently unknown if there is a direct cause and effect relationship between periodontal disease and COPD [60]. Nonetheless, since both NBP and COPD have high mortality rates, periodontal treatment and dental care provide a cost effective way to help prevent these life threatening diseases.

#### 1.1.4.5 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disorder that can lead to severe disability and increased risk of mortality [61]. The relationship between periodontitis and RA has been recognized since the 1820s [62]. Similarly to other diseases, the link is likely due to the similarities in underlying inflammatory mechanisms. There is also speculation that periodontal pathogens might induce RA because they have been found in joints of patients with RA [63]. A clear cause and effect

relationship, however, has not been established. One study has shown that anti TNF-α therapy is more effective in RA patients without periodontitis [64].

It is evident that periodontal disease needs to be evaluated in the context of overall health. Clinicians and researchers need to be aware of the links between specific diseases and periodontitis in order to identify those at risk for periodontal destruction and to develop treatment plans. Importantly, periodontal therapy may involve the management of related conditions and conversely, the management of many chronic diseases may involve the treatment of periodontitis.

### 1.1.5 Sanative Therapy for the Treatment of Periodontal Disease

Sanative therapy (ST) is a conservative and cost-effective first line of treatment to manage periodontal infections. The goal of ST is to remove plaque and calculus from the root surfaces and to provide a root surface that is compatible with reattachment [65]. ST is performed using mechanical scaling instruments to remove bacteria that have invaded deep into the periodontal pocket below the gingival margin. ST is done by a process known as scaling and root planing. Scaling is the "instrumentation of the crown and root surfaces of the teeth to remove plaque, calculus, and stains from these surfaces" and root planing is "a treatment procedure designed to remove cementum or surface dentin that is rough, impregnated with calculus, or contaminated with toxins or microorganisms" [66]. Scaling and root planing are performed with mechanical debridement instruments and sonic or ultrasonic scalers that use vibration accompanied by a stream of water to remove adherent deposits from teeth. The procedure is invasive and requires the use of local anesthesia. Once sub-gingival plaque and bacteria are under control, the immune

response can resolve. This means a reduction in inflammation, inflammatory cytokines, ROS, MMP activity and osteoclast activity. Once these destructive processes have subsided, healing and regeneration can take place. Successful ST results in a decrease in probing depths by reducing gingival swelling and allowing for new soft tissue formation to occur. There will be an increase in collagen fiber formation resulting in improved health of the periodontal ligament and junctional epithelium, ultimately leading to greater tooth attachment [67]. Although ST therapy can prevent further alveolar bone loss, unlike soft tissue, there is minimal regeneration of alveolar bone [68].

### 1.1.6 Periodontal Healing: Regeneration and Repair

Periodontal healing is comprised of two distinct but simultaneously occurring processes, periodontal regeneration and periodontal repair. In an ideal situation, regeneration would outweigh repair, however, this is not usually the case.

#### 1.1.6.1 Periodontal Regeneration

Periodontal regeneration is defined as "restoration of lost periodontium" implying the restoration of architecture (alveolar bone, cementum, periodontal ligament and gingivae) and function [66]. Periodontal regeneration is a complex process involving many different cell types working in a coordinated sequence. The periodontal wound consists of two margins, the mineralized root surface and the connective tissue of the gingival flap. The root surface has been scaled and planed in order to provide a surface free of bacterial colonization and therefore compatible with reattachment. The first step in healing is the formation of a blood clot at the interface between the tooth and the gingival

flap [69]. A fibrin clot is developed on the tooth surface within minutes. The blood clot (platelets and fibrin) is responsible for providing a scaffold to begin tissue regeneration and to release growth factors such as platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), and transforming growth factor (TGF) [70]. These factors are critical for recruiting and activating the cells needed for the subsequent steps in the regeneration process. Following the formation of the blood clot, there are two inflammatory phases [69]. The early phase of inflammation occurs within an hour and is predominated by inflammatory cells such as neutrophils and monocytes at the tooth surface. The cells in this phase are responsible for removing any pathogenic organisms and debris left in the periodontal wound. They are also responsible for releasing cytokines and chemokines needed to initiate the next phase. The late phase of inflammation occurs within 3 days and is predominated by macrophages, which further release growth factors [PDGF, IGF, TGF, fibroblast growth factor (FGF)] that recruit and activate periodontal ligament cells and osteoblasts. The effects of growth factors on periodontal ligament cells and osteoblast are summarized in **Table 1.1.6.** Regeneration of the periodontal ligament requires the presence of specific cells types and therefore the nature of the regeneration/repair process will depend on which cells populate the exposed root surface [71]. It appears that only cells from the periodontal ligament and not the gingival connective tissue or bone are capable of initiating new attachment [72, 73]. Using modern cell sorting technologies, periodontal ligament stem cells that can differentiate into cementum and collagen forming cells have been identified and characterized [74]. By day 7 of the regeneration process, granulation tissue has formed and new connective tissue attachment is visible at the tooth surface. Studies in canines

have shown that small periodontal wounds gain functional integrity (measured as the tensile strength between the tooth and gingival flap interface) by about 2 weeks [75]. Although periodontal ligament formation occurs and clinical attachment level is gained following scaling and rooting planing, there is usually not complete regeneration of the periodontal apparatus [76]. For example, in treating sites with a PD of 7.4 mm, there is on average a gain in attachment of only 1.2 mm [77]. The remaining amounts of PD reduction are due to a gain in recession of 2.0 mm on average. Similarly, although the recruitment and activation of osteoblasts would suggest regeneration of alveolar bone, there is actually very little, if any, hard tissue regeneration [68]. For example, one year after treatment, there is only, on average, 0.9% gain in radiographic bone level with most individuals showing no gain and some showing a loss. It is therefore evident that while periodontal regeneration is possible and does occur to a certain extent, this process is limited and does not completely restore the periodontium to its pre-periodontal disease state. Thus, current periodontal research is seeking ways to enhance and optimize the regeneration process.

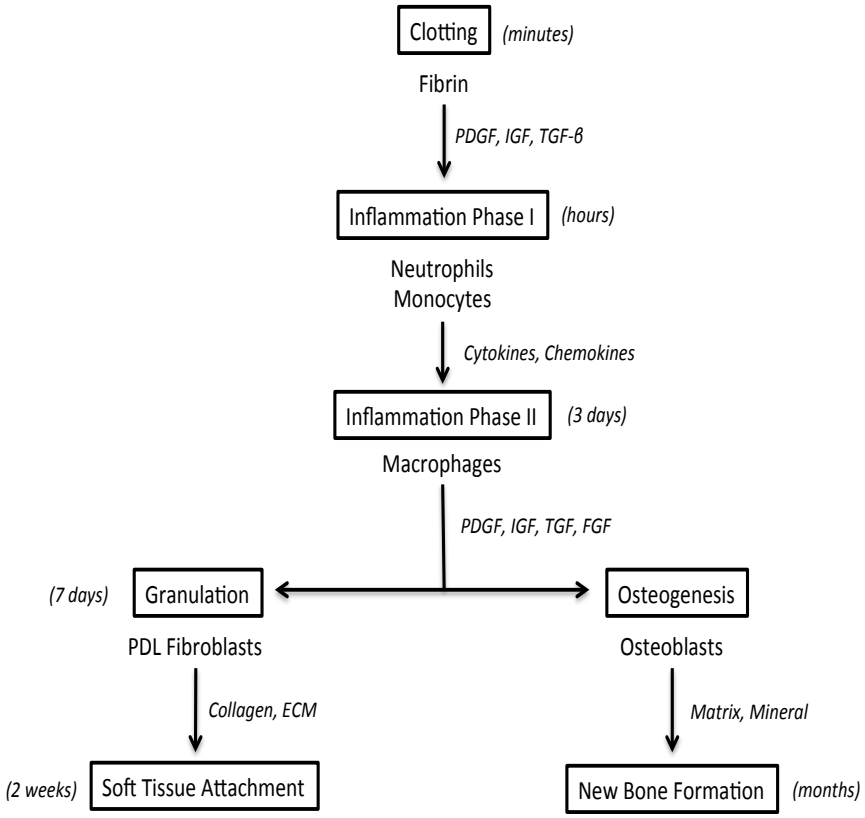


**Figure 1.4.** Periodontal regeneration

**Table 1.1** The effects of growth factors on osteoblast and periodontal ligament cells

| Growth Factor | Source | Effects on Osteoblasts | Effects on Periodontal Ligament Cells |
|---|---|---|---|
| PDGF | Platelets<br>Endothelial cells<br>Smooth muscles<br>Macrophages<br>Fibroblasts | ↑ Chemotaxis<br>↑ Cell division | ↑ Chemotaxis<br>↑ Cell division |
| IGF | Macrophages<br>Osteoblasts<br>Plasma stored in bone<br>Platelets | ↑ Cell division<br>↑ Bone formation | ↑ DNA synthesis in PDL fibroblasts |
| TGF | Platelet α granules<br>Macrophages<br>Osteoblasts | ↑ Bone matrix<br>Coupling bone resorption with deposition | ↑ Cell division (weakly) |
| FGF | Macrophages<br>Osteoblasts<br>Bone | Regulation | ↑ Chemotaxis<br>↑ Cell division |

### 1.1.6.2 Periodontal Repair

The American Academy of Periodontology defines repair as "Healing of a wound by tissue that does not fully restore the architecture or the function of the part" [66]. In periodontal repair there is healing that takes place (reduction in probing depth) but with less gain in clinical attachment. Periodontal repair is characterized by the formation of a long junctional epithelium [78]. The epithelial cells of the gingival flap will migrate apically (toward the root) along the instrumented root surface essentially blocking periodontal regeneration. It was eventually determined that epithelial migration results when there is disruption of the fibrin clot [79]. This has lead to the belief that periodontal repair happens in place of regeneration under non-ideal conditions such as wound instability and population of the wound by the wrong cell types.

#### 1.1.6.3 Healing and Inflammation

Inflammation has important implications for wound healing. In cutaneous wounds, there is significant evidence suggesting that inflammation negatively affects the quality of wound healing. For example, fetal wounds are characterized by an absence of inflammation and they heal without the formation of scar tissue [80]. When inflammation is induced in the fetal wound, the wound then heals with formation of scar tissue. Impaired wound healing in the presence of inflammation is thought to occur due to an imbalance in proteolytic activity and oxidative stress. Chronic wounds have higher levels of MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 and MMP-11 [81, 82] and higher levels of neutrophil-derived serine proteases such as cathepsin G, urokinase-type plasminogen activator and neutrophil elastase [83]. These proteases are detrimental to wound healing as they degrade extracellular materials such as fibronectin and vitronectin [84]. Proteases also degrade important healing growth factors such as vascular endothelial growth factor [85]. Therefore, in order to shift the balance toward healing and tissue formation, the destructive forces caused by inflammation need to be minimized. Thus, the resolution of inflammation is crucial for healing to occur. Although most of the evidence for this relationship is derived from studies in cutaneous wounds, the healing processes underlying the healing of oral wounds are generally quite similar [86]. The mechanisms of periodontal healing impaired by inflammation have not been specifically studied because the cause of the periodontal wound is inflammation itself and it is therefore obvious that a reduction in inflammation is needed for healing to occur. One recent abstract however has reported that periodontal ligament cells in vitro have reduced proliferation and adhesion in the presence of inflammation [87]. It is therefore evident

that the resolution of inflammation should be an effective way to promote periodontal healing. Future research in this area will likely focus on anti-inflammatory agents that can be delivered alongside conventional periodontal therapy in order to optimize periodontal healing.

### 1.1.7 Importance of Successful Periodontal Therapy

If sanative therapy is not successful, soft and hard tissue destruction will continue and patients with periodontitis will ultimately end up losing their teeth. Tooth loss has profound effects that extend beyond oral health. Firstly, tooth loss is associated with poorer nutritional status [88-93]. Individuals, especially older ones, with fewer teeth consume less protein, less fruits and vegetables and are more likely to be underweight. Tooth loss is also associated with lower quality of life scores [94]. As the number of natural teeth decrease, individuals score worse on the Oral Health Impact Profile (OHIP), a tool that documents the functional and psychosocial impacts of oral health disorders and assesses issues such as pain, problems with eating and sleeping, concerns about appearance and difficulties in social situations. Lastly, in some populations, tooth loss has even been associated with increased risk of total mortality and death from upper gastrointestinal cancer, heart disease, and stroke [95, 96].

The benefits of successful periodontal therapy also extend beyond the prevention of tooth loss as numerous systemic health benefits have been reported [97]. Current evidence suggests that periodontal intervention may improve glycemic control in diabetics, reduce systemic markers of inflammation, lower cholesterol levels and improve vascular endothelial function. Due to the similar inflammatory mechanisms

underlying the pathogenesis of periodontal disease and many other chronic diseases such as diabetes, osteoporosis, cardiovascular disease, respiratory diseases, rheumatoid arthritis and obesity, there exists great potential for periodontal therapy to modulate these complex relationships [98].

Nutrition and diet may also play an important role in ensuring the success of periodontal therapy. There is growing evidence that diet and nutritional supplement use can optimize periodontal outcomes after sanative therapy. The remaining portions of the literature review will focus on this evidence supporting a link between nutrition, periodontal health and periodontal healing after therapy.

### 1.2 Nutrition and Periodontal Disease

In recent years, researchers and clinicians have begun to better understand the role of nutrition in periodontal health. A number of studies have uncovered relationships between specific nutrients and risk of periodontal disease. A small number of studies have also looked at the relationship between diet and periodontal status in a clinical population or the relationship between diet and periodontal therapy [99]. Based on the current literature, three nutrients that might have important clinical implications are vitamin C, vitamin D and omega-3 fatty acids. Though other nutrients have been investigated in isolated studies, these three form the majority of the research concerning nutrition and periodontal disease. Firstly, each has been associated with reduced risk for periodontal disease in the general population [100-102]. This means that they play a protective role in periodontal health and may be useful in helping patients who have undergone periodontal treatment to maintain their periodontal health in the long-term.

Secondly, vitamin C, vitamin D and omega-3 fatty acids might improve healing after periodontal procedures. Thus, they may be useful adjuncts to optimize periodontal treatment procedures such as sanative therapy. Each of these nutrients will be reviewed in sections 1.2.1, 1.2.2, and 1.2.3, respectively.

### 1.2.1 Vitamin C

Vitamin C is a water-soluble vitamin found primarily in fruits and vegetables. Plants and animals synthesize vitamin C from glucose; however, humans, primates and guinea pigs have lost the ability to synthesize vitamin C due to a non-functional gene for the L-gulonolactone oxidase enzyme (required for the final step in vitamin C synthesis), making dietary vitamin C intake essential. Vitamin C is absorbed in the small intestine by sodium dependent vitamin C transporters (SVCTs) and is transported unbound in the bloodstream. Vitamin C is not stored in high amounts in the body and excess is excreted in the urine. Therefore, plasma vitamin C levels tend to reflect recent intake and are not necessarily a good measure of usual vitamin C intake. However, fasting plasma vitamin C can indicate short-term vitamin C status, especially at lower intakes. Plasma vitamin C levels are considered adequate when > 28 micromole/liter (μmol/L), suboptimal when 11-28 μmol/L and deficient when < 11μmol/L. Plasma vitamin C, however, is not strongly associated with vitamin C intake at higher levels of intake, especially through supplements, because a plateau in plasma vitamin C is achieved. The recommended dietary allowance (RDA) for vitamin C is 90 milligrams (mg) per day for males and 75 mg/day for females over 19 years of age. The RDA for smokers also calls for an additional 35 mg of vitamin C per day due to the oxidative stress caused by smoking.

Vitamin C is an essential cofactor for α-ketoglutarate-dependent dioxygenases such as prolyl hydroxylases, which are needed for the biosynthesis of collagen [103]. Vitamin C also provides protection against oxidative stress-induced cellular damage by acting as a water-soluble endogenous antioxidant. Vitamin C will readily donate an electron to potentially damaging oxidizing radicals such as hydroxyl radical [$HO^{\bullet}$], alkoxyl radical [$RO^{\bullet}$], peroxyl radical [$LOO^{\bullet}$], thiol radical [$GS^{\bullet}$], and tocopheroxyl radicals [$TO^{\bullet}$] [104]. The resulting ascorbate radical is relatively unreactive and can be reduced back to vitamin C by NADH/NADPH dependent reductases or pH-dependent disproportionate reactions.

The relationship between vitamin C and oral health has been known since the start of the twentieth century when vitamin C deficiency was determined to be the causative agent of scurvy [105]. Vitamin C may play a duel role in periodontal health. Firstly, because vitamin C is an antioxidant, it may help attenuate the oxidative stress associated with periodontitis. Secondly, because vitamin C is essential for collagen formation, it might promote healing and regeneration of soft tissues after periodontal procedures.

#### 1.2.1.1 Vitamin C and Periodontal Disease Risk

Data from NHANES III has shown a relationship between reduced dietary vitamin C intake and increased risk for periodontal disease in the general population [100]. The odds ratios (OR) for having periodontal disease (defined as having a mean CAL ≥ 1.5 mm) after adjusting for age, gender, smoking, and bleeding was 1.30, 1.26, 1.21 and 1.16 for subjects who consumed 0-29 mg, 30-59 mg, 60-99 mg, and 100-179 mg of dietary vitamin C per day respectively. Also, when vitamin C intake was used as a

continuous predictor, the odds ratio was 1.21 for current smokers, 1.28 for former smokers and 1.06 for non-smokers indicating a higher risk for smokers and former smokers with lower vitamin C intakes. To compare with dietary recommendations, increased periodontal disease risk was most apparent in individuals who had intakes well below the RDA (75 mg/day for females and 90 mg/day for males). Smokers would be even further from meeting the RDA as they require an additional 35 mg/day of vitamin C. Thus, risk seems to be conferred in this case by deficiencies in vitamin C intake. At risk individuals must have been consuming very few fruits and vegetables as the RDA can be met with a few commonly consumed fruits and vegetables (1/2 cup of orange juice has 66 mg, 1/2 a grapefruit has 47 mg, a medium potato has 27 mg and 1/2 cup of cooked peppers has > 100 mg) [106].

Three related studies have investigated the relationship between dietary vitamin C intake, serum ascorbic acid and periodontal disease risk in an elderly Japanese population [107-109]. Compared to individuals consuming 22-80 mg of vitamin C a day, those consuming 81-100 mg and 101-196 mg had incident rate ratios of 0.76 and 0.72, respectively, for periodontal disease progression (defined as having ≥ 3 mm of attachment loss over the two year study period) [109]. In the same population, there was also a small but significant correlation ($r = -0.23$) between fasting serum ascorbic acid levels and clinical attachment loss [107]. Similarly, when participants were classified into tertiles of ascorbic acid levels, those in the middle [5.9-8.9 micrograms (μg) per milliliter (mL)] and lowest (0.2 -5.9 μg/mL) tertiles had relative risks of 1.12 and 1.30 respectively for periodontal disease progression compared to those in the highest tertile (8.5-22.6 μg/mL) [108].

Another small study in Amsterdam found that plasma ascorbic acid was lower in patients with periodontitis compared to controls matched for age, gender, race and smoking habits (8.3 vs. 11.3 mg/l) [110]. They also observed an inverse relationship between polymorphonuclear neutrophilic leucocyte ascorbic acid levels and probing depth. Since the amount of vitamin C in neutrophils (as opposed to plasma levels) is associated with disease severity (probing depth), this suggests that the relationship between vitamin C and periodontal disease is at least in part mediated by the amount of vitamin C in these cells, which have a higher antioxidant demand due to the oxidizing environment they create during inflammatory processes.

#### 1.2.1.2 Human Intervention Studies for Periodontal Health

In a case-matched study, patients with gingivitis (and who were vitamin C sufficient) receiving professional cleaning were given 1000 mg of ascorbic acid daily for 6 weeks. No significant differences were observed in probing depth, attachment level, gingival inflammation, and plaque level [111]. The lack of effect may be due to the fact that patients were vitamin C sufficient at baseline or that patients with gingivitis do not have enough periodontal tissue destruction to see a benefit from extra vitamin C.

Two unique studies using chewing gum or whole food, however, have shown more promising results with vitamin C supplementation. In one study, healthy patients who tend to form calculus received 60 mg of vitamin C in sugar free chewing gum 5 times daily for 3 months [112]. Vitamin C gum chewers showed a reduction in the number of bleeding sites while the non-vitamin C chewers did not. In the other study, patients with chronic periodontitis were given 2 grapefruits daily for 2 weeks, which

resulted in increased serum vitamin C, a lower sulcus-bleeding index, but no difference in probing depth and plaque index [113]. The effects may be due to vitamin C but they could also be due to a number of other bioactive compounds found in grapefruit, which might have antioxidant activity [114].

#### 1.2.1.3 Human Intervention Studies for Periodontal Healing

In a recent study, patients undergoing non-surgical periodontal therapy were given 2000 mg of vitamin C for 4 weeks and then re-examined at 1 and 3 months post-treatment [115]. There were no significant differences in PD, CAL or BOP between the treatment and control group but this may have been due to a small sample size (n = 15), a short intervention period and because the authors failed to control for the baseline probing depth and clinical attachment loss of patients.

#### 1.2.1.4 *In Vitro* and Animal Experiments

Recent cell culture work has shown that vitamin C can induce periodontal ligament progenitor cells (a type of mesenchymal stem cell) to differentiate into osteoblasts (a bone forming cell) [116]. These findings have highlighted the potential use of ascorbic acid in regenerative medicine and dentistry. Although vitamin C has traditionally been thought of as a modulator of ROS and soft tissue formation, it may also play a role in hard tissue formation although we do not know the relevance of these findings *in vivo*.

One study in animals supports a role for vitamin C in promoting soft tissue health. During natural aging in Wistar rats, vitamin C supplementation (1g/L in drinking water)

increased the number of type 1 collagen fibres resulting in a thicker, healthier periodontal ligament at 14 months of age [117].

To summarize, vitamin C intake and vitamin C status have consistently been associated with risk for periodontal disease, while results from clinical trials are mixed. As vitamin C is an important antioxidant and essential cofactor, a plausible mechanistic link exists between this nutrient and periodontal disease risk and possibly periodontal healing. Vitamin C is therefore an excellent candidate to investigate as a potential modulator of healing following sanative therapy.

### 1.2.2 Vitamin D

Vitamin D is a fat-soluble vitamin that has been associated with numerous health outcomes. Vitamin D is synthesized in the skin upon expose to ultraviolet light. It is also obtained in the diet though few foods are high in vitamin D. Fatty fish, eggs, fortified milk and margarine are among the few foods that provide a significant amount of vitamin D. Vitamin D is transported through the blood stream primarily bound to the vitamin D binding protein and is carried to the liver where it is converted to 25-hydroxyvitamin D (25(OH)D). 25(OH)D has a half-life of about 3 weeks and is thus used as the standard biomarker of vitamin D status [118]. 25(OH)D eventually undergoes a second hydroxylation to 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], which is the active form of vitamin D. This activation step is performed by the enzyme 1$\alpha$-hydroxylase, which is expressed in many tissues including the kidneys, skin, lungs, breast, intestine, prostate, endocrine glands, cells of the immune system (macrophages, T cells, B cells, dendritic

cells), osteoblasts and chondrocytes [119]. The half-life of $1,25(OH)_2D$ is about 4 hours and its levels are regulated hormonally according to the calcium needs of the body. Vitamin D works by binding to nuclear receptors and modulating gene expression. The classical role of vitamin D is to regulate calcium levels by increasing intestinal absorption, kidney reabsorption and bone release of calcium. Vitamin D, along with calcium is therefore critical in the prevention of skeletal disorders such as rickets, osteomalacia and osteoporosis. Vitamin D metabolism is outlined in **Figure 1.2.2**.

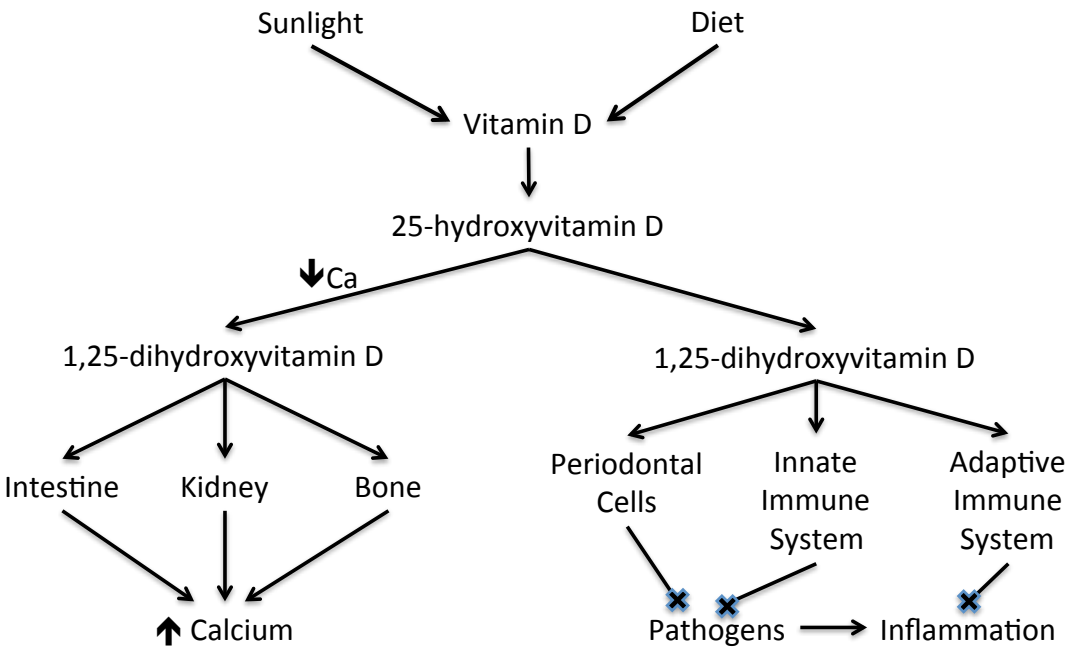


**Figure 1.5** Vitamin D metabolism.

The classical role of 1,25-dihydroxyvitamin D is depicted on the left side of the figure where 1,25-dihydroxyvitamin D is produced in response to low calcium resulting in increased intestinal absorption, kidney reabsorption and bone resorption. The right side of the figure depicts the immunomodulatory effects of 1,25-dihydroxyvitamin D. 1,25-dihydroxyvitamin D can enhance antibacterial activity in periodontal cells and innate immune cells, as well as inhibit inflammation from the adaptive the adaptive immune system. Lines ending with an arrow indicate a stimulatory effect while lines ending with an "X" indicate an inhibitory effect.

The RDA for vitamin D for males and females age 19-70 is 600 international units (IUs)/day and 800 IU/day for older individuals [120]. Health Canada states that most individuals have sufficient vitamin D when their serum 25(OH)D is 50 nmol/L [20 ng/mL] or greater. Symptoms of severe deficiency such as rickets and osteomalacia appear when serum 25(OH)D levels are below 30 nmol/L [12 ng/mL]. The exact cut-off for vitamin D sufficiency, however, is currently under debate. Many researchers believe that 25(OH)D levels should be at least 75 nmol/L (30ng/mL) to promote multiple health outcomes including fewer falls, better tooth attachment, less colorectal cancer, improved depression and wellbeing [121]. This debate largely stems from epidemiological evidence and will likely not be resolved until human clinical trials are completed. Nonetheless, some organizations such as the Canadian Cancer Society and Osteoporosis Canada recommend people take 1000 IU of vitamin D a day [122, 123].

Recent enthusiasm in vitamin D now stems from our understanding of its extra skeletal effects. For example, vitamin D has come to be known as an anti-inflammatory agent due to its ability to regulate both the innate and adaptive immune systems [124]. Vitamin D has several innate immune targets. Firstly, when bacterial pathogens are detected via toll like receptor on myeloid cells, there is an up regulation of 1$\alpha$-hydroxylase (the enzyme that converts 25(OH)D to 1,25$(OH)_2$D, which is the active form of vitamin D). Activated vitamin D has been shown to enhance antimicrobial activity by inducing expression of beta defensins [125] and cathelicidins [126] in cells such as monocytes and neutrophils. These molecules help eliminate bacterial pathogens by destabilizing their cell membranes. By enhancing the innate immune response to pathogens, they are more quickly eliminated, which reduces the amount of chronic

bacterial exposure. Another important innate target of vitamin D is the dendritic cell, a cell type that is crucial to the coordination of the adaptive immune system with the innate immune system. Vitamin D has been shown to enhance the tolerogenic properties of myeloid dendritic cells by allowing them to preferentially recruit regulatory T cells [127]. Vitamin D also has direct effects on the adaptive immune system by acting specifically on B and T cells. In B cells, vitamin D can inhibit the generation of memory and plasma B cells and promote the apoptosis of immunoglobulin-producing B cells [128]. T cells constitute a diverse population but in general vitamin D acts on T cells by inhibiting the production of pro-inflammatory Th1 cytokines (IL-2, INF-γ, TNF-α) and promoting the production of more anti-inflammatory Th2 cytokines (IL-3, IL-4, IL-5, IL-10) [129].

As the immunomodulatory effects of vitamin D have been well documented, vitamin D may play a duel role in periodontal health by enhancing the initial immune attack against pathogenic organisms and by modulating the immune response in order to minimize chronic inflammation. In addition, since the resolution of inflammation is vital to periodontal healing, vitamin D may also enhance healing after periodontal therapies by acting as an anti-inflammatory agent.

#### 1.2.2.1 Vitamin D and Periodontal Disease Risk

Two large cross sectional studies using NHANES III data have looked at the relationship between 25(OH)D and periodontal disease. The first found an inverse relationship between serum 25(OH)D and clinical attachment loss in men and women over 50 years of age [101]. Men with serum 25(OH)D levels 40.4-53.7 nmol/L and < 40.4 nmol/L had 0.23 mm and 0.39 mm more attachment loss respectively compared to

men with serum 25(OH)D ≥ 85.6 nmol/L. Women with serum 25(OH)D levels < 40.4 nmol/L had 0.26 mm more attachment loss compared to women with serum 25(OH)D ≥ 85.6 nmol/L. There was no relationship between attachment loss and serum 25(OH)D for subjects under 50 years of age. The fact that the relationship was apparent only in individuals over 50 years of age may be because periodontal disease is more prevalent in older populations. The second study found that subjects that have never smoked in the highest quintile of serum 25(OH)D (average 106 nmol/L) were 20% less likely to bleed on probing [130].

Two studies have also investigated the relation between 25(OH)D and periodontal disease risk specifically among postmenopausal women. The first found that postmenopausal women who were vitamin D sufficient (>50 nmol/L) had 33% lower odds of having periodontal disease [131]. The second study used NHANES III data to look at the interaction of hormone replacement therapy (HRT) and vitamin D [132]. They found decreased odds of moderate to severe periodontitis among HRT users who were vitamin D sufficient (>50 nmol/L). They hypothesize that vitamin D enhances the anti-inflammatory effects of estrogen and progesterone.

There are a couple of studies that investigated vitamin D intake in patients with periodontal disease. In the first, the use of calcium and vitamin D supplements was investigated among older patients (50 years of age and older) who had previously undergone periodontal treatment and were on maintenance therapy. Those who took calcium and vitamin D supplements (at least 1000 mg calcium and 400 IU vitamin D) daily for at least 18 months showed a trend for shallower probing depths, fewer bleeding sites, lower gingival index values, fewer furcation involvements, less attachment loss, and

less alveolar crest height loss compared to those not taking such supplements but the results were not significant [133].

The second study investigated vitamin D status in relation to healing after periodontal therapy. This study found that patients with severe periodontitis undergoing open flap debridement surgery who were vitamin D sufficient (>50 nmol/L) had better clinical attachment level gain and probing depth reduction [134]. Although these results are promising, the study had a very small sample size (n = 7) and the findings are compounded by the fact that more than half of the vitamin D deficient group were smokers. Study after study show that smokers have compromised healing after periodontal treatment resulting in less attachment gain and less probing depth reduction [33]. Therefore, the impaired healing seen in the vitamin D deficient group may be exaggerated due to the effects of smoking. These findings need to be replicated by analyzing smokers and non-smokers separately to determine if impaired healing is associated with vitamin D status rather than smoking status.

#### 1.2.2.2. Vitamin D Intervention Studies in Humans

Two vitamin D intervention studies whose primary outcome was to measure bone loss at non-oral sites (whole body, hip and spine) have provided valuable insight into the relationship between vitamin D, calcium and tooth loss. The first study investigated tooth loss in postmenopausal women. It was found that neither 400 IUs of vitamin D with 377 mg of calcium daily for 1 year or 700 IUs of vitamin D with 500 mg of calcium daily for 2 years could reduce the incidence of tooth loss [54]. However, a similar study where elderly subjects (≥ 65 years of age) received 700 IUs of vitamin D and 500 mg of calcium

daily for 3 years found there were lower odds of tooth loss in the treatment group but with no differences in probing depth [135]. These findings suggest a role for vitamin D in alveolar bone maintenance. To our knowledge, there are currently no intervention studies with vitamin D in patients with periodontal disease.

#### 1.2.2.3 *In Vitro* and Animal Experiments

In the last few years, advances in basic science have helped uncover a potential role for vitamin D in the periodontium. First, it is now known that gingival fibroblasts and periodontal ligament cells express 1α-hydroxylase, enabling them to locally generate the active form of vitamin D [136]. Notably, vitamin D has been shown to enhance the innate immune response to *Aggregatibacter actinomycetemcomitans* (a periodontal pathogen) by increasing the expression of innate immune genes such as LL-37, CD14, TREM-1 and IL-8 in human gingival epithelial cells [137]. In contrast though, vitamin D has been shown to decrease IL-8 expression in response to *Porphyromonas ginigivalis* bacteria in human periodontal ligament cells [138]. Based on the limited number of studies, it is clear that more research is needed to determine the direct effects of vitamin D on the periodontium.

Few studies have looked at vitamin D in animals with experimental periodontitis. One group studying diabetic mice with periodontitis have found that 25(OH)D injections lowered fasting glucose levels, glycosylated hemoglobin, serum TNF-α levels, and decrease alveolar bone loss [139, 140]. These findings have not yet been replicated in non-diabetic mice with periodontitis.

To date, there is potential for vitamin D to play a significant role in periodontal health. The associations between vitamin D and periodontal health have been well documented and there exists plausible mechanistic links showing its immune enhancing activity and anti-inflammatory activity. Based on the current literature, there is a need for more studies to determine if vitamin D can be useful for maintaining periodontal health and promoting healing after periodontal therapies.

### 1.2.3 Polyunsaturated Fatty Acids

Omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) are essential fats that must be obtained from the diet [141]. Linoleic acid (LA) is the primary omega-6 PUFA and α-linolenic acid (ALA) is the primary omega-3 PUFA obtained in the diet. The RDA for LA is 17 g/day (males 19-50), 14 g/day (males 51+), 12 g/day (females 19-50) and 11 g/day (females 51+), while the RDA for ALA is 1.6 g/day for males 19+ and 1.1 g/day for females 19+. LA and ALA can be converted to longer chain fatty acids. LA can be converted to arachidonic acid (AA), while ALA can be converted to eicosapentanoic acid (EPA) and docosahexanoic acid (DHA). The conversion of ALA into EPA or DHA, however, is very low in humans ranging from 1% to 10% [142]. Substantial levels of EPA and DHA can only be obtained by eating fatty fish, certain seafood or taking dietary supplements. Currently, Health Canada does not have an RDA for EPA and DHA.

EPA and DHA have received a significant amount of attention due to a number of purported health benefits. They are most notably known for their cardio-protective effects: decreases in blood pressure, inflammation, triglycerides, insulin resistance, and improved vascular function [143]. However, EPA and DHA may be efficacious in

improving or preventing a number of health conditions such as obesity, metabolic syndrome [144], osteoporosis [145], rheumatoid arthritis [146] and cancer [147], although these relationships continue to be studied. Many of the health benefits of EPA and DHA are likely due to their ability to modulate inflammation. Long chain fatty acids are incorporated into cell membrane phospholipids and serve as precursors for eicosanoids. AA and EPA are converted by phospholipase A2, cyclooxygenase (COX) and lipooxygenase (LOX) to eicosanoids such as prostaglandins (PG), thromboxanes (TX), leukotrienes (LT) and hydroxyl-fatty acids [148]. In general, AA-derived eicosanoids are pro-inflammatory while those derived from omega-3 long chain fatty acids are less inflammatory (but not always). It has also been shown that EPA and DHA are precursors for a number of biologically active agents such as resolvins, protectins and mareins [149], which help resolve inflammation by inhibiting the production of inflammatory cytokines and decreasing leukocyte recruitment. Omega-6 and omega-3 PUFA metabolism is illustrated in **Figure 1.2.3**. Because omega-6 and omega-3 fatty acids are metabolized through similar pathways, the ratio of omega-6 to omega-3 may be an important determinant of inflammation. In today's Western diet, a ratio of omega-6 to omega-3 ratio of 15:1 may promote the development of a number of chronic diseases [150]. In contrast for example, a lower ratio of 4:1 has been associated with a 70% decrease in risk for total mortality from cardiovascular disease [151]. Current Health Canada recommendations for the ratio of omega-6 to omega-3 fatty acids is approximately 10:1, which falls between these two extremes.

There are exceptions to the general trend of omega-6 PUFA being pro-inflammatory. Gamma-linolenic acid (GLA), for example, is an omega-6 PUFA that is

thought to have anti-inflammatory properties. GLA is believed to compete with AA to form the less saturated prostaglandin E1 resulting in reduced production of AA derived metabolites [152]. *In vitro*, GLA has been shown to suppress superoxide generation [153] and inflammatory cytokine production [154].

Due to their anti-inflammatory properties, there exists a plausible link between omega-3 PUFA intake and periodontal disease. In fact, there are a number of studies demonstrating this relationship and there have even been a few successful intervention studies using these nutrients to manage periodontal disease.

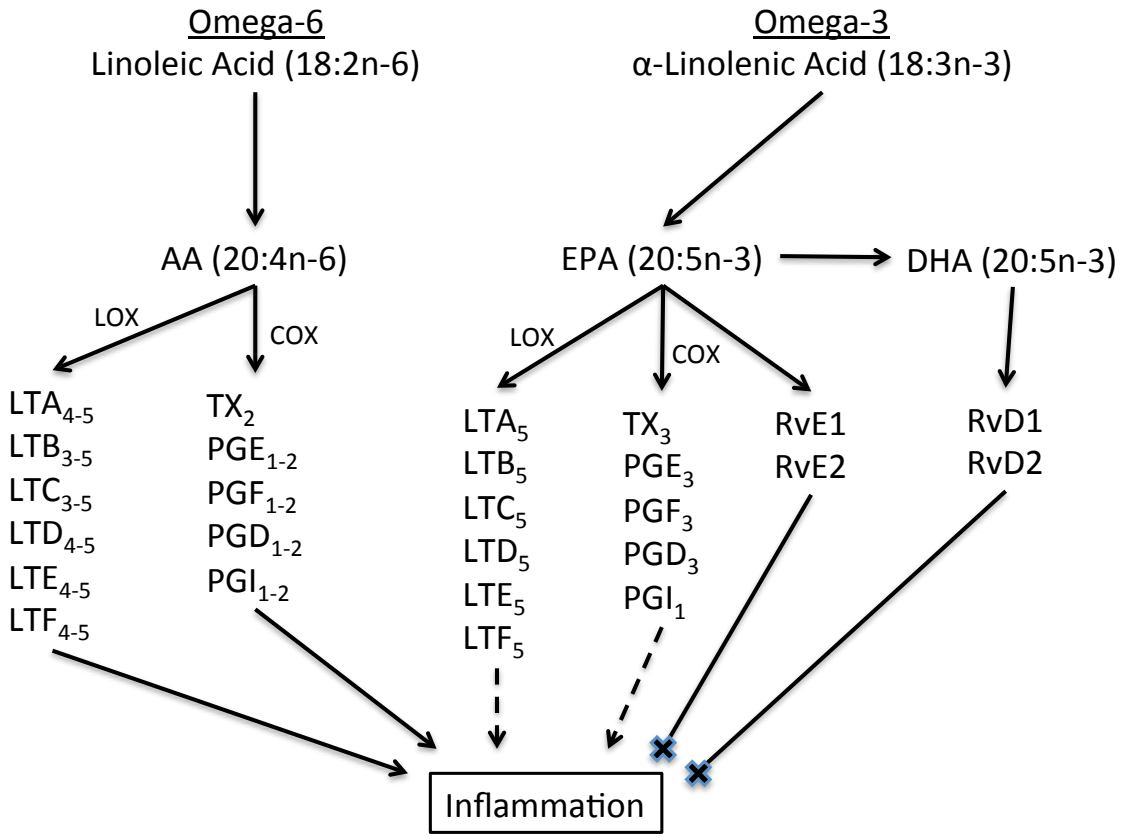


**Figure 1.6** Omega-6 and omega-3 PUFA metabolism (modified from Albertazzi and Coupland, 2002 [155]). Abbreviations: AA (arachidonic acid), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), LOX (lipooxygenase), COX (cyclooxygenase), LT (leukotriene), TX (thromboxane), PG (prostaglandin), Rv (resolvin). A line ending in an arrow indicates a stimulatory effect while a line ending in an "X" indicates an inhibitory effect. A hatched line indicates a weaker effect compared to a solid line.

#### 1.2.3.1 PUFAs and Periodontal Disease Risk

Data from NHANES III has been used to examine the relationship between omega-3 PUFAs and periodontal disease risk. It was found that subjects who consumed 0-40 mg/day and > 40 mg/day of DHA had 0.80 and 0.69 odds ratio, respectively, of having periodontal disease (defined as > 4mm pocket depth or > 3mm attachment loss in

any one tooth) compared to those who did not consume any DHA [102]. However, there were no associations with EPA or ALA intake. Interestingly, the associations remained consistent even when those taking PUFA supplements were excluded from the analysis.

A couple of prospective studies have investigated PUFA intake in relation to periodontal disease in an elderly Japanese population. In one study, those in the lowest tertile of DHA intake had increased incidence of periodontal progression (incidence rate ratio = 1.49) but no associations were found with EPA [156]. A second study found that those in the highest tertile of total omega-6 fatty acids compared to total omega-3 fatty acids intake (ratios 4.02:1 – 5.67:1) were at a greater risk of periodontal disease events (measured as number of sites with CAL >3 mm) [157]. The highest tertile of omega-6/omega-3 in this study is still quite low compared to North American diets (~15:1) making it difficult to know if these findings could be replicated in a North American population.

#### 1.2.3.2 PUFA Intervention Studies in Humans

To date, only a couple of small intervention studies have been performed in humans, but with promising results. In a pilot study, adults with periodontitis received 3 g of fish oil (~ 390 mg EPA and 540 mg DHA), or 3 g of borage oil (~ 600 mg GLA, an omega-6 PUFA) or 1.5 g of each for 12 weeks. Interestingly, improvements in probing depth and gingival inflammation were seen in the borage oil group, while only a trend for improvement was seen in the fish oil and combination groups [158]. These findings are quite impressive as the sample size was quite small (n = 5-7) and improvements were seen without the use of adjunctive periodontal therapy.

In another promising study, patients undergoing sanative therapy were supplemented with fish oil (900 mg/day EPA + DHA) and low dose aspirin (81 mg/day) for six months. The treatment group had significantly fewer deep pockets at 3 and 6 months compared to the control group and significantly lower levels of salivary RANKL and MMP-8 at 6 months [159]. It will be interesting to see if these results can replicated using fish oil alone without low dose aspirin.

#### 1.2.3.3 *In Vitro* and Animal Studies

Diets rich in omega-3 PUFA have been found to be beneficial in animals with experimental periodontitis. A fish oil diet reduces expression of IL-1β and TNF-α and increases expression of antioxidant enzymes (catalase and superoxide dismutase) in gingival tissue of rats treated with *Porphyromonas gingivalis* [160]. Administration of EPA and DHA has also been shown to inhibit MMP-8 expression in gingival tissue of rats treated with *E.coli* endotoxin [161]. Furthermore, omega-3 PUFA rich diets have been shown to decrease alveolar bone loss in both rat [162] and mouse [163] models of periodontitis.

One group has been able to demonstrate direct effects of omega-3 PUFA metabolites in periodontal biopsies [164]. They have shown that treatment of periodontal ligament cells and monocytes with resolvin D1, a downstream metabolite of DHA, can inhibit production of prostaglandin E2 and up regulate production of lipoxin A4. This ultimately resulted in increased periodontal ligament fibroblast proliferation and wound closure.

In summary, intakes of EPA and DHA show promise as potential modulators of periodontal healing. Like vitamin C and vitamin D, intakes of long chain omega-3 PUFA have been associated with reduced risk of periodontal disease. EPA and DHA intervention studies, though only pilot studies, also show promising results. Mechanistic studies suggest that EPA and DHA modulate healing, as they are required for the resolution of inflammation. Their use to optimize periodontal outcomes after sanative therapy warrants further investigation.

### 1.3 Summary and Rationale

All of the available studies linking vitamin C, vitamin D and omega-3 PUFAs to periodontal health and periodontal healing are summarized in **Table 1.2**. There exists a substantial amount of evidence demonstrating a link between lower risk of periodontal disease and higher intakes of vitamin C, vitamin D and DHA. It is clear from the epidemiological studies that higher intakes of these nutrients are associated with decreased risk of periodontitis. Interestingly, protective levels are often higher than current recommendations. For example, in the NHANES studies, vitamin C intakes >180 mg/day (compared to the RDA of 75 or 90 mg/day) were the most protective [100]. Similarly, serum 25(OH)D levels ≥ 85 nmol/L (compared to Health Canada's 50 nmol/L cut-off) were the most protective [101]. Fortunately since higher DHA intakes (≥ 0.04 g/day) have been associated with reduced incidence of periodontal disease [102], Canada's Food Guide recommendation to consume two servings of fish per week [165] should easily help individuals meet these levels. For example, two 75.0 g servings of salmon (approximately the size and thickness of the palm of a hand) a week would

provide approximately 2 g of DHA resulting in an average daily intake of approximately 0.30 g/day.

Despite these links, very few studies, if any, have properly investigated the relationship between vitamin C, vitamin D, omega-3 PUFAs and periodontal outcomes after sanative therapy. There is the study by Bashutski et al. [134] showing improved healing when patients are vitamin D sufficient. However, study design issues such as small sample size (n = 7) and lack of control for important confounders (i.e. smoking) casts doubt on the significance of these findings.

There is also currently a lack of clinical intervention studies with vitamin C, vitamin D and omega-3 PUFAs in patients undergoing periodontal disease treatment. Only one intervention study with vitamin C has been reported and it did not find any benefit in PD, CAL or BOP after sanative therapy [115]. However, this study had a small sample size (n = 15), a short follow-up time (1 month) and most importantly, failed to control for outcomes at baseline. There are currently no intervention studies with vitamin D. There is also only one study looking at omega-3 PUFA supplementation in conjunction with sanative therapy [159]. Although this study had positive results, the omega-3 PUFAs were administered with low dose aspirin and therefore we do not know the unique impact of the omega-3 PUFAs.

Nonetheless, the animal and cell culture studies do suggest that these nutrients will be able to promote periodontal health and improve healing after periodontal therapy. Vitamin C may reduce oxidative stress and promote collagen synthesis in order to repair the periodontal ligament. Meanwhile, vitamin D and omega-3 PUFAs may help resolve inflammation in order to allow healing to occur. The potential mechanisms by which they

might optimize periodontal healing are illustrated in **Figure 1.3**. These hypothesized mechanisms are based on the idea that they will promote healing by tilting the balance between destruction and repair toward the repair processes. By helping to fight infection and minimize chronic inflammation, they help reduce the amount of periodontal destruction. Similarly, by reducing inflammation, they may allow healing to proceed because healing cannot take place as long as inflammatory destruction is still occurring. Lastly, nutrients like vitamin C and D may further favor a balance toward healing by enhancing the regenerative processes of soft and hard tissue formation.

Due to the potential of vitamin C, vitamin D and omega-3 PUFAs to help resolve periodontitis and promote healing after sanative therapy, we propose a study to investigate the relationship between intakes of these nutrients and periodontal status, specifically PD, after sanative therapy.

**Table 1.2** Summary of available studies. The numbers of studies in each area of research are listed along with the main findings of the studies.

| | **Vitamin C** | **Vitamin D** | **EPA and DHA** |
|---|---|---|---|
| **Association Studies** | - 5 studies<br>(↑ Risk of having periodontal disease with low intakes of vitamin C and low ascorbic acid levels) | - 4 studies<br>(↑ Risk for periodontal disease with lower serum 25(OH) D)<br>- 1 study on suppl. use in periodontal maintenance patients (trend for ↓PD, CAL, BOP) | - 2 studies investigating EPA, DHA and Periodontal Disease (↓ risk with ↑DHA intake but not with EPA)<br>- 1 PUFA study (↓ risk of periodontal disease with ↓ n-6: n-3 ratio) |
| **Association Studies with Sanative Therapy** | - None | - 1 study in patients undergoing open flap debridement (patients with 25(OH)D ≥ 50 nmol had ↑ PD and CAL reduction) | - None |
| **Intervention Studies** | - 2 studies, one with vitamin C chewing gum and one with grapefruit<br>(Both showed ↓ BOP) | -3 tooth loss studies (one found that individuals ≥ 65 taking 700IU vitamin D + 500mg calcium had ↓ risk of tooth loss) | - 1 study where periodontal patients received 3000 mg/d of fish oil for 12 weeks (trend for ↓PD) |
| **Intervention Studies with Sanative Therapy** | - 1 study with 1000 mg/d in patients with gingivitis<br>- 1 study with 2000 mg/d in patients with periodontitis<br>(No effects but poor study designs) | - None | - 1 study with fish oil (900 mg EPA and DHA) + low dose aspirin (↓ PD and ↓ salivary RANKL and MMP-8) |
| **Preclinical and Mechanistic: Animal Studies** | - 1 study with vitamin C in drinking water (↑ periodontal ligament collagen fibers in very old rats) | - 2 studies treating diabetic mice with 25(OH)D injections (↓ HbA1c, ↓ serum TNF-α and ↓ alveolar bone loss) | - 4 studies with omega-3 rich diets in rodents with experimental periodontitis (↓ gingival TNFα, IL-1β and MMP-8, ↓ alveolar bone loss) |
| **Preclinical and Mechanistic: Cell Culture Studies** | - 1 study treating periodontal ligament progenitor cells with vitamin C (Induced differentiation into osteoblasts) | - 1 study showing presence of 1α-hydroxylase in periodontal cells<br>- 2 studies treating periodontal cells with vitamin D (enhanced response to pathogens) | - 1 study treating periodontal ligament cells with resolvin D1 (↓ inflammation, ↑ fibroblast proliferation and closure of experimental wounds) |

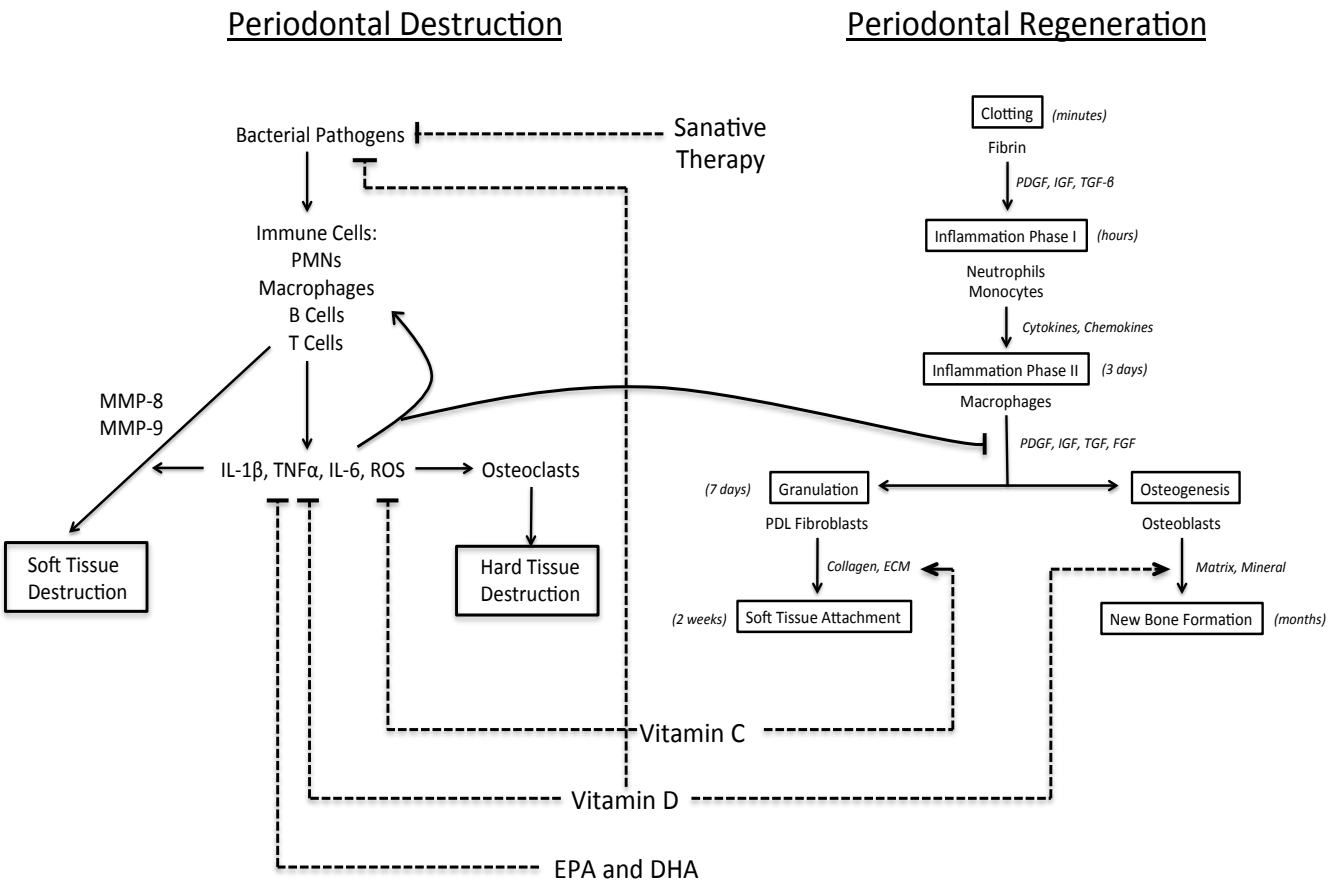


**Figure 1.7** Potential mechanisms for improved periodontal healing after sanative therapy with vitamin C, vitamin D, EPA and DHA by inhibition of periodontal destruction and promotion of periodontal regeneration. On the left is a replicate of figure 1.3 depicting periodontal destruction and on the right is a replicate of figure 1.4 depicting periodontal regeneration.. Processes (e.g. soft/hard tissue destruction/formation) are enclosed in a box while cells/mediators are not enclosed. A line with an arrow indicates a stimulatory effect while lines ending in a bar indicate an inhibitory effect. A hatched line indicates intervention by sanative therapy and nutrition. Sanative therapy inhibits bacterial pathogens (by mechanically removing them) on the destruction side of the figure. Vitamin C inhibits reactive oxygen species on the destruction side and promotes collagen synthesis on the regeneration side. Vitamin D inhibits inflammation and periodontal pathogens on the destruction side and promotes bone formation on the regeneration side. EPA and DHA inhibits inflammation on the destruction side.

# Chapter Two

# OBJECTIVES AND HYPOTHESES

## 2.1 Objectives

The main objectives of this study were to determine:

1. if higher intakes of vitamin C, vitamin D, EPA and DHA, either through diet and/or supplement use, are associated with improved periodontal outcomes after sanative therapy;
2. if higher serum 25-hydroxyvitamin D levels are associated with a greater reduction in probing depth after sanative therapy. A biomarker for vitamin D was deemed necessary in order to account for endogenous vitamin D synthesis from sunlight exposure (a phenomenon that does not occur for other nutrients).

## 2.2 Hypotheses

1. Higher intakes of vitamin C, vitamin D, EPA, DHA, either through diet and/or supplement use, will be associated with a greater reduction in probing depth eight weeks after sanative therapy.
2. Higher serum 25-hydroxyvitamin D levels will be associated with a greater reduction in probing depth eight weeks after sanative therapy.

# Chapter Three

# MATERIALS AND METHODS

### 3.1 Experimental Design

This study was approved by the Human Bioscience Research Ethics Board at Brock University, St. Catharines, Ontario (File #12-068-Ward, **Appendix 8.1**). The study was conducted at a periodontal clinic in southern Ontario. All patients who had sanative therapy prescribed as part of their treatment plan were invited to participate in the study. Patients had moderate to severe periodontitis, defined as having ≥ 2 interproximal sites with PD ≥ 5 mm. No other exclusion criteria were applied. The study design is shown in **Figure 3**. Prior to enrollment, patients attended a consultation where a baseline periodontal examination was completed. Patients then returned approximately 2 months later for sanative therapy at which point they were enrolled in the study. Patients were given a letter of invitation (**Appendix 8.2**) and they signed a consent form (**Appendix 8.3**). During the appointment where sanative therapy was performed, a blood sample was taken and body weight, height and waist and hip circumferences were measured. Patients were then given a food frequency questionnaire, a supplement use questionnaire and a physical activity questionnaire to complete at home. The physical activity questionnaire was administered because low levels of physical activity have been associated with periodontal disease and therefore physical activity is a potential confounder of the relationship between diet and periodontal healing. Patients returned to the clinic approximately 8 weeks later for their re-exam. During their re-exam, a periodontal examination was performed, a blood sample was taken and body weight, height and waist and hip circumferences were measured. Participants were later provided with a personalized report containing the result of their vitamin D and dietary analyses (**Appendix 8.8**).

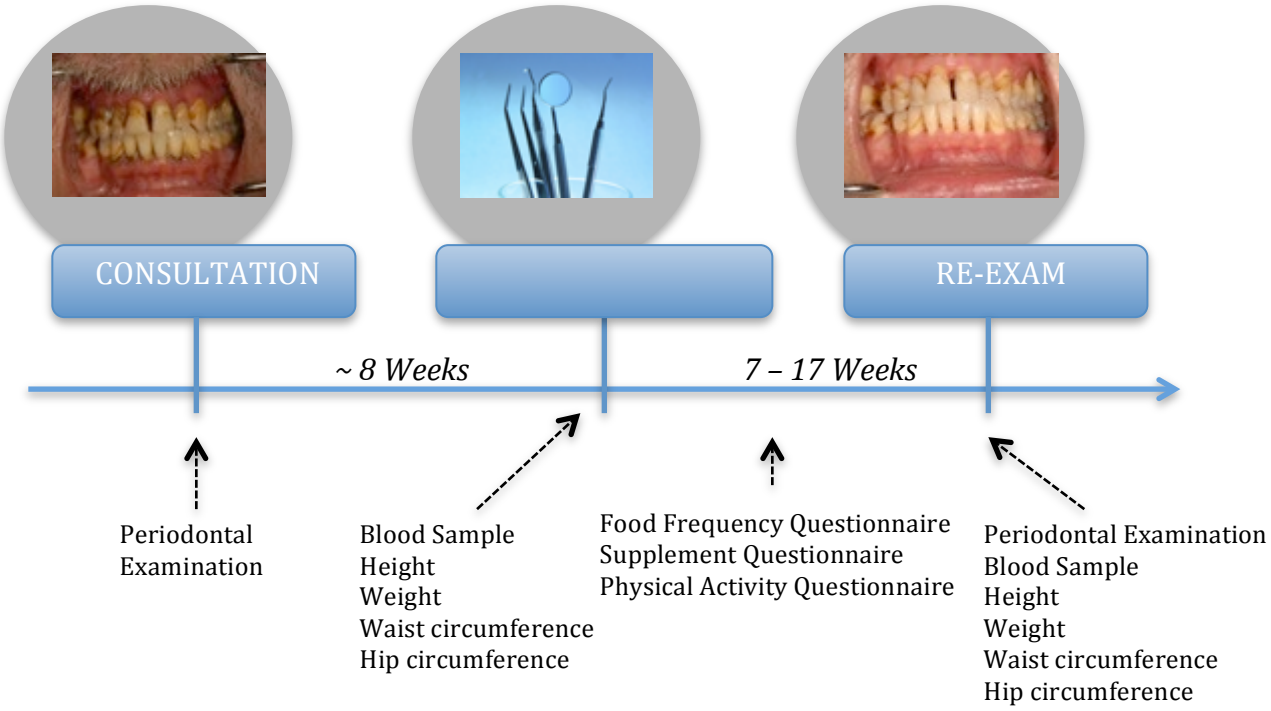


**Figure 3.1** Experimental outline

### 3.2 Periodontal Examination

The baseline periodontal examination was completed by one periodontist and the re-exam periodontal examination was completed by one of four calibrated hygienists. Prior to the study, the hygienists were calibrated for probing pressure. Probing pressure was simulated by having each hygienist probing against an electronic scale to ensure that they each probed with a pressure of 0.25 Newtons. The examination included measurement of probing depth (PD), bleeding on probing (BOP), and plaque score. PD was measured using a UNC15 Probe (Hu-Friedy) at six sites per tooth (mesial buccal, buccal, distal buccal, mesial lingual, lingual and distal lingual) and rounded to the nearest millimeter. The PD of all healthy sites was estimated as 3 mm since the clinicians did not

routinely record the PD of healthy sites. Subsequently, an average PD was calculated for the whole mouth including healthy sites, which is standard in the literature. BOP was assessed by visual inspection after probing and is expressed as % sites with bleeding. Plaque score was assessed by visual inspection and is expressed as % teeth with visible plaque.

### 3.3 Nutrient Intake Estimates

To estimate daily nutrient intakes from diet, patients completed the 2005 Block Food Frequency Questionnaire (FFQ) at home and returned the questionnaire at their re-exam (**Appendix 8.4**). The Block FFQ is a commonly used tool to estimate usual dietary intakes [166-168]. The 2005 Block FFQ queries 110 food items selected from the United States National Health and Nutrition Examination Survey (NHANES). Respondents are asked how often each item is consumed and to specify the portion size. Pictures are provided to enhance the accuracy of quantification. A series of "adjustment" questions are also asked to provide greater accuracy in assessing fat and carbohydrate intake [169]. The adjustment questions, for example, ask respondents whether or not they consume low fat or low carbohydrate options for foods where low fat and low carbohydrate options are commonly available. Nutrient intakes are then quantitatively assessed using a nutrient database developed from the U.S. Department of Agriculture Food and Nutrient Database for Dietary Studies [170]. A FFQ was used in lieu of other methods (such as a diet record) in order to minimize patient burden.

Energy-adjusted nutrient intakes were then calculated using the residual method [171]. The use of energy-adjusted nutrient intakes has become standard in

epidemiological research because it minimizes reporting errors and it isolates the effect of a nutrient or food independent of energy intake [172, 173]. Because nutrient intakes are highly correlated to total energy intake, errors due to over and under reporting can be minimized by standardizing nutrient intakes to one level of energy intake. The residual method involves performing a regression to predict nutrient intakes from total energy intake. The residuals (difference between raw nutrient intakes and predicted nutrient intakes) can then be added to a constant (the nutrient intake predicated from the mean total energy intake) to derive energy-adjusted nutrient intakes. In this data set, nutrient intakes are standardized to 2000 kilocalories (Kcal).

Patients also completed a supplement use questionnaire (**Appendix 8.5**). From a list, they were asked to check any supplements they use with an option to record supplements not on the list. They were also asked to record the dose, brand, frequency and duration of use. The nutrient composition of each supplement taken (including multivitamins) was recorded. If the exact brand was not reported, the most commonly used brand was used.

### 3.4 Blood Collection

A study nurse collected blood by venipuncture before sanative therapy and at the re-exam. 5 mL of blood was collected into a glass red top serum vacutainer tube with no additive (BD-Canada, Mississauga, ON). Tubes were placed in a refrigerator (2-8°C) and allowed to clot for at least an hour. Blood was centrifuged at room temperature in a table top centrifuge (Hettich EBA 270) for 15 minutes at 1,268 x *g*. Serum was collected in 200 microliter (μL) and 600 μL aliquots and stored at -80°C.

### 3.5 Vitamin D Status

Frozen baseline serum samples were delivered to Life Labs Medical Laboratory Services (St. Catharines, ON). Serum 25-hydroxyvitamin D levels were measured according to their standardized medical laboratory procedures using the Liaison chemiluminescence system (DiaSorin, Mississauga, ON). The Liaison system is currently used in many clinical and research laboratories [174-176].

### 3.6 Patient Information

Patient information including age, gender, smoking status (recorded as never, former or current), allergies, health conditions and medication use were collected from the patient's medical history form (**Appendix 8.6**).

### 3.7 Anthropometry and Physical Activity

Body dimensions and physical activity were measured, as they may be potential confounders. Before sanative therapy and at the re-exam, patients met with a study nurse to have their height and weight (Health o Meter Professional) measured without shoes in order to calculate their BMI. Patients had their waist circumference (measured at the top of the iliac crest) and their hip circumference (measured at the widest part of the hips) measured with a non-stretchable measuring tape in order to calculate their waist-to-hip ratio. Patients also completed the Godin Leisure-Time Exercise Questionnaire (**Appendix 8.7**), a validated tool that measures leisure time exercise behavior [177]. Among BMI, waist-to-hip ratio and physical activity, waist-to-hip ratio was found to be the best

predictor of periodontal disease outcomes (data not shown) and thus was the only one of the three included as a covariate in regression analyses.

### 3.8 Statistical Analysis

The relationship between dietary intakes and probing depth was investigated using hierarchical multiple linear regression (MLR). Dietary intakes of vitamin C, EPA and DHA and serum 25(OH)D were each used as continuous predictors and re-exam PD was used as a continuous outcome.

Before investigating the effects of diet, a basic model was created to evaluate periodontal healing (**Table 3.1**). Baseline PD was included in the model because we were interested in the response to sanative therapy and therefore needed variation due to differences in PD before sanative therapy removed. Large effect size clinical variables that might obscure the effects of diet (smoking status, follow-up time and hygienist performing sanative therapy) were then included in the model to determine if they would need to be controlled for. Baseline PD, current smoking, follow-up time, and hygienist performing sanative therapy were all significant predictors of re-exam PD ($p < 0.05$). In order to adjust for these effects, current smokers (n = 19) were excluded from further analyses since smoking undoubtedly reduces a patient's response to sanative therapy [33] and because smokers have different nutritional requirements than non-smokers [178]. Ideally, stratified analyses would have been performed, however, there was an insufficient number of current smokers to do that. In order to minimize the effect of follow-up time, participants who took longer than four months to return for their re-exam were excluded (n = 4). This was a reasonable cut-off point as relapse of periodontal

disease begins within less than half a year [179]. Thus, too late of a re-examination would not be an accurate assessment of the initial response to sanative therapy. Finally, as there were differences in PD based on which hygienist performed sanative therapy, control for hygienist was included in statistical models. All MLR models were therefore adjusted for baseline PD, follow-up time and hygienist before looking for associations with diet variables.

For each dietary predictor, a second MLR model was created, which further adjusted for age (years), sex (male or female), waist-to-hip ratio, former smoking (yes or no), plaque (% teeth), and dietary supplement use (yes or no) in order to control for possible confounders. Although other possible confounders exist such as health conditions, medication use and allergies, they were not controlled for because we set a limit of ten predictors in our regression models because we had a modest sample size and wanted to avoid over-parameterization of the data and loss of power. Values reported are: $R^2$ (amount of variance explained by the model), $R^2_{Adj}$ (generalizability of the model to other populations), $\Delta R^2$ (amount of unique variance explained by the predictor of interest), B (regression coefficient: change in outcome for each change in unit of the predictor) and $\beta$ (standardized regression coefficient to compare the effect size of different predictors). Predictors and outcomes were verified for normality. An outcome was considered normally distributed if its skewness was less than 3.29 [180]. Non-normal data was transformed into normal scores using Blom's rank-based normalization formula. Models were also verified for homoscedasticity, independence of errors (Durbin-Watson $> 1$ and $< 3$), the absence of mullticollinearity (tolerance $> 0.2$ and variance inflation factor $<< 10$) and the absence of multivariate outliers using Mahalanobis distance.

Dietary supplement use was investigated using analysis of covariance (ANCOVA). Individuals who took various types of supplements (any supplement, multivitamin, vitamin C, vitamin D and omega-3) were compared to a control group (individuals who did not report taking any supplements). Re-exam PD was used as a continuous outcome and baseline PD, follow-up time and hygienist were used as covariates in the model. Adjusted means ± stander errors of the mean (SEM) and $\eta_p^2$ (a measure of effect size) are reported. The statistical assumptions of homogeneity of variance, univariate normal distribution, independence of covariate and treatment, and homogeneity of regression were verified.

All statistical procedures were performed using SPSS version 20. Statistical significance was defined as $p < 0.05$.

**Table 3.1** Model for predicting re-exam PD

| | ***B* ± *SE* (mm)** | ***β*** | ***P* value** |
|---|---|---|---|
| Baseline PD (mm) | 0.19 ± 0.02 | 0.65 | < 0.001 |
| Follow-up time (months) | 0.03 ± 0.01 | 0.17 | 0.008 |
| Dental hygienist | | | |
| Hygienist 1 (*n* = 19) | Reference | Reference | - |
| Hygienist 2 (*n* = 31) | - 0.07 ± 0.03 | - 0.16 | 0.034 |
| Hygienist 3 (*n* = 21) | 0.01 ± 0.04 | 0.02 | NS |
| Hygienist 4 (*n* = 8) | - 0.03 ± 0.05 | - 0.05 | NS |
| Smoking status | | | |
| Never (*n* = 32) | Reference | Reference | - |
| Former (*n* = 28) | 0.03 ± 0.03 | 0.06 | NS |
| Current (*n* = 19) | 0.13 ± 0.04 | 0.28 | < 0.001 |

$R^2 = 0.72$, $R^2_{Adj} = 0.69$, $p < 0.001$

**Table 3.2** Variables used in each statistical model

| Model 1 | Model 2 |
|---|---|
| Baseline probing depth (mm) | Baseline probing depth (mm) |
| Hygienist (1,2,3,4) | Hygienist (1,2,3,4) |
| Follow-up time (days) | Follow-up time (days) |
| | Age (years) |
| | Sex (male or female) |
| | Waist-to-hip ratio |
| | Former smoker (yes or no) |
| | Plaque score (% teeth) |
| | Dietary supplement use (yes or no) |

# Chapter Four

# RESULTS

In total, 91 patients participated in the study. Of those, 12 did not return their food frequency questionnaire and were thus excluded. Current smokers (n = 19) and those who took longer than 4 months to return for their re-exam (n = 4) were excluded leaving of final sample size of 56. The participant characteristics, clinical outcomes and dietary intakes are described in **Table 4.1**.

**Table 4.1** Participant characteristics, clinical outcomes and dietary intakes

| | |
|---|---|
| Age (years) | 59 ± 13 (23 – 90) |
| Sex [n (%)] | |
| Males | 26 (46) |
| Females | 30 (54) |
| BMI [n (%)] | |
| Normal (18.5 – 24.9) | 9 (16) |
| Overweight (25.0 – 29.9) | 30 (54) |
| Obese (30.0 +) | 17 (30) |
| Waist-to-hip ratio - Males | 0.93 ± 0.06 (0.78 – 1.04) |
| Waist-to-hip ratio - Females | 0.84 ± 0.06 (0.72 – 0.95) |
| Smoking Status [n (%)] | |
| Never smoked | 30 (54) |
| Former smokers | 26 (46) |
| Diabetics [n (%)] | 5 (9) |
| Baseline Clinical Measures | |
| No. Teeth | 24 ± 4 (12 – 32) |
| Probing depth (mm) | 3.96 ± 0.63 (3.07 – 5.80) |
| Sites 4-6 mm (n) | 75 ± 33 (7 – 139) |
| Sites > 7 mm (n) | 8 ± 12 (0 – 53) |
| Bleeding on probing (%) | 47 ± 29 (0 – 100) |
| Plaque score | 72 ± 28 (10 – 100) |
| Re-Exam Clinical Measures | |
| Probing depth (mm) | 3.12 ± 0.11 (3.00 – 3.41) |
| Sites 4-6 mm (n) | 12 ± 10 (0 – 40) |
| Sites > 7 mm (n) | 0 ± 0.5 (0 – 2) |
| Bleeding on probing (%) | 4 ± 5 (0 – 23) |
| Plaque score | 32 ± 22 (0 – 100) |
| Follow-up time (days) | 74 ± 15 (50 – 116) |
| Nutrient Intakes | |
| Total energy (Kcal) | 1550 ± 600 (719 – 3158) |
| Dietary vitamin C (mg) | 135 ± 57 (37 – 352) |
| Total vitamin C (mg) | 318 ± 364 (69 – 2172) |
| Dietary vitamin D (IU) | 181 ± 112 (29 – 707) |
| Total vitamin D (IU) | 1047 ± 1211 (31 – 6705) |
| Dietary EPA (mg) | 39 ± 32 (0 – 172) |
| Total EPA (mg) | 95 ± 176 (0 – 944) |
| Dietary DHA (mg) | 61 ± 45 (0 – 244) |
| Total DHA (mg) | 97 ± 120 (0 – 659) |
| 25-hydroxyvitamin D (nmol/L) | 66 ± 24 (13 – 118) |
| Daily dietary supplement use [n (%)] | |
| No supplements | 19 (34) |
| Any supplement | 37 (66) |
| Multivitamin | 20 (36) |
| Vitamin C (≥ 500 mg/day) | 12 (21) |
| Vitamin D (≥ 1000 IU/day) | 22 (39) |
| Fish Oil | 10 (14) |
| Omega 3/6/9 | 2 (4) |

Data are expressed as mean ± SD (range) for continuous variables and counts (%) for categorical variables.

The regression analyses by dietary intakes and total intakes (diet + supplements) of vitamin C, vitamin D, EPA and DHA, as well as serum 25(OH)D concentrations are summarized in **Table 4.2**. Dietary intakes of nutrients and serum 25(OH)D were normally distributed while total intakes were positively skewed and therefore transformed into normal scores.

**Vitamin C Intake**

Hierarchical MLR was performed to predict re-exam PD using vitamin C intake as a continuous predictor. After controlling for baseline PD, hygienist and follow-up time, there was a trend for lower re-exam PD with higher dietary intakes of vitamin C but it did not reach statistical significance ($\Delta R^2 = 0.03$, $\beta = -0.18$, $p = 0.07$). The strength of the association remained consistent after adjustment for baseline PD, hygienist, follow-up time plus potential confounders including age, sex, waist-to-hip ratio, former smoking, plaque score, and dietary supplement use ($\Delta R^2 = 0.03$, $\beta = -0.18$, $p = 0.07$). There were no significant associations with total (diet + supplement) intake of vitamin C.

**Vitamin D Intake and Serum 25(OH)D**

Hierarchical MLR was performed to predict re-exam PD using vitamin D intake as a continuous predictor. After controlling for baseline PD, hygienist and follow-up time, there were no significant associations with either dietary or total intake of vitamin D and re-exam PD. Similarly after controlling for baseline PD, hygienist, follow-up time plus potential confounders including age, sex, waist-to-hip ratio, former smoking, plaque

score, and dietary supplement use, there were no significant associations with either dietary or total intake of vitamin D and re-exam PD.

The average serum 25(OH)D was 66 ± 24 nmol/L and ranged from 13 – 118 nmol/L. 82% of the population had serum 25(OH)D ≥ 50 nmol/L and 29% had serum 25(OH)D ≥ 75 nmol/L. Serum 25(OH)D was not significantly associated with dietary intake of vitamin D ($r = 0.01$, $p = 0.92$) or total (diet + supplemental) intake of vitamin D ($r = 0.21$, $p = 0.12$). Vitamin D supplement users (dose ≥ 1000 IU/day) had a higher serum 25(OH)D compared to non-users (78 ± 24 nmol/L vs. 59 ± 21 nmol/L, $t = -3.15$, $p = 0.003$). Hierarchical MLR was performed to predict re-exam PD using serum 25(OH)D as a continuous predictor. After controlling for baseline PD, hygienist and follow-up time, there was no significant associations with 25(OH)D and re-exam PD. Similarly after controlling for baseline PD, hygienist, follow-up time plus potential confounders including age, sex, waist-to-hip ratio, former smoking, plaque score, and dietary supplement use, there was no significant associations with serum 25(OH)D and re-exam PD.

**EPA and DHA Intake**

After controlling for baseline PD, hygienist and follow-up time, there were no significant associations between re-exam PD and any of dietary EPA, total EPA, dietary DHA or total DHA. This remained consistent when the models were adjusted for baseline PD, hygienist, follow-up time plus potential confounders including age, sex, waist-to-hip ratio, former smoking, plaque score, and dietary supplement use.

**Table 4.2** Regression of re-exam PD (mm) by intakes of vitamin C, D, EPA and DHA

| | $R^2$ | $R^2_{adj}$ | $\Delta R^2$ | $B \pm SE$ (mm) | $\beta$ | $P$ value |
|---|---|---|---|---|---|---|
| Vitamin C - DIET (100 mg) | | | | | | |
| Model 1 | 0.62 | 0.57 | 0.03 | - 0.03 ± 0.02 | - 0.18 | 0.07 |
| Model 2 | 0.64 | 0.54 | 0.03 | - 0.04 ± 0.02 | - 0.18 | 0.07 |
| Vitamin C – DIET + SUPP | | | | | | |
| Model 1 | 0.59 | 0.54 | 0.00 | - 0.01 ± 0.01 | - 0.05 | NS |
| Model 2 | 0.61 | 0.50 | 0.00 | - 0.01 ± 0.01 | - 0.05 | NS |
| Vitamin D – DIET (100 IU) | | | | | | |
| Model 1 | 0.59 | 0.54 | 0.00 | 0.00 ± 0.01 | 0.04 | NS |
| Model 2 | 0.61 | 0.50 | 0.00 | 0.00 ± 0.01 | 0.03 | NS |
| Vitamin D – DIET + SUPP | | | | | | |
| Model 1 | 0.59 | 0.54 | 0.00 | - 0.01 ± 0.01 | - 0.04 | NS |
| Model 2 | 0.61 | 0.50 | 0.00 | - 0.01 ± 0.02 | - 0.09 | NS |
| Serum 25(OH)D (10 nmol/L) | | | | | | |
| Model 1 | 0.59 | 0.54 | 0.00 | 0.00 ± 0.01 | - 0.04 | NS |
| Model 2 | 0.61 | 0.50 | 0.00 | 0.00 ± 0.01 | - 0.03 | NS |
| EPA – DIET (1 g) | | | | | | |
| Model 1 | 0.60 | 0.55 | 0.01 | - 0.30 ± 0.33 | - 0.09 | NS |
| Model 2 | 0.62 | 0.51 | 0.01 | - 0.36 ± 0.36 | - 0.11 | NS |
| EPA – DIET + SUPP | | | | | | |
| Model 1 | 0.61 | 0.56 | 0.02 | - 0.01 ± 0.01 | - 0.13 | NS |
| Model 2 | 0.63 | 0.52 | 0.02 | - 0.06 ± 0.01 | - 0.14 | NS |
| DHA – DIET (1 g) | | | | | | |
| Model 1 | 0.60 | 0.55 | 0.01 | - 0.20 ± 0.24 | - 0.08 | NS |
| Model 2 | 0.62 | 0.51 | 0.01 | - 0.25 ± 0.26 | - 0.10 | NS |
| DHA – DIET + SUPP | | | | | | |
| Model 1 | 0.61 | 0.56 | 0.02 | - 0.02 ± 0.01 | - 0.15 | NS |
| Model 2 | 0.63 | 0.52 | 0.02 | - 0.02 ± 0.01 | - 0.16 | NS |

Model 1: Adjusted for baseline PD, hygienist and follow-up time.
Model 2: Adjusted for baseline PD, hygienist, follow-up time, age, sex, former smoking, waist-to-hip ratio, plaque score, and supplement use.
Regression coefficients are expressed in terms of the unit shown in brackets for each predictor. For example, there is a 0.03 ± 0.02 mm reduction in PD for every 100 mg of vitamin C.
Predictors listed without a unit of measurement are normal scores.

**Dietary Supplement Use**

Dietary supplement users were compared to non-users using ANCOVA to control for baseline PD, hygienist and follow-up time (summarized in **Table 4.3**). The adjusted re-exam PD for non-users (n = 19) was 3.12 ± 0.02 mm and this group comprises the control group for all ANCOVA comparisons. The adjusted re-exam PD for those who reported taking any kind of dietary supplement (n = 37) was 3.12 ± 0.01 mm. There was no significant difference in re-exam PD between supplement users and the control group. Multivitamin supplement users (n = 20) had an adjusted re-exam PD of 3.12 ± 0.02, which was not significantly different from the control group. Vitamin C supplement users (n = 12) had an adjusted re-exam PD of 3.10 ± 0.02 mm but they were not significantly different from the control group. Vitamin D supplement users (n = 22) had an adjusted re-exam PD of 3.11 ± 0.02 mm but they were not significantly different from the control group. Omega-3 (fish oil + omega3/6/9) supplement users (n = 12) had an adjusted re-exam PD of 3.11 ± 0.02 mm but they were not significantly different from the control group.

**Table 4.3** Comparison of re-exam PD (mm) between supplement users and non-users

| | *n* | **PD**: Mean ± SE (mm) | *Comparison to control* | |
|---|---|---|---|---|
| | | | $\eta_p^2$ | *P* value |
| Non-users (control) | 19 | 3.12 ± 0.02 | - | - |
| Any Supplement | 37 | 3.12 ± 0.01 | 0.00 | 0.73 |
| Multivitamin | 20 | 3.12 ± 0.02 | 0.00 | 0.87 |
| Vitamin C | 12 | 3.10 ± 0.02 | 0.03 | 0.40 |
| Vitamin D | 22 | 3.11 ± 0.02 | 0.02 | 0.45 |
| Omega-3 | 10 | 3.11 ± 0.02 | 0.00 | 0.82 |

Covariates are baseline PD, hygienist, and follow-up time.

**Exploratory Analyses**

Due to the fact that this is the first study to investigate the relationship between diet and periodontal outcomes after sanative therapy, we further explored associations with variables provided in the nutrition report. Due to the nature of exploring associations without *a priori* hypotheses, we acknowledge the possibility of finding associations by chance. Additionally, due to the relatively modest sample size in this study, it would be underpowered to detect any associations if a correction (such as Bonferroni) was used for multiple comparisons. Therefore, findings from these exploratory analyses are for the purpose of guiding future research projects and separate studies with the proper *a priori* hypotheses will be needed to validate any findings.

**Polyunsaturated Fatty Acids**

Since EPA and DHA intakes were relatively low in the study population, we decided to investigate possible associations with essential fatty acids, which were consumed in higher amounts by the study population. Intakes of LA, ALA and the omega-6 to omega-3 ratio were investigated as potential predictors of re-exam PD.

Hierarchical MLR was performed to predict re-exam PD using either LA, ALA, or omega-6/omega-3 intake as a continuous predictor.

After controlling for baseline PD, hygienist and follow-up time, there was a significant association between higher intakes of dietary LA and lower re-exam PD ($\Delta R^2 = 0.03$, $\beta = -0.19$, $p < 0.05$). This association remained consistent when the model was adjusted for baseline PD, hygienist, follow-up time plus potential confounders including

age, sex, waist-to-hip ratio, former smoking, plaque score, and dietary supplement use ($\Delta R^2 = 0.04$, $\beta = -0.22$, $p = 0.04$). In the fully adjusted model, LA intake could uniquely explain 4% of the variance in re-exam PD and for each 10 grams of LA consumed there was a 0.10 ± 0.05 mm reduction in PD. This association is depicted visually in **Figure 4.1**.

After controlling for baseline PD, hygienist and follow-up time, there was also a significant association between higher dietary intakes of ALA and lower re-exam PD ($\Delta R^2 = 0.05$, $\beta = -0.22$, $p = 0.01$). This association remained consistent when the model was adjusted for baseline PD, hygienist, follow-up time plus potential confounders including age, sex, waist-to-hip ratio, former smoking, plaque score, and dietary supplement use ($\Delta R^2 = 0.06$, $\beta = -0.26$, $p = 0.009$). In the fully adjusted model, ALA intake could uniquely explain 6% of the variance in re-exam PD and for each gram of ALA consumed there was a 0.09 ± 0.03 mm reduction in PD. This association is depicted visually in **Figure 4.2**.

After controlling for baseline PD, hygienist and follow-up time, there was a significant association between a larger total omega-6 to omega-3 ratio and greater re-exam PD ($\Delta R^2 = 0.03$, $\beta = 0.18$, $p < 0.05$). This association remained consistent when the model was adjusted for baseline PD, hygienist, follow-up time plus potential confounders including age, sex, waist-to-hip ratio, former smoking, plaque score, and dietary supplement use ($\Delta R^2 = 0.04$, $\beta = 0.21$, $p = 0.04$). In the fully adjusted model, the omega-6 to omega-3 ratio could uniquely explain 4% of the variance in PD and for each unit increase there was a 0.02 ± 0.01 mm increase in PD. This association is depicted visually in **Figure 4.3.**

**Table 4.4** Summary of significant associations between PD and essential fatty acid intake

| | $R^2$ | $R^2_{adj}$ | $\Delta R^2$ | $B \pm SE$ (mm) | $\beta$ | *P* value |
|---|---|---|---|---|---|---|
| LA – DIET (10 g) | | | | | | |
| Model 1 | 0.62 | 0.58 | 0.03 | - 0.08 ± 0.04 | - 0.19 | 0.05 |
| Model 2 | 0.65 | 0.55 | 0.04 | - 0.10 ± 0.05 | - 0.22 | 0.04 |
| ALA – DIET (1 g) | | | | | | |
| Model 1 | 0.64 | 0.60 | 0.05 | - 0.08 ± 0.03 | - 0.23 | 0.01 |
| Model 2 | 0.67 | 0.58 | 0.06 | - 0.09 ± 0.03 | - 0.26 | 0.009 |
| Total Dietary Omega-6:Omega-3 | | | | | | |
| Model 1 | 0.62 | 0.56 | 0.03 | 0.02 ± 0.01 | 0.18 | 0.05 |
| Model 2 | 0.65 | 0.55 | 0.04 | 0.02 ± 0.01 | 0.21 | 0.04 |

Model 1: Adjusted for baseline PD, hygienist and follow-up time
Model 2: Adjusted for baseline PD, hygienist, follow-up time, age, sex, former smoking, waist-to-hip ratio, plaque score, and supplement use
Regression coefficients are expressed in terms of the unit shown in brackets for each predictor. For example, there is a 0.10 ± 0.05 mm reduction in PD for 10 g of LA.

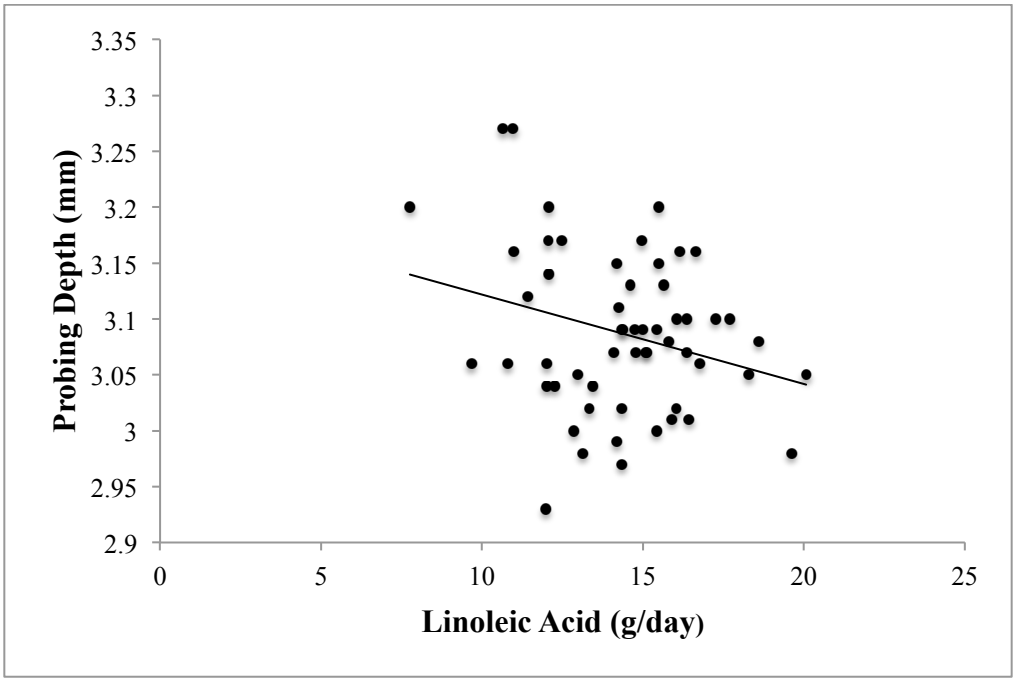


**Figure 4.1** Relationship between LA intake and re-exam PD (adjusted for baseline PD, hygienist and follow-up time), $R^2 = 0.03$, $p = 0.04$

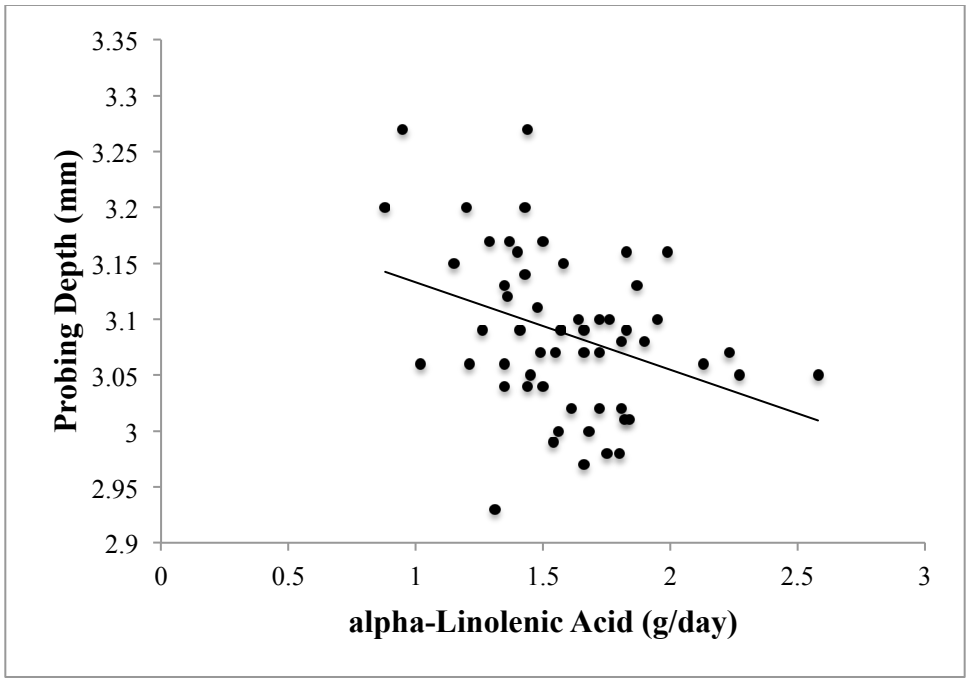


**Figure 4.2** Relationship between ALA intake and re-exam PD (adjusted for baseline PD, hygienist and follow-up time), $R^2 = 0.05$, $p = 0.01$

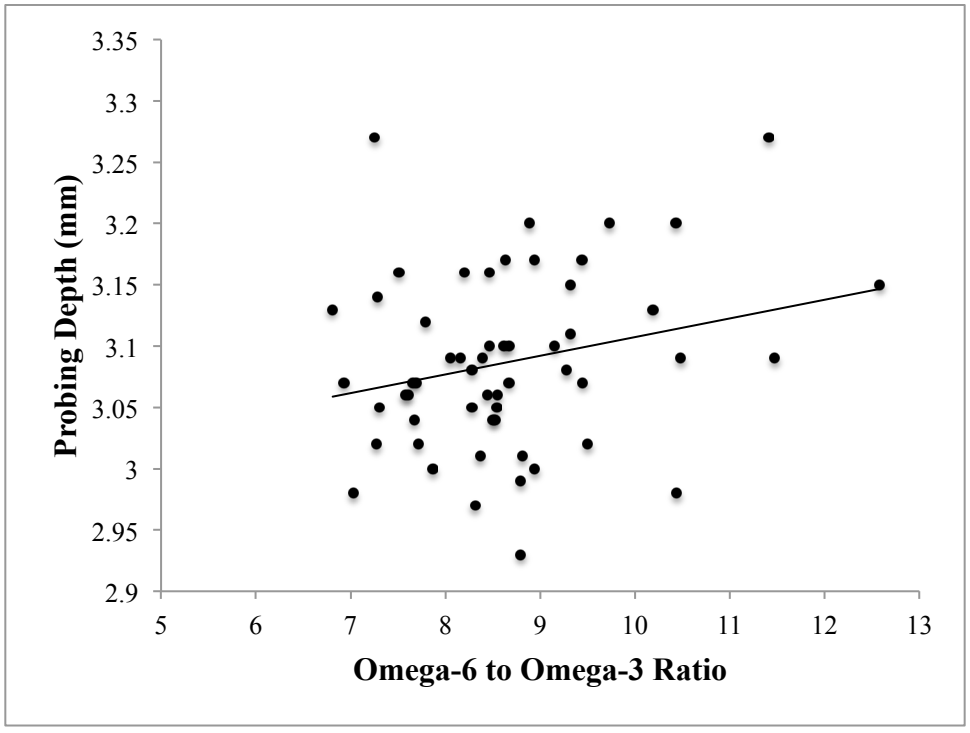


**Figure 4.3** Relationship between the omega-6 to omega-3 ratio and re-exam PD (adjusted for baseline PD, hygienist and follow-up time), $R^2 = 0.03$, $p = 0.04$

**Fruit, Vegetable, Dairy and Milk Intake**

A second approach to the exploratory analyses was to investigate potential relationships with specific food groups. Because of the relationship between dietary intakes of vitamin C and periodontal disease, food sources of vitamin C including fruits and vegetables were investigated as potential predictors of periodontal healing. Similarly, because of the relationship between vitamin D and periodontal disease, foods that contain significant amounts of vitamin D were investigated. Overall dairy intakes as well as milk intake (which is the only dairy food that is actually fortified with vitamin D) were investigated as potential predictors of periodontal healing.

Hierarchical MLR was performed to predict re-exam PD using either fruit, vegetable, dairy or milk intake as a continuous predictor. After adjusting for baseline PD, hygienist and follow-up time, neither fruit, nor dairy, nor milk intakes were significant predictors of re-exam PD. These results remained consistent when the models were further adjusted for potential confounders including age, sex, waist-to-hip ratio, former smoking, plaque score, and dietary supplement use.

After adjusting for baseline PD, hygienist and follow-up time, it was found that higher intakes of total vegetables were associated with lower re-exam PD ($\Delta R^2 = 0.05$, $\beta = -0.23$, $p = 0.02$). This association remained consistent when the model was adjusted for baseline PD, hygienist, follow-up time plus potential confounders including age, sex, waist-to-hip ratio, former smoking, plaque score, and dietary supplement use ($\Delta R^2 = 0.06$, $\beta = -0.26$, $p = 0.01$). In the fully adjusted model, total vegetable intake uniquely explained 6% of the variance in PD and there was a 0.02 ± 0.01 mm reduction in PD for

each daily serving (1/2 cup/125 mL) of vegetables consumed. This association is depicted visually in **Figure 4.4.**

To further explore this association we analyzed by vegetable subgroup. The subgroups are based on the 2005 Dietary Guidelines for Americans. These groups were created to ensure adequate intake of all vitamins and minerals as the nutrient composition varies between subgroups [181]. American guidelines were used (as opposed to Canadian ones) because the company that processes the Block FFQ is American-based and only provides dietary assessments based on American guidelines. However, Canada's Food Guide categorizes vegetables into similar groups as they recommend individuals to consume dark leafy green and orange vegetables every day. The subgroups tested were: deep yellow orange vegetables (carrots, sweet potatoes, winter squash, and pumpkin), dark green leafy vegetables (broccoli, spinach, romaine, collard, turnip, and mustard greens), potatoes (white potatoes), starchy vegetables (corn and green peas), tomatoes, legumes (pinto beans, kidney beans, lentils, chickpeas, tofu) and other vegetables (in the Block FFQ: squash, cauliflower, okra, cooked peppers). After adjusting for baseline PD, hygienist and follow-up time, the subgroups that were significantly associated with re-exam PD (in order of decreasing effect size) were other vegetables ($\Delta R^2$ = 0.08, β = -0.28, p = 0.002), deep yellow orange vegetables ($\Delta R^2$ = 0.05, β = - 0.24, p = 0.01) and legumes ($\Delta R^2$ = 0.04, β = -0.19, p = 0.04). These associations remained consistent when the models were adjusted for baseline PD, hygienist, follow-up time plus potential confounders including age, sex, waist-to-hip ratio, former smoking, plaque score, and dietary supplement use. Associations for deep yellow orange vegetable, legume and other vegetable intake are depicted visually in **Figures 4.5**, **4.6** and **4.7** respectively.

In order to determine possible nutrient and/or bioactive food component associations we further analyzed associations with nutrients and bioactives that are relatively abundant in vegetables. We tested associations with vitamin A, α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, quercetin, α-tocopherol, vitamin K, and potassium. After adjustment for baseline PD, hygienist and follow-up time, β-carotene ($\Delta R^2$ = 0.05, β = -0.24, p = 0.01) and β-cryptoxanthin ($\Delta R^2$ = 0.04, β = -0.22, p = 0.02) were significantly associated with re-exam PD. These associations remained consistent when the models were adjusted for baseline PD, hygienist, follow-up time plus potential confounders including age, sex, waist-to-hip ratio, former smoking, plaque score, and dietary supplement use. Associations for β-carotene and β-cryptoxanthin are depicted visually in **Figures 4.8** and **4.9** respectively. Statistics regarding vegetable subgroups and bioactives that were significant predictors of re-exam PD are summarized in Table **4.5**.

For the exploratory analyses, a summary of intakes and their respective effects are summarized in **Table 4.6**.

**Table 4.5** Summary of significant associations between PD and vegetable intake

| | $R^2$ | $R^2_{adj}$ | $\Delta R^2$ | $B \pm SE$ (mm) | $\beta$ | $P$ value |
|---|---|---|---|---|---|---|
| Total Vegetables (1 serv./125mL) | | | | | | |
| Model 1 | 0.64 | 0.59 | 0.05 | - 0.02 ± 0.01 | - 0.23 | 0.02 |
| Model 2 | 0.67 | 0.57 | 0.06 | - 0.02 ± 0.01 | - 0.26 | 0.01 |
| Yellow Orange (1 cup/250 mL) | | | | | | |
| Model 1 | 0.64 | 0.60 | 0.05 | - 0.14 ± 0.05 | - 0.24 | 0.01 |
| Model 2 | 0.67 | 0.58 | 0.06 | - 0.16 ± 0.06 | - 0.28 | 0.006 |
| Legumes (1 cup/250 mL) | | | | | | |
| Model 1 | 0.63 | 0.58 | 0.04 | - 0.71 ± 0.33 | - 0.19 | 0.04 |
| Model 2 | 0.66 | 0.56 | 0.05 | - 0.89 ± 0.36 | - 0.24 | 0.02 |
| Other Vegetables (1 cup/250 mL) | | | | | | |
| Model 1 | 0.67 | 0.63 | 0.08 | - 0.09 ± 0.03 | - 0.28 | 0.002 |
| Model 2 | 0.69 | 0.61 | 0.08 | - 0.10 ± 0.03 | - 0.30 | 0.002 |
| β-Carotene (1000 μg) | | | | | | |
| Model 1 | 0.64 | 0.60 | 0.05 | - 0.01 ± 0.01 | - 0.24 | 0.01 |
| Model 2 | 0.67 | 0.58 | 0.06 | - 0.01 ± 0.01 | - 0.28 | 0.006 |
| β-Cryptoxanthin (0.1 mg) | | | | | | |
| Model 1 | 0.63 | 0.59 | 0.04 | - 0.02 ± 0.01 | - 0.22 | 0.02 |
| Model 2 | 0.65 | 0.55 | 0.04 | - 0.02 ± 0.01 | - 0.22 | 0.03 |

Model 1: Adjusted for baseline PD, hygienist and follow-up time
Model 2: Adjusted for baseline PD, hygienist, follow-up time, age, sex, former smoking, waist-to-hip ratio, plaque score, and supplement use
Regression coefficients are expressed in terms of the unit shown in brackets for each predictor. For example, there is a 0.02 ± 0.01 mm reduction in PD for 1 serving of vegetables.

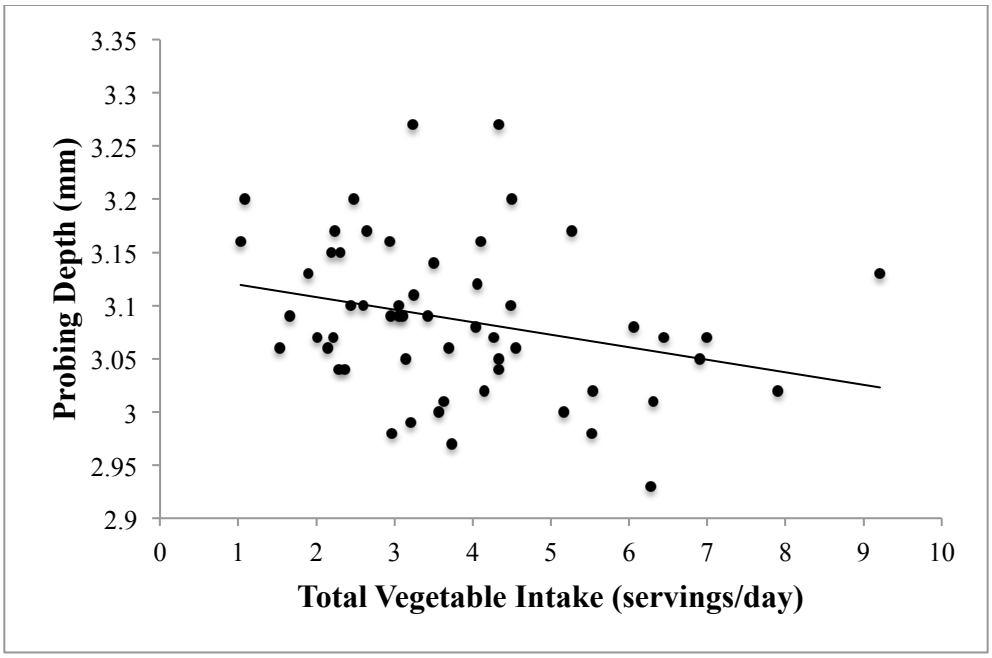


**Figure 4.4** Relationship between total vegetable intake and re-exam PD (adjusted for baseline PD, hygienist and follow-up time), $R^2 = 0.05$, $p = 0.02$. One serving is ½ cup or 125 mL.

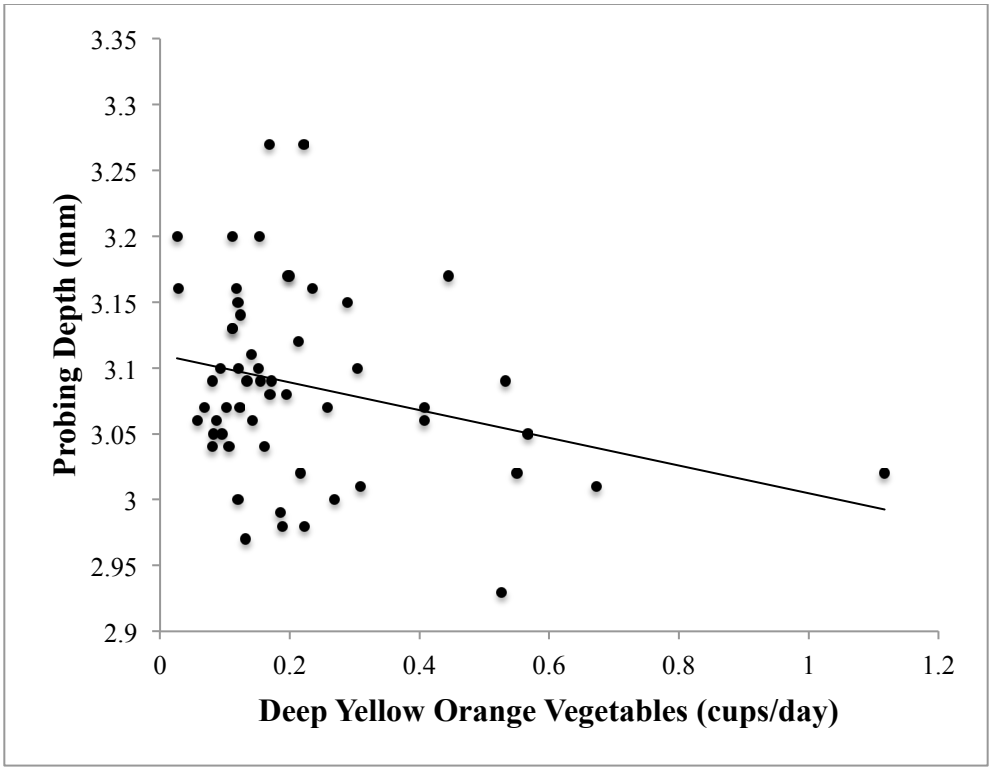


**Figure 4.5** Relationship between deep yellow orange vegetable intake and re-exam PD (adjusted for baseline PD, hygienist and follow-up time), $R^2 = 0.05$, $p = 0.01$

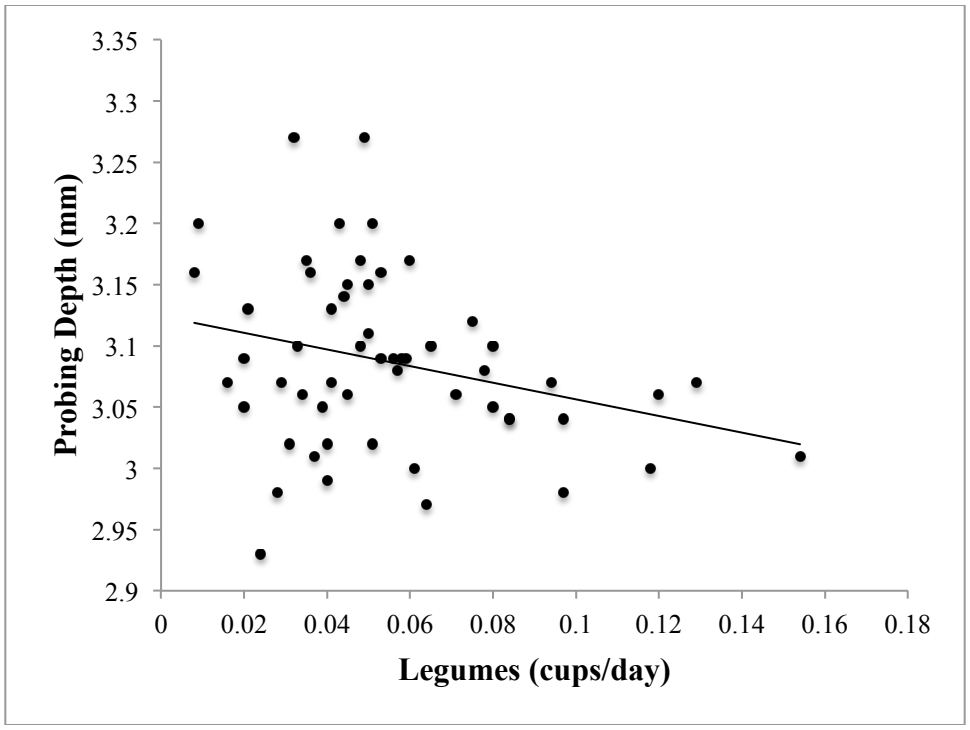


**Figure 4.6** Relationship between legume intake and re-exam PD (adjusted for baseline PD, hygienist and follow-up time), $R^2 = 0.04$, $p = 0.04$

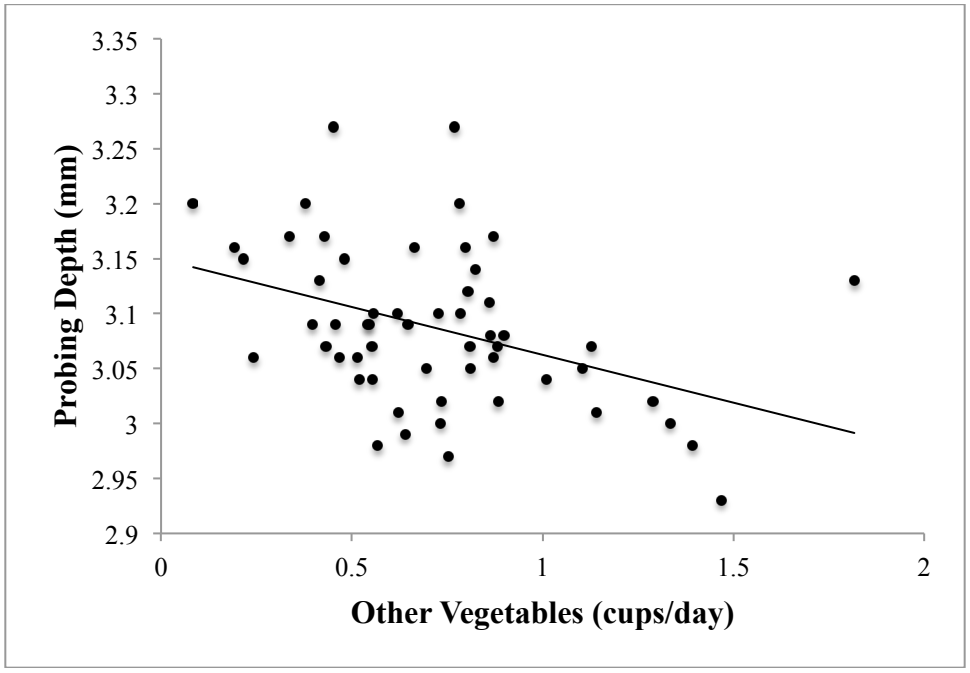


**Figure 4.7** Relationship between other vegetable intake and re-exam PD (adjusted for baseline PD, hygienist and follow-up time), $R^2 = 0.08$, $p = 0.002$

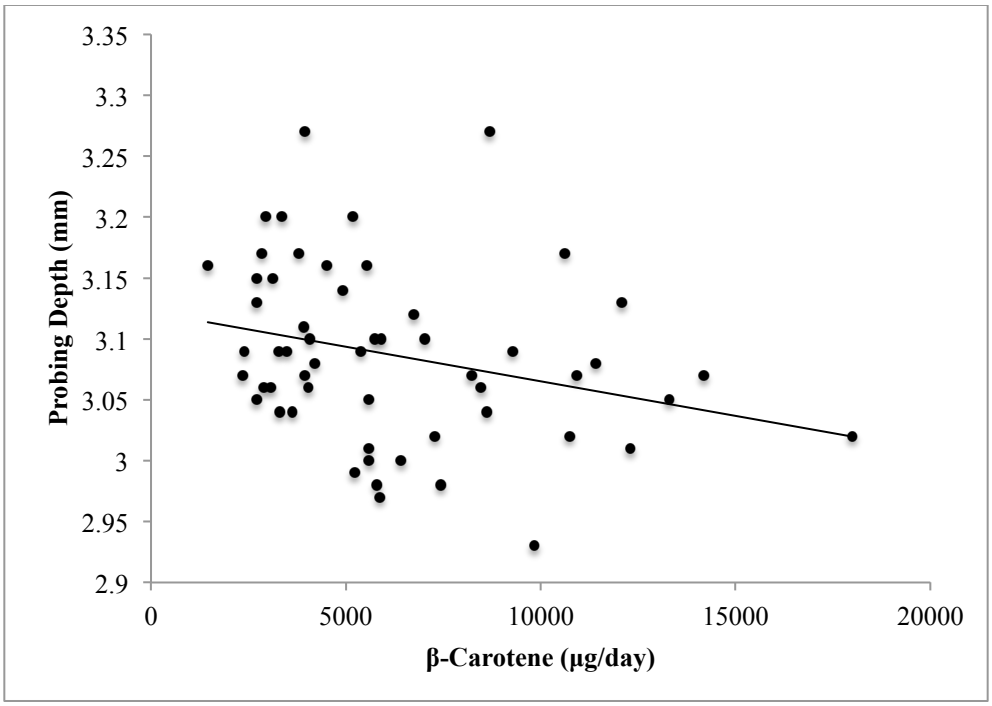


**Figure 4.8** Relationship between β-carotene intake and re-exam PD (adjusted for baseline PD, hygienist and follow-up time), $R^2 = 0.05$, $p = 0.01$

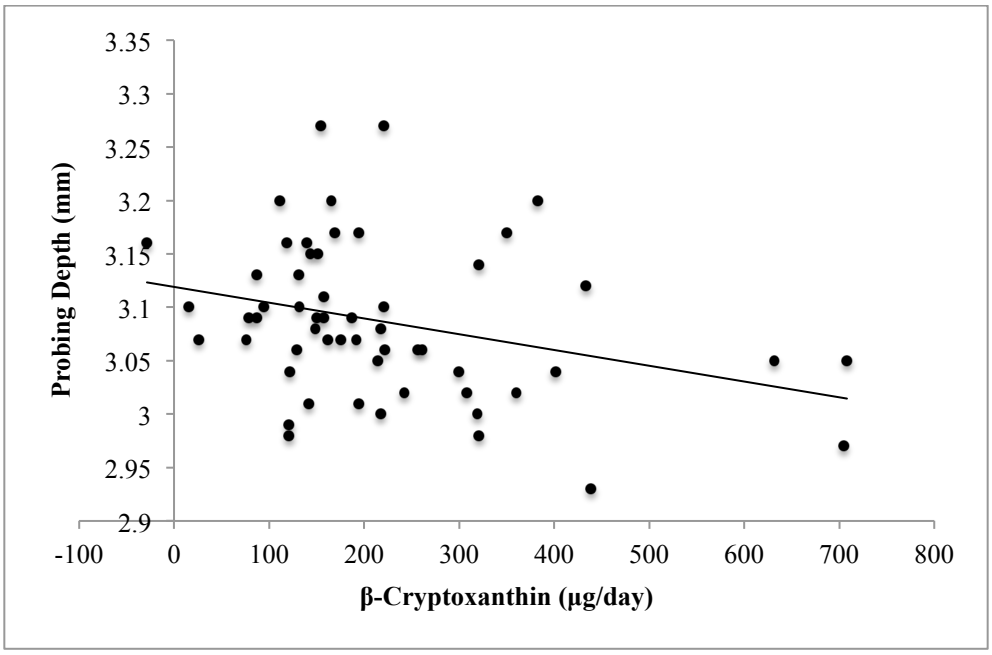


**Figure 4.9** Relationship between β-cryptoxanthin intake and re-exam PD (adjusted for baseline PD, hygienist and follow-up time), $R^2 = 0.04$, $p = 0.02$

**Table 4.6** Summary of dietary intakes and their respective effects on mean PD after sanative therapy

| **Food/Nutrient/Bioactive** | **Daily Intakes of the population** Mean ± SD (range) | **Effect** |
|---|---|---|
| Linoleic Acid (g) | 14.4 ± 2.4 (7.8 – 20.1) | 0.10 ± 0.05 mm reduction in PD for every 10 g |
| α-Linolenic Acid (g) | 1.6 ± 0.03 (0.9 – 2.6) | 0.09 ± 0.03 mm reduction in PD for ever 1 g |
| Omega-6 to Omega-3 Ratio | 8.6 ± 2.2 (6.8 – 12.6) | 0.02 ± 0.01 mm increase in PD for ever unit |
| Total Vegetables (Serving = ½ cup or 125 mL) | 3.8 ± 1.7 (1.0 – 9.2) | 0.02 ± 0.01 mm reduction in PD for every serving |
| Deep Yellow Orange Vegetables (1 cup or 250 mL) | 0.22 ± 0.19 (0.03 – 1.12) | 0.16 ± 0.06 mm reduction in PD for every cup |
| Legumes (1 cup or 250 mL) | 0.06 ± 0.03 (0.01 – 0.15) | 0.89 ± 0.36 mm reduction in PD for every cup |
| Other Vegetables (1 cup or 250 mL) | 0.72 ± 0.33 (0.08 – 1.8) | 0.10 ± 0.03 mm reduction in PD for every cup |
| β-Carotene (mg) | 6.2 ± 3.5 (1.5 – 18.0) | 0.01 ± 0.01 mm reduction in PD for every mg |
| β-Cryptoxanthin (μg) | 219 ± 151 (0 – 708) | 0.02 ± 0.01 mm reduction in PD for every 100 μg |

# Chapter Five

# DISCUSSION AND CONCLUSIONS

## 5.1 Discussion

This study was the first to investigate the relationship between diet and periodontal healing following sanative therapy. Although we had hypothesized that higher intake of specific nutrients such as vitamin C, vitamin D, EPA and DHA, either through diet or supplement use, would be associated with lower PDs, these findings were not observed. Similarly, we had hypothesized that higher serum 25(OH)D would be associated with lower PDs, but this finding was not observed. However, diets rich in essential fatty acids (linoleic acid and α-linolenic acid) and rich in vegetables were associated with a better response to sanative therapy, suggesting that a diet incorporating these elements may be an effective strategy to optimize healing after periodontal treatment.

Our study population was fairly similar to the general Canadian population in terms of health behaviours including body weight and smoking. From 2009 to 2011, Statistics Canada reports that for males 40 to 79, 42 – 45 % of the population is overweight and 31 – 36 % of the population is obese [182]. Similarly for females age 40 – 79, 30 – 35 % of the population is overweight and 28 – 32 % of the population is obese. These proportions are similar to those observed in our study population, which had slightly more overweight individuals (54 %) but a similar proportion of obese individuals (30 %). In terms of smoking, 20.3 % of Canadians over 12 years of age were current smokers in 2012 [183]. Of those that do not currently smoke in Canada, about half are former smokers. The proportion of smokers in the study population was slightly higher with 26 % of patients being current smokers with a comparable proportion of former smokers (50% of non-smokers).

In addition to associations with nutrition, we observed other significant predictors of periodontal healing including the number of days between treatment and follow-up, and which hygienist the patient saw. The relationship between follow-up time and mean PD is consistent with the literature. After the initial healing and reduction in PD there is a natural tendency for some relapse to occur within six months of treatment [179]. To compensate in this study, patients at the extreme end (longer than 4 months for follow-up) were removed and follow-up time was then controlled for statistically. Also in this study, patients who saw hygienist number 2 had a significantly lower mean probing depth compared to the other hygienists. This could have resulted either due to greater efficacy in performing sanative therapy or due to differences in technique when measuring follow-up PD. Differences in measurement of PD is a probable explanation as measuring PD can be quite variable and depends on factors such as probing force and probing position [3]. Although we attempted to minimize any systematic differences in measurement technique by calibrating the hygienists prior to the study, difference still occurred. Since it was not feasible to discard patients seen by hygienists number 2 due to sample size, the best compromise was to control statistically for differences that might have occurred due to which hygienist the patient saw.

**Vitamin C**

Vitamin C was hypothesized to improve the response to sanative therapy by acting as an anti-oxidant and promoting collagen synthesis. Although this study did not find a significant association between vitamin C intake and PD, one explanation for this result could be an inadequate sample size. There was an almost significant trend (p =

0.07) for reduced PD with higher dietary intakes of vitamin C and therefore we still consider dietary vitamin C to be potentially associated with periodontal healing. The current analysis has a power of 0.38. To detect this association with $\alpha = 0.05$ and power $= 0.80$ would require a sample size of 189. Eighty percent power is, however, quite high, and a power of 0.50 requiring 84 participants would likely detect a significant association. Significant associations may also have been more readily detected using a different method of measuring vitamin C intake or a direct measure of vitamin C status. Block FFQ estimates of vitamin C intake have been compared to both intake estimates from multiple diet records (two four-day records) [169] and plasma vitamin C [184] resulting in modest correlations of $r = 0.57$ and $r = 0.43$ respectively. There is not yet an agreement on which is the best method as each has its advantages and disadvantages. For example, a FFQ measures usual intakes and therefore might not be as precise as a diet record. A diet record may be more precise for a given time period but may not reflect usual intakes. Similarly, plasma vitamin C is a good indicator of present vitamin C status but may not reflect past or usual vitamin C status. Ideally both a measure of vitamin C intake and status would have been useful and thus remains a limitation of this study.

As this is the first study to look at vitamin C intake in relation to sanative therapy, there are no other studies with which to directly compare these results. Several association studies in the general population have found that higher dietary intakes of vitamin C reduce an individual's risk for periodontitis [100, 107-109]. Dietary intakes between 100 and 179 mg/day were found to be the most protective in the United States NHANES study. Comparatively, those in the highest tertile of vitamin C intake in our study were consuming 151 to 352 mg/day. This might suggest that even higher vitamin C

intakes are required to promote periodontal healing compared to only providing protection from periodontitis. Intake levels that possibly promote periodontal healing appear higher than recommendation for healthy individuals (RDA 90 mg/d for males and 75 mg/day for females).

Interestingly, there did not appear to be a benefit to sanative therapy with use of vitamin C supplements (minimum dose of 500 mg/day). Although vitamin C supplement users tended to have lower probing depths, the difference was very small (0.02 mm) and thus not significantly different. This effect size is so small that it would require 318 supplement users for 80% power and 156 supplement users for 50% power. It is therefore reasonable to conclude that vitamin C supplements are not likely to improve outcomes after sanative therapy. This finding agrees with the study by Abou-Sulaiman [115], who supplemented patients undergoing sanative therapy with 2000 mg of vitamin C a day and found no differences in clinical outcomes. Although these findings are in agreement, there should be caution in drawing any definite conclusion as the Abou –Sulaiman study had significant limitations (mostly failure to control for baseline status).

In terms of potential mechanisms with dietary vitamin C intake, we hypothesized that vitamin C would optimize periodontal healing by first scavenging reactive oxygen species thereby reducing periodontal destruction and inflammation, which would allow healing to take place, and secondly by acting as an essential cofactor to optimize collagen synthesis and aid in periodontal regeneration (**Figure 1.3**). However, the fact that there was no benefit with vitamin C supplements suggests that there are aspects not yet identified in this proposed mechanism, as high doses of vitamin C alone do not seem to produce an effect. Since fruits and vegetables are a rich source of vitamin C, associations

with vitamin C intake might simply be reflecting associations with fruit and vegetable intake, which is supported by the results of this study. It may therefore be erroneous to try and attribute the positive effects of a healthy fruit and vegetable rich diet to a single nutrient, a concept that will be further discussed shortly.

**Vitamin D**

We had hypothesized that higher serum vitamin D levels would optimize healing after sanative therapy by boosting the innate immune response to clear pathogenic bacteria and by acting as an anti-inflammatory agent to help resolve inflammation in order for healing to proceed (**Figure 1.3**). However, we did not find an association between serum vitamin D levels and reduced probing depth. Similarly, there was no association between vitamin D intake or vitamin D supplement use and reduced probing depth. At the time the present study was planned, there was one study by Bashutski et al. [134] who found that patients undergoing open flap debridement had greater reductions in probing depth when their serum 25(OH)D was greater than 50 nmol/L. Thus, this study was used somewhat as a basis for the present study. However, we had suspected problems with their findings as 4/7 (57%) of their control group were smokers and only 2/13 (15%) of the high vitamin D group were smokers. Additionally, because their sample size was so small, the findings would have been highly susceptible to sampling error. Our data would suggest that the differences in periodontal healing they claim to be due to vitamin D status may be due to effects of smoking, as smoking may hinder the response of a patient to sanative therapy.

In retrospect, the lack of association between vitamin D status and periodontal healing is not that surprising as we found no literature on the effects of vitamin D and soft tissue healing. There is some evidence demonstrating a positive effect of vitamin D on fracture healing in animal studies [185], however, that is likely due to the osteogenic effects of vitamin D. To date though, there does not appear to be any such studies with soft tissue healing. Although the negative effects of inflammation on cutaneous wound healing have been well documented [186], that link has not been extended to include any modulatory effect of vitamin D.

Although we did not find a relationship between vitamin D and periodontal outcomes after sanative therapy, that does not exclude the possibility of a role for vitamin D in the periodontal clinic. As the epidemiological evidence suggests, high serum vitamin D levels (> 85 nmol/L) may serve a protective role in periodontal health [101, 130]. This is also supported by cell culture studies showing effects of vitamin D on the innate immune response to oral pathogens [137]. Vitamin D may therefore be useful in periodontal maintenance patients. In our sample, only 11/56 (20%) of the population had serum 25(OH)D > 85 nmol/L with a small number (18%) still below 50 nmol/L. This suggests that there is room for improving the vitamin D status of our patient population. To achieve levels comparable to the epidemiological studies would likely require vitamin D supplementation as patients taking at least 1000 IU of vitamin D per day had a mean serum 25(OH)D of 78 ± 24 nmol/L compared to 59 ± 21 nmol/L in patients not taking vitamin D supplements. Ensuring adequate vitamin D levels might help patients who have undergone sanative therapy maintain their periodontal health and prevent relapses of the disease. Indeed, Miley et al. [133] tested this idea by comparing periodontal health of

patients who were in a maintenance program (frequency of visits not reported but at least 6 months since initial sanative therapy) and were or were not taking vitamin D supplements. They found that vitamin D supplement users had lower probing depths and lower levels of attachment loss. So although our study did not see a benefit to sanative therapy with higher vitamin D levels, the possible long-term role of vitamin D in patients undergoing periodontal treatment warrants further investigation.

**EPA and DHA**

We had hypothesized that higher intakes of EPA and DHA would be associated with reduced PD due to their ability to help resolve inflammation (**Figure 1.3**), however, neither EPA nor DHA intakes were associated with re-exam PD. The lack of association could be due to a number of potential reasons. Firstly, intakes of EPA and DHA may have been too low to exert a measurable effect. In this study the average intake of EPA was 39 ± 32 mg/day (range 0 – 172) and the average intake of DHA was 61 ± 45 mg/day (range 0 - 244). In the study by El-Sharkawy et al. [159] patients undergoing sanative therapy had greater PD reduction and attachment gain with 900 mg of fish oil per day + low dose aspirin. This intervention would have provided roughly an additional 120 mg of EPA and 160 mg of DHA per day making intakes higher than those in our study. Similarly, in the pilot study by Rosenstein et al. [158], a trend for reduced PD was seen in patients taking 3000 mg/day of EPA, which was significantly higher than reported in our study. Even some of the dietary observational studies have reported considerably higher intakes of EPA and DHA than seen in this seen in this study. For example, in the study by Iwasaki et al. [156], those in the highest tertile of DHA intake had reduced risk for

periodontal disease, however, the average intake of DHA for that whole population was 1086 ± 731 mg/day for males and 656 ± 327 mg/day for females. Thus, it is quite possible that participants in our study were simply not consuming high enough amounts of EPA and DHA to modulate periodontal therapy. It is possible that lower intakes, such as the ones seen in this study, might positively affect the response to sanative therapy; however, the effect size would be quite small. For example, to detect an association with EPA would require 224 participants for 50% power and 509 participants for 80% power and to detect an association with DHA intake would require 271 participants for 50% power and 617 participants for 80% power. It is also possible that the associations in our study were attenuated by inaccuracies in the quantification of EPA and DHA intake. Most dietary EPA and DHA come from fish intake and therefore the majority of their estimated intakes come from only three questions in the Block FFQ (Appendix 8.4, page 4). Also, novel foods containing relatively high concentrations of DHA are now more common and this is not captured in the Block 2005 FFQ. The novel foods, including eggs (15 mg DHA/egg), milk (10 mg DHA/cup) and margarine (30 mg DHA/teaspoon), would considerably increase intake among individuals who would otherwise have low DHA intakes [187]. Indeed some researchers have shown that food frequency questionnaires that are targeted toward long chain fatty acids more accurately quantify EPA and DHA intake compared to general FFQs (this may be the case for other nutrients but that has not been directly studied). Meyer et al. [188] compared the validity of the Anti-Cancer Council of Victoria Dietary Questionnaire (ACCDQ), a tool similar to the Block FFQ in Australia, against a PUFA-targeted FFQ. Using erythrocyte EPA as a reference, the validity of the PUFA FFQ was 0.92 compared to only 0.19 in the ACCDQ. Similarly,

using plasma as a reference, the validity of the PUFA FFQ was 0.96 compared to 0.49 in the ACCDQ. Thus it is quite possible that in our study, which used a general FFQ, EPA and DHA were not quantified accurately enough. Therefore, the relationship between EPA and DHA intake and periodontal therapy may still warrant further investigation, but may require different tools for measuring nutrient intakes.

In addition to the lack of association with EPA and DHA dietary intakes, we also did not find an association between omega-3 supplement use and PD reduction. Although this may suggest no effect of omega-3 supplements, these results should not be taken very definitively. There were very few omega-3 supplement users in the population. Only 10 patients reported using fish oil supplements while only 2 reported using omega 3/6/9 supplements. There was a large amount of heterogeneity in the composition and dosages of the supplements. For example, omega-3 supplements provided anywhere from 25 to 600 mg of DHA. Thus, it is difficult to consider the omega-3 supplement user group as a homogenous group such as one you would see in a clinical trial. So although we did not find evidence for a benefit with omega-3 supplementation, this study is not equipped to show that there is no effect. Since omega-3 supplement use was uncommon in the study population, a clinical trial with omega-3 supplements would be a faster approach to determine if they optimize periodontal healing after sanative therapy.

**Linoleic Acid, α-Linolenic acid and omega-6 to omega-3 ratio**

Originally, we had not hypothesized associations between LA or ALA intake and periodontal healing because the anti-inflammatory mechanisms of PUFAs are thought to predominately occur through the action of the longer chain fatty acids EPA and DHA.

Nonetheless, there were significant associations between PD and LA or ALA. Individuals consuming more LA and ALA had greater reductions in PD compared to those consuming less. In this population, the mean energy adjusted LA intake was 14.4 ± 2.4 g/day and the mean energy adjusted ALA intake was 1.6 ± 0.3 g/day. The highest tertile of intake, and thus the level most associated with optimized periodontal healing, ranged from 15 - 20 g/day for LA and 1.7 – 2.6 g/day for ALA. Compared to the RDAs [LA: 14-17 g/d (males), 11-12 g/d (females), ALA 1.6 g/d (males) and 1.1 g/d (females)], these levels are slightly exceeding recommended intakes although it is difficult to compare directly due to energy adjustment.

This is the first study to report a link between LA, ALA and periodontal outcomes. Interestingly, the epidemiological evidence from NHANES showed associations between periodontal disease and DHA intake but not with ALA intake [102]. That finding seems intuitive as omega-3 PUFAs are known to modulate inflammation through EPA, DHA, and resolvins, with very low conversion of ALA into EPA and DHA (about 10%) [189]. There then exists a mystery of why, in this study, ALA intake was associated with periodontal healing while EPA and DHA, the more active metabolites, were not. One possible explanation is that the lack of EPA and DHA association was due to relatively low amounts in the diet, as was previously discussed. Perhaps then high enough levels of ALA might be able to generate sufficient quantities of endogenous EPA and DHA despite low conversion rates. Goyens et al. [189] report conversion rates in humans between 6 and 10%. For example then, if only 10% of 1.6 g of ALA was converted to EPA, that would represent 160 mg of EPA, which is still higher than average EPA intakes in this population (39 ± 32 mg). Research into other chronic diseases is

revealing differential effects of ALA compared to EPA and DHA. Similarly to our study, Farina et al. [190] found associations between reduced risk of hip fracture in older adults and higher intakes of ALA but not but not with higher intakes of EPA, DHA or fish. They explained this finding to be the result of very low intakes of dietary EPA and DHA in that population. Another possible explanation is that ALA acts through pathways that are not dependent on conversion to EPA and DHA. Very recent research using computer modeling suggests that ALA might have anti-inflammatory activity itself by inhibition of COX-2, although more work is needed to verify this claim [191].

Nonetheless, evidence from this study suggests that ALA rich diets may be beneficial for periodontal healing after sanative therapy. Dietary interventions could increase ALA intake with foods such as flaxseed (2.4 g/tsp.), canola oil (0.42 g/tsp.), flaxseed oil (2.58 g/tsp.), almonds (0.15 g/0.25 cup) and soybeans (0.76 g/0.75 cup). These foods could be incorporated into a diet modification plan in order to test this hypothesis. Many clinical studies have reported benefits with flaxseed intervention. In hypertensive patients, flaxseed intervention has provided clinically significant reductions in systolic (- 10 mm Hg) and diastolic (- 7 mm Hg) blood pressure [192]. Furthermore, circulating levels of ALA correlated significantly with both systolic and diastolic blood pressure. In overweight and obese individuals with pre-diabetes, daily flaxseed consumption has been shown to decrease fasting glucose and insulin levels while improving insulin sensitivity [193]. Lastly, one study showed that daily flaxseed oil consumption for 4 months lowered inflammation (measured as C-reactive protein levels) in chronic hemodialysis patients [194]. Although flaxseed is rich in other biologically

active compounds such as lignans and fibre, the high content of ALA in flaxseed likely plays an important role in mediating these effects.

Another surprising finding was that higher LA intake was associated with better healing after sanative therapy. LA has received a considerable amount of negative attention due to suspected conversion of LA into AA and subsequent conversion into pro-inflammatory eicosanoids [150]. However, in recent years, this hypothesis has not been substantiated. Fifteen randomized, placebo controlled intervention studies with LA in healthy adults found no change in multiple markers of inflammation such as C-reactive protein, fibrinogen, plasminogen activator inhibitor type 1, cytokines, soluble vascular adhesion molecules, or TNF-α [195]. Similarly, 36 intervention studies have found no changes in serum, plasma or erythrocyte AA concentrations with either decreased or increased LA intake [196]. Thus, there is no evidence to support a link between increased LA intake and inflammation in humans. LA intake has been of particular interest in the cardiovascular disease literature. Higher LA intakes have been associated with reduced risk for coronary heart disease in several large prospective cohort studies such as the Health Professionals Follow-up Study [197], the Kupio Heart Study [198] and the Nurses' Health Study [199]. In studies with vascular endothelial cells, omega-6 PUFA had anti-inflammatory properties, suppressing the production of adhesion molecules, chemokines, and interleukins, although not as strongly as omega-3 PUFA [200]. With this evidence in mind, the American Heart Association maintains that higher LA intake reduces risk for cardiovascular diseases [201]. Similarly, higher intakes of LA may be beneficial for periodontal healing by similar mechanisms. Higher intakes of LA are likely

not resulting in higher levels of pro-inflammatory eicosanoids and may actually have an anti-inflammatory effect.

Another finding that adds complexity to the fatty acid story is that, despite beneficial associations with both higher ALA and LA intakes, a higher ratio of total omega-6 to omega-3 was associated with a less favorable reduction in PD. The association between the omega-6 to omega-3 ratio and periodontitis has been reported by Iwasaki et al. [157]. They found that a higher ratio was associated with a greater risk for the development of periodontal disease. Their lowest tertile of n-6:n-3, and thus the most protective level, ranged from 1.9 to 3.4. In our study, the lowest tertile ranged from 6.8 to 8.2. This range is higher than the Iwasaki study but still lower than ratios obtained by following current recommendations in Canada, which suggest an LA:ALA ratio of ~ 10:1. The traditional explanation for this effect is that a lower omega-6 to omega-3 ratio would result in a shift from the production inflammatory omega-6 derived eicosanoids to the production of the less inflammatory omega-3 derived eicosanoids as well as more available substrate for the formation of resolvin molecules (**Figure 1.6**). However, based on recent evidence surrounding LA intake, this hypothesized mechanism may not be accurate. The component of this ratio contributing to inflammation may actually be lower intake of omega-3 PUFAs and not higher intake of omega-6 PUFAs. Support for this idea is found in intervention studies that change either omega-6 or omega-3 PUFA intake while holding the other one constant. Hwang et al. [202] showed that at constant fish oil intake, increasing LA had no effect on $TXB_2$ or fibrinogen concentrations while conversely, increasing fish oil intake at constant LA intake decreased $TXB_2$ and fibrinogen concentrations. Similarly, increasing omega-6 PUFA at constant omega-3

intake [203] and constant ALA intake [204] has not resulted in changes in C-reactive protein concentrations. Thus, the optimal way to decrease inflammation might be to maintain both higher omega-6 PUFA and omega-3 PUFA intake while taking particular attention to ensure sufficient omega-3 PUFA intake. Dietary modification that ensures adequate omega-6 and omega-3 PUFA may therefore be useful in optimizing sanative therapy.

**Vegetable Intake**

The relationship between vegetable intake and periodontal healing following sanative therapy is a relatively novel one. We showed that greater vegetable intake was associated with a greater reduction in probing depth. Intakes of vegetables in the range of 4 to 8 servings (1 serving = 1/2 cup or 125 mL) per day for a 2000 kcal diet (highest tertile) were the most beneficial. Canada's Food Guide recommends 7-8 servings of fruits and vegetables per day for females 19-50 and 7 servings per day for females over 50. Likewise, 8-10 servings per day are recommended for men 19-50 and 7 servings per day for men over 50 [165]. Assuming sufficient intakes of fruit, these individuals were therefore meeting and possibly exceeding current recommendations for fruit and vegetable intake. Furthermore, we were able to show that the associations with vegetable intake were stronger for particular types of vegetables, namely yellow/orange vegetables (carrots, sweet potatoes, winter squash, and pumpkin), legumes (pinto beans, kidney beans, lentils, chickpeas, tofu) and other vegetables. The "other vegetable" group is not well defined but according to the foods surveyed in the Block 2005 FFQ, they include cauliflower, okra and peppers.

Chapple et al. have documented the benefits of vegetable intake on periodontal outcomes in a recent study [205]. They supplemented patients undergoing sanative therapy with fruit and vegetable capsules made from blended fruit and vegetable pulp and juice powder concentrate. Those receiving the fruit and vegetable intervention had greater reductions in PD two months after treatment thus showing that compounds found in fruits and vegetables have the capability of optimizing healing after sanative therapy. Clinical trials with fruits and vegetables have also effectively modulated inflammation under different conditions. For example, 8 servings a day of carotenoid-rich fruits and vegetables for 4 weeks has been shown to reduce systemic inflammation (measured as c-reactive protein levels) in healthy non-smoking men [206]. Similarly, a flavonoid-rich fruit and vegetable intervention (6 servings per day) in men at risk for cardiovascular disease has been shown to decrease C-reactive protein levels and improve markers of endothelial function [207]. Although, fruit and vegetable intervention studies are currently sparse, more and more trials are under way to determine if fruit and vegetable intake modulates disease activity in a number of pathologies, and periodontal disease should be no exception.

Vegetable intake may be optimizing periodontal healing through the action of many bioactive compounds. Firstly, vegetables are a rich source of antioxidants. Similar to vitamin C, other dietary antioxidants are able to accept unpaired reactive electrons becoming oxidized in the process by forming a molecule that is more stable than the original oxidizing molecule. The decrease in oxidative stress would help resolve inflammation and allow healing to proceed. The role for dietary antioxidants from vegetables is supported by our data, as β-carotene and β-cryptoxanthin intake were

associated with better periodontal healing after sanative therapy. β-Carotene and β-cryptoxanthin are both carotenoids with antioxidant activity [208]. Iwasaki et al. have previously reported an association between dietary β-carotene and periodontal disease progression in individuals over 70 years of age [109]. Similarly, the associations with various vegetable subgroups might also suggest antioxidant and inflammatory activity from bioactives in vegetables. Since orange and yellow vegetable intake was associated with improved periodontal healing, these vegetables were likely the source of many of the antioxidant compounds such as β-carotene and β-cryptoxanthin. Orange and deep yellow vegetables are rich sources of antioxidant carotenoids. The β-carotene content of these foods is 5054 μg for 1 medium carrot, 11062 μg for 1 medium sweet potato, 6050 μg for 1 cup of winter squash and 3800 μg for 1 cup of pumpkin. Although there are no dietary reference intakes for carotenoids, 5000 μg of β-carotene would provide 417 μg of retinol activity equivalents, which alone would meet half the RDA for vitamin A (900 μg for males and 700 μg for females of retinol activity equivalents). Thus, even one deep yellow orange vegetable per day would provide significant amounts of antioxidant carotenoids such as β-carotene. The most significantly correlated vegetable subgroup was the "other vegetable" subgroup. One of the foods in that subgroup are peppers, which are not only high in antioxidants such as carotenoids and ascorbic acid but also contain capsaicinoids and capsinoids [209]. Preliminary *in vitro* and animal studies have demonstrated the anti-inflammatory capacity of capsaicinoids and capsinoids [210, 211]. However, caution should be taken in trying to attribute the mechanism of a diverse and complex food group to single nutrients or compounds. For example, this lesson was learned in the ATBC (α-tocopherol, β-carotene) cancer prevention trial in smokers [212].

Epidemiological evidence had suggested a decreased risk of lung cancer with higher dietary intakes of β-carotene; however, supplementation with purified β-carotene actually produced the opposite effect and increased lung cancer risk. The benefits seen with vegetable intake and chronic disease, including periodontal disease, are therefore likely due to a vast number of bioactive compounds. There are over 5000 phytochemicals present in plant-based foods with many more still undiscovered [213]. Furthermore, it is likely that the synergistic effects of many bioactives are responsible for the health benefits seen with higher fruit and vegetable intake [214]. For example, the total antioxidant capacity of 1 gram of apples is the equivalent to 1500 mg of vitamin C. That is, vitamin C only accounts for 0.04% of the antioxidant capacity in one gram of apples [215]. The bottom line message is that future nutritional research into chronic disease prevention and management will likely need to focus on whole foods rather than purified nutrients, and periodontal disease is no exception. Data from our study corroborates this idea as improved periodontal outcomes were seen with higher vegetable intake while no benefits were seen with use of dietary supplements. Therefore, vegetable intake should be further researched as a potentially positive modulator of periodontal healing following sanative therapy.

**Summary**

The findings from this study broadly reflect the nutritional recommendations for healthy eating set by organizations such as Health Canada. It is likely not a coincidence that the dietary intake levels identified in this study are similar to the dietary recommended intakes and the dietary guidelines. Patients undergoing sanative therapy

can therefore be directed to follow current guidelines for essential fatty acid intake and for fruit and vegetable intake, which will have multiple benefits. Firstly, through possible optimization of sanative therapy, patients may experience greater improvements in periodontal healing and decreased risk of tooth loss. Secondly, patients will experience benefits to overall health and decrease their risk for chronic diseases such as diabetes, cardiovascular disease and osteoporosis as the dietary guidelines have been designed to address multiple health outcomes. This speaks to the broadening scope of dental practice where clinicians can, in addition to improving oral health, improve the overall health and well being of their patients.

### 5.2 Conclusions

Higher intakes of vitamin C, vitamin D, EPA and DHA, either through diet and/or supplement use was not associated with improved periodontal outcomes after sanative therapy. Similarly, higher serum 25-hydroxyvitamin D levels were also not associated with improved periodontal outcomes after sanative therapy. However, higher intakes of LA, ALA and vegetables (particular carotenoid rich orange yellow vegetables, legumes, and other vegetables such as peppers) as well as a lower total omega-6 to omega-3 ratio were associated with greater reductions in probing depth following sanative therapy. Dietary strategies that increase vegetable intake and ALA intake while maintaining LA intake may help improve periodontal healing following sanative therapy, ultimately leading to greater tooth retention and greater overall health.

# Chapter Six

# FUTURE DIRECTIONS

This was one of the first studies to investigate the relationship between diet and periodontal outcomes following sanative therapy and thus provides a strong basis for planning future research in this area.

### 6.1 Future Observational Studies

One possible route might be to confirm and elaborate on the findings of this study. As this is the first study to report better periodontal healing with higher intakes of vegetables and ALA, an important step would be to be able to replicate these findings. The findings should also be replicated using different methods to quantify dietary intakes. In the current study, a food frequency questionnaire was used to quantify usual dietary intakes and therefore a future study might want to look more specifically at quantifying what was eaten during the two-month healing period. For that, a method such as a diet record could be useful or even new types of technology that are currently being developed to monitor food intake could be used. For example, the Remote Food Photography Method, where participants take photographs of food selection and plate waste, has been shown to be much more accurate than self-reported food intake [216]. Intakes of fruit, vegetables and specific fatty acids can also be assessed through biomarkers. Measuring a panel of biomarkers in plasma such as α- and β-carotene, vitamin C, lutein, zeaxanthin and β-cryptoxanthin may be useful in predicting fruit and vegetable intake [217] and there are many options available to assess fatty acid status [218]. In addition to quantifying dietary intakes, a future study could also increase the number of clinical outcomes measured to more fully characterize the effects of diet. For example it would be useful to measure clinical attachment loss in order to look at the

association between diet and periodontal regeneration as well as overall healing. It would also be interesting to know the long-term effects of diet, which could be accomplished by following patients to a later date. Long-term follow-up would also allow for the monitoring of alveolar bone loss or gain using dental radiographs. Dental measurements could also be refined by taking baseline PD and CAL measurements at the start of the study (currently they are measured at a consultation). This would allow for the same hygienist who takes the re-exam measurements to take the baseline measurements thus reducing differences due to examiners. It would also remove any possible inaccuracies due to changes in clinical outcomes between the consultation and the treatment. Finally, possible mechanisms could be elucidated by measuring antioxidant capacity [219] and inflammation (e.g. C-reactive protein) in serum samples.

### 6.2 Future Intervention Studies

In the literature, one of the major knowledge gaps identified was the lack of intervention studies in patients with periodontal disease. Although our study did not directly address this gap, this study establishes the framework for future intervention studies looking at optimization of periodontal healing through nutrition. Findings from this study will enhance the efficacy at which new dietary strategies are uncovered in clinical trials. For example, a clinical trial should focus on increasing consumption of whole foods rather than supplement use as dietary supplements were not found to be beneficial in this study. A variety of fruits and vegetables should be consumed although particular attention might want to be given to specific types of vegetables identified in this study such as ones rich in carotenoids. ALA intake could also be increased with

foods such as flaxseed, canola oil, flaxseed oil, almonds and soybeans. A simple study might include providing dietary guidelines to a treatment group while also providing a way of tracking diet to ensure modification occurs. A more costly but likely more effective study would be to provide a treatment group with a defined amount of fruit and vegetables and/or ALA rich foods. Intervention studies using fruits and vegetables have been successfully completed where different amounts of fruits and vegetables were provided to participants [207]. Similarly dietary modification to increase ALA intake can be accomplished by providing foods with flaxseed such as bars, muffins, bagels, tea biscuits or pasta [220]. Although studies with dietary supplements are attractive due to practicality, the results from this study suggest a whole food approach would be more effective. It might however be of interest to compare a whole food approach to a dietary supplement (like the fruit and vegetable capsule used by Chapple et al. [205]) in a parallel study design. Any of the intervention studies could use nutritional biomarkers to assess compliance with treatments and the same details in the periodontal examination that were described in section 6.1 could be taken into consideration. Results from such a trial would be essentially in determining if the dietary components identified in the current study do in fact modulate periodontal outcomes. This would be a large step forward in creating dietary strategies to optimize periodontal healing following sanative therapy, which would ultimately lead to greater tooth retention and greater overall health.

# Chapter Seven

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# Chapter Eight

# APPENDICES

## Appendix 8.1 Certificate of Ethics Clearance

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

### Certificate of Ethics Clearance for Human Participant Research

| | | | |
|---|---|---|---|
| DATE: | 11/1/2012 | | |
| PRINCIPAL INVESTIGATOR: | WARD, Wendy - Kinesiology | | |
| FILE: | 12-068 - WARD | | |
| TYPE: | Masters Thesis/Project/Faculty Research | STUDENT: | David Dodington |

TITLE: Dietary Intake and Periodontal Outcomes after Sanative Therapy

**ETHICS CLEARANCE GRANTED**

Type of Clearance: NEW Expiry Date: 11/29/2013

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from 11/1/2012 to 11/29/2013.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 11/29/2013. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:

Brian Roy, Chair
Bioscience Research Ethics Board

**Note:** Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

## Appendix 8.2 Letter of Invitation

Brock

Faculty of Applied Health Sciences
Department of Kinesiology &
Department of Community Health Sciences

January 2013

**Letter of Invitation**

| | |
|---|---|
| Project Title: | **Dietary Intakes and Periodontal Outcomes after Sanative Therapy** |
| Faculty Investigator: | **Dr. Wendy E. Ward**, Associate Professor & Canada Research Chair in Bone and Muscle Development,<br>Faculty of Applied Health Sciences, Brock University |
| Co-Investigator: | **Dr. Peter C. Fritz**, Periodontist & Implant Surgeon,<br>Reconstructive Periodontics and Implant Surgery Clinic, Fonthill, ON<br>Adjunct Assistant Professor, Department of Kinesiology, Brock University |
| Student Investigator: | **Mr. David Dodington**, M.Sc. Candidate, Faculty of Applied Health Sciences,<br>Brock University |

I, Wendy Ward, Associate Professor & Canada Research Chair in Bone and Muscle Development, from the Department of Kinesiology, Brock University, invite you to participate in a research project entitled, ***Dietary Intakes and Periodontal Outcomes after Sanative Therapy***. Please note that this study is in addition to your scheduled appointment and that it is your choice to participate or not participate in this research study. **Your decision will not impact the standard of care that you will receive.**

You are here today at Dr. Fritz's clinic for a sanative cleaning (also referred to as a "deep cleaning") to improve the health of your gums and teeth. ***The purpose of this study is to determine if specific dietary patterns or intakes of a specific nutrient allows a patient to achieve healthier gums and teeth after sanative therapy.*** While the cleaning you receive today will improve the health of your gums, there is some evidence that specific nutrients may further improve the health of gums.

Participation will result in your regularly scheduled appointment requiring an additional 15-20 minutes. You will also need 30-40 minutes to complete a take-home dietary questionnaire at your convenience. We will record certain information from your confidential patient information and medical history form. Specifically we will be recording your age, the list of medications taken, the list of mineral, vitamin and/or herbal supplements taken, any allergies (as this may alter response to sanative therapy), smoking status and history, and any existing health conditions. You will have your body weight, height, waist and hip circumference measured. A blood sample will be obtained (5 mL, approximately 1 teaspoon) to measure vitamin D status and inflammation. Crevicular fluid (the fluid between the gum and a tooth) will be sampled at 3 teeth with the most extensive periodontal disease. Your crevicular fluid samples will be analyzed for markers of inflammation.

When you return for your follow-up visit that is scheduled 8 weeks after today's appointment, body weight and height will be measured, a final blood sample and crevicular fluid samples will be obtained.

Your participation will help us to develop dietary strategies that may enhance the recovery of gum tissue, allowing you to keep your natural teeth, after a sanative cleaning. Healthier gums and teeth are associated with better overall health including a healthier and stronger skeleton.

If you have any pertinent questions about your rights as a research participant, please contact the Brock University Research Ethics Officer (905-688-5550 ext. 3035, reb@brocku.ca)

If you have any questions, please feel free to contact me.

Thank you,

Wendy E. Ward

**Principal Investigator:**
**Dr. Wendy E. Ward, Associate Professor & Canada Research Chair in Bone and Muscle Development**
**Faculty of Applied Health Sciences**
**Brock University**
**905-688-5550 (x3024)**
**wward@brocku.ca**

**Co-Investigator:**
**Dr. Peter C. Fritz, Periodontist & Implant Surgeon**
**Reconstructive Periodontics and Implant Surgery Clinic**
**165 Highway 20 West, Suite 1**
**Fonthill, ON**
**905 892 0800**
**Adjunct Assistant Professor, Faculty of Applied Health Sciences, Brock University**
**peter.fritz@utoronto.ca**

**Student Investigator:**
**Mr. David Dodington, M.Sc. Candidate**
**Faculty of Applied Health Sciences**
**Brock University**
**905-382-3308**
**dd12vw@brocku.ca**

**This study has been reviewed and received ethics clearance through the Brock University Ethics Board (file #12-068)**

## Appendix 8.3 Consent Form

Faculty of Applied Health Sciences
Department of Kinesiology &
Department of Community Health Sciences

### Informed Consent

Date: January 2013

Project Title: **Dietary Intakes and Periodontal Outcomes after Sanative Therapy**


| Principal Investigator: | Co-Investigator: |
|---|---|
| Dr. Wendy E. Ward, Associate Professor & Canada Research Chair in Bone and Muscle Development<br>Faculty of Applied Health Sciences<br>Brock University<br>905-688-5550 X3024<br>wward@brocku.ca | Dr. Peter C. Fritz, Periodontist & Implant Surgeon<br>Reconstructive Periodontics and Implant Surgery Clinic<br>165 Highway 20 West, Suite 1<br>Fonthill, Ontario<br>905-892-0800<br>Adjunct Assistant Professor<br>Faculty of Applied Health Sciences<br>Brock University<br>peter.fritz@utoronto.ca |
| Student Investigator:<br><br>Mr. David Dodington, M.Sc. Candidate<br>Faculty of Applied Health Sciences<br>Brock University<br>905-382-3308<br>dd12vw@brocku.ca | |


**INVITATION**

You are invited to participate in a study that examines if your usual diet is associated with periodontal outcomes. Specifically, we are trying to finding out if specific dietary patterns or intakes of specific nutrients are associated with healthier gums and overall improved tooth health after sanative cleaning.

**WHAT'S INVOLVED**

We will record certain information from your confidential patient information and medical history form. Specifically we will be recording your age, the list of medications taken, the list of mineral, vitamin and/or herbal supplements taken, any allergies (as this may alter response to sanative therapy), smoking status and history, and any existing health conditions. You will be asked to complete a dietary questionnaire, a physical activity questionnaire. Immediately before your sanative cleaning and at your regularly scheduled 8-week follow-up appointment you will have your body weight, height, waist and hip circumference measured. You will also be asked to have a blood sample taken (5 mL, approximately 1 teaspoon) and have three samples of crevicular fluid (fluid between the gum and the tooth) taken at sites with the most extensive periodontal disease. Crevicular fluid is collected by placing a small paper point into the site. Your blood will be analyzed for serum 25-hydroxvitamin, a marker of vitamin D status because we cannot accurately measure vitamin D status using the dietary questionnaire. Your blood will also be analyzed for markers of inflammation (c-reactive protein, interleukin-6 and tumor necrosis factor-alpha). Crevicular fluid will only be analyzed for markers of inflammation. Specifically, crevicular fluid samples will be analyzed for gene expression of several markers of inflammation (interleukin-1beta, interleukin-6, tumor necrosis factor-alpha, receptor activator of nuclear factor-kappa B, receptor activator of nuclear factor-kappa-B and matrix metalloproteinase-8). A decrease in the levels of these markers of inflammation is associated with healthier gums. Blood and crevicular fluid samples will be stored in a locked freezer in Dr. Ward's laboratory for a maximum of 10 years after data are published. It is ideal for the study if your dietary habits remain similar

throughout the study. Participation will require approximately 15-20 minutes in addition to your regularly scheduled clinic visits, immediately prior to the sanative cleaning and at the follow-up appointment that occurs 8 weeks after this cleaning. 30-40 minutes will also be required to complete the food frequency and physical activity questionnaire and these can be completed at home.

**POTENTIAL BENEFITS AND RISKS**

Your participation will help us to develop dietary strategies that may enhance tooth health through the recovery of gum tissue after sanative therapy. Healthier gums are associated with better tooth health as well as overall health including a healthier and stronger skeleton. There are no anticipated physical risks related to participation in this study. There is a small risk that there may be a small bruise or mild discomfort associated with the blood sampling.

**CONFIDENTIALITY**

Any information that arises from participants will be treated with confidentiality. Your name will not be included or, in any other way, associated with the data collected in the study. Data collected during this study will be stored in a locked filing cabinet in Dr. Ward's office at Brock University or Dr. Fritz's office and will be destroyed 10 years after your participation in the study. Access to this data will be restricted to the principal investigator, coinvestigator, and their research team.

**VOLUNTARY PARTICIPATION**

Participation in this study is voluntary. If you wish, you may decline to answer any questions or participate in any component of the study. You may withdraw from the study at any time. Withdrawal from the study will not affect your clinical care. Non-participation will not adversely affect your clinical care.

**PUBLICATION OF RESULTS**

Results of this study may be published in professional journals and presented at conferences. You will receive a summary of results by mail when the study is completed.

**CONTACT INFORMATION AND ETHICS CLEARANCE**

If you have any questions about this study or require further information, please contact the Principal Investigator (Dr. Wendy Ward) or the Co-Investigator (Dr. Peter Fritz) using the contact information provided above. This study has been reviewed and received ethics clearance through the Brock University Ethics Board (file #12-068). If you have any comments or concerns about your rights as a research participant, please contact the Research Ethics Office at 905-688-5550 ext. 3035, reb@brocku.ca.

Thank you for your assistance in this project. Please keep a copy of this form for your records.

**CONSENT FORM**

I agree to participate in this study described above. I have made this decision based on the information I have read in the Consent Letter. I have had the opportunity to receive any additional details I wanted about the study and understand that I may ask questions in the future. I understand that I may withdraw this consent at any time.

Name: _____ (please print)

Signature: _____

Date: _____

**Appendix 8.4 Block 2005 Food Frequency Questionnaire**

# FOOD QUESTIONNAIRE

RESPONDENT ID #

TODAY'S DATE

| | DAY | YEAR |
|---|---|---|
| Jan | | |
| Feb | | |
| Mar | | 2005 |
| Apr | | 2006 |
| May | | 2007 |
| Jun | | 2008 |
| Jul | | 2009 |
| Aug | | 2010 |
| Sep | | 2011 |
| Oct | | 2012 |
| Nov | | 2013 |
| Dec | | 2014 |

## ABOUT THIS SURVEY

**This form is about the foods you usually eat.**
**It will take about 30 - 40 minutes to complete.**
**Please answer each question as best you can.**
**Estimate if you aren't sure.**

- **USE ONLY A NO. 2 PENCIL.**
- **Fill in the circles completely, and erase completely if you make any changes.**

**Please write your name in this box.**

## ABOUT YOU

**SEX**
- Male
- Female

**AGE**

**WEIGHT** pounds

**HEIGHT** ft. in.

**If female, are you pregnant or breast feeding?**
- No
- Yes
- Not female

## INSTRUCTIONS

**There are usually two kinds of questions to answer for each food.**

1. **HOW OFTEN,** on average, did you eat the food during the past year?
   *Please DO NOT SKIP any foods. Mark Never if you did not eat any of the food in the question.
2. **HOW MUCH** did you usually eat of the food?
   *Sometimes we ask how many you eat, such as 1 egg, 2 eggs, etc., ON THE DAYS YOU EAT IT.
   *Sometimes we ask "how much" as A, B, C or D. **LOOK AT THE ENCLOSED PICTURES.**
   For each food, pick the picture (bowls or plates) that looks the most like the serving size you usually eat. (If you don't have pictures: A=1/4 cup, B=1/2 cup, C=1 cup, D= 2 cups.)
3. **EXAMPLE:** This person drank apple juice twice a week, and had one glass each time.
   Once a week he ate a "C"-sized serving of rice (about 1 cup).

| | HOW OFTEN IN THE PAST YEAR | | | | | | | | | HOW MUCH ON THOSE DAYS (SEE PORTION SIZE PICTURES FOR A-B-C-D) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NEVER | A FEW TIMES per YEAR | ONCE per MONTH | 2-3 TIMES per MONTH | ONCE per WEEK | 2 TIMES per WEEK | 3-4 TIMES per WEEK | 5-6 TIMES per WEEK | EVERY DAY | | | | |
| Apple juice | ○ | ○ | ○ | ○ | ○ | ● | ○ | ○ | ○ | How many glasses each time | 1 ● | 2 ○ | 3 ○ | 4 ○ |
| Rice | ○ | ○ | ○ | ○ | ● | ○ | ○ | ○ | ○ | How much each time | A ○ | B ○ | C ● | D ○ |

PLEASE DO NOT WRITE IN THIS AREA

Block 2005.1 ©2005 BDDS Phone 510-704-8514 www.nutritionquest.com

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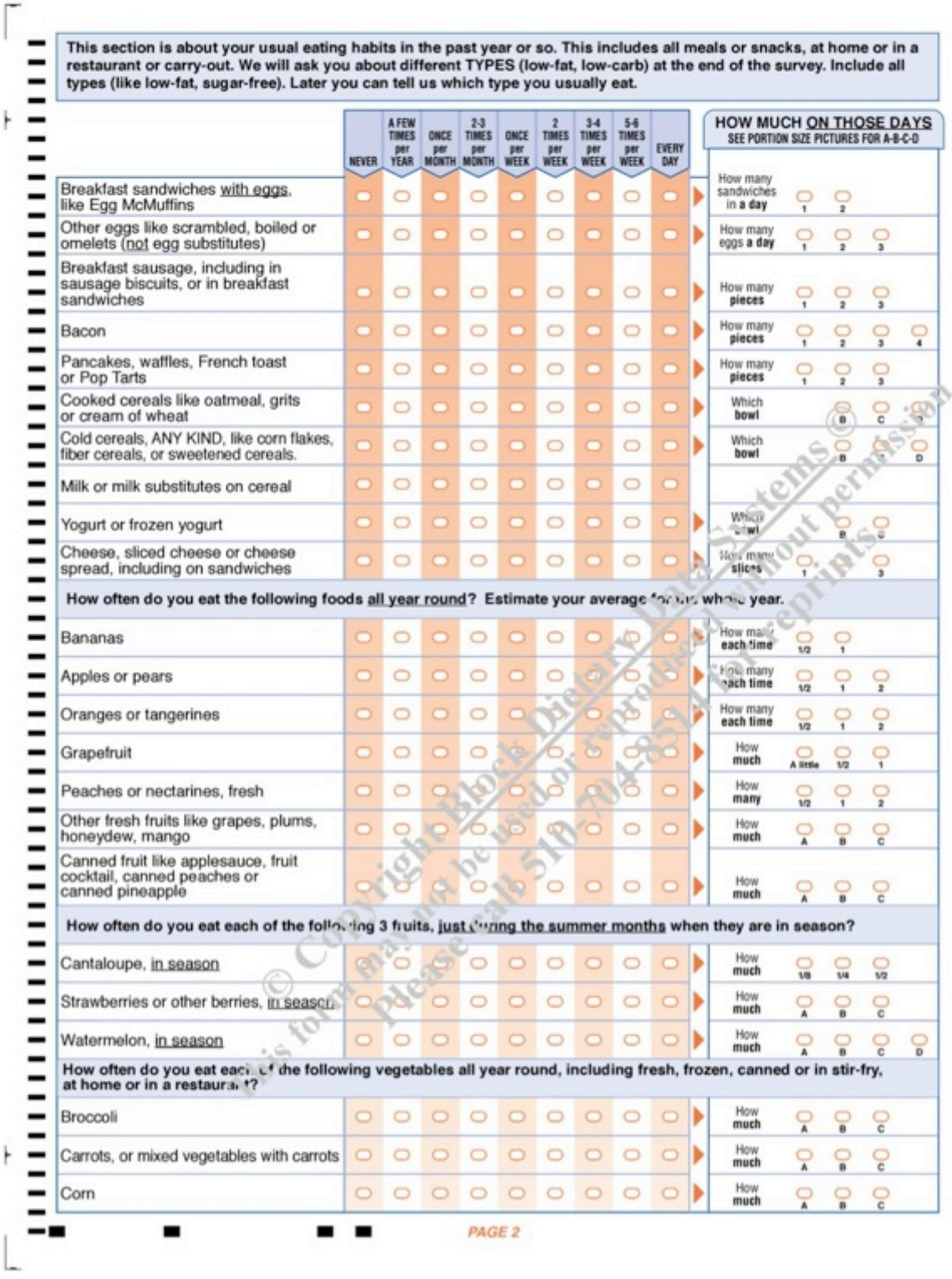

**This section is about your usual eating habits in the past year or so. This includes all meals or snacks, at home or in a restaurant or carry-out. We will ask you about different TYPES (low-fat, low-carb) at the end of the survey. Include all types (like low-fat, sugar-free). Later you can tell us which type you usually eat.**

| | NEVER | A FEW TIMES per YEAR | ONCE per MONTH | 2-3 TIMES per MONTH | ONCE per WEEK | 2 TIMES per WEEK | 3-4 TIMES per WEEK | 5-6 TIMES per WEEK | EVERY DAY | HOW MUCH ON THOSE DAYS (SEE PORTION SIZE PICTURES FOR A-B-C-D) |
|---|---|---|---|---|---|---|---|---|---|---|
| Breakfast sandwiches with eggs, like Egg McMuffins | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many sandwiches in a day: 1 2 |
| Other eggs like scrambled, boiled or omelets (not egg substitutes) | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many eggs a day: 1 2 3 |
| Breakfast sausage, including in sausage biscuits, or in breakfast sandwiches | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many pieces: 1 2 3 |
| Bacon | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many pieces: 1 2 3 4 |
| Pancakes, waffles, French toast or Pop Tarts | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many pieces: 1 2 3 |
| Cooked cereals like oatmeal, grits or cream of wheat | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | Which bowl: B C |
| Cold cereals, ANY KIND, like corn flakes, fiber cereals, or sweetened cereals. | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | Which bowl: B D |
| Milk or milk substitutes on cereal | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | |
| Yogurt or frozen yogurt | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | Which bowl: B |
| Cheese, sliced cheese or cheese spread, including on sandwiches | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many slices: 1 3 |
| **How often do you eat the following foods all year round? Estimate your average for the whole year.** | | | | | | | | | | |
| Bananas | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many each time: 1/2 1 |
| Apples or pears | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many each time: 1/2 1 2 |
| Oranges or tangerines | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many each time: 1/2 1 2 |
| Grapefruit | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much: A little 1/2 1 |
| Peaches or nectarines, fresh | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many: 1/2 1 2 |
| Other fresh fruits like grapes, plums, honeydew, mango | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much: A B C |
| Canned fruit like applesauce, fruit cocktail, canned peaches or canned pineapple | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much: A B C |
| **How often do you eat each of the following 3 fruits, just during the summer months when they are in season?** | | | | | | | | | | |
| Cantaloupe, in season | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much: 1/8 1/4 1/2 |
| Strawberries or other berries, in season | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much: A B C |
| Watermelon, in season | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much: A B C D |
| **How often do you eat each of the following vegetables all year round, including fresh, frozen, canned or in stir-fry, at home or in a restaurant?** | | | | | | | | | | |
| Broccoli | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much: A B C |
| Carrots, or mixed vegetables with carrots | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much: A B C |
| Corn | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much: A B C |

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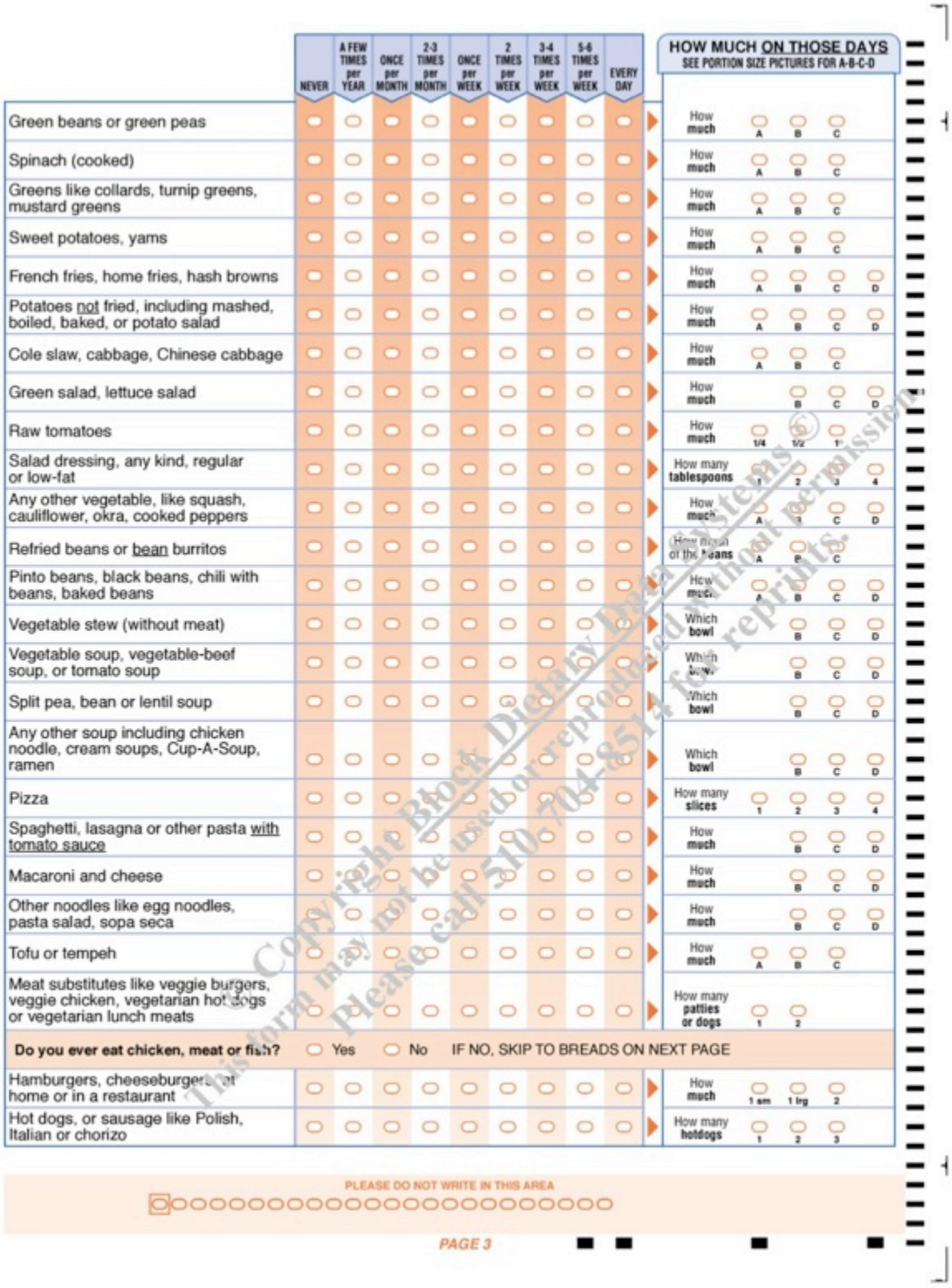

| | NEVER | A FEW TIMES per YEAR | ONCE per MONTH | 2-3 TIMES per MONTH | ONCE per WEEK | 2 TIMES per WEEK | 3-4 TIMES per WEEK | 5-6 TIMES per WEEK | EVERY DAY | HOW MUCH ON THOSE DAYS (SEE PORTION SIZE PICTURES FOR A-B-C-D) |
|---|---|---|---|---|---|---|---|---|---|---|
| Green beans or green peas | | | | | | | | | | How much A B C |
| Spinach (cooked) | | | | | | | | | | How much A B C |
| Greens like collards, turnip greens, mustard greens | | | | | | | | | | How much A B C |
| Sweet potatoes, yams | | | | | | | | | | How much A B C |
| French fries, home fries, hash browns | | | | | | | | | | How much A B C D |
| Potatoes not fried, including mashed, boiled, baked, or potato salad | | | | | | | | | | How much A B C D |
| Cole slaw, cabbage, Chinese cabbage | | | | | | | | | | How much A B C |
| Green salad, lettuce salad | | | | | | | | | | How much B C D |
| Raw tomatoes | | | | | | | | | | How much 1/4 1/2 1 |
| Salad dressing, any kind, regular or low-fat | | | | | | | | | | How many tablespoons 1 2 3 4 |
| Any other vegetable, like squash, cauliflower, okra, cooked peppers | | | | | | | | | | How much A C D |
| Refried beans or bean burritos | | | | | | | | | | How much of the beans A B C |
| Pinto beans, black beans, chili with beans, baked beans | | | | | | | | | | How much A B C D |
| Vegetable stew (without meat) | | | | | | | | | | Which bowl B C D |
| Vegetable soup, vegetable-beef soup, or tomato soup | | | | | | | | | | Which bowl B C D |
| Split pea, bean or lentil soup | | | | | | | | | | Which bowl B C D |
| Any other soup including chicken noodle, cream soups, Cup-A-Soup, ramen | | | | | | | | | | Which bowl B C D |
| Pizza | | | | | | | | | | How many slices 1 2 3 4 |
| Spaghetti, lasagna or other pasta with tomato sauce | | | | | | | | | | How much B C D |
| Macaroni and cheese | | | | | | | | | | How much B C D |
| Other noodles like egg noodles, pasta salad, sopa seca | | | | | | | | | | How much B C D |
| Tofu or tempeh | | | | | | | | | | How much A B C |
| Meat substitutes like veggie burgers, veggie chicken, vegetarian hot dogs or vegetarian lunch meats | | | | | | | | | | How many patties or dogs 1 2 |
| **Do you ever eat chicken, meat or fish?** | ○ Yes | ○ No | IF NO, SKIP TO BREADS ON NEXT PAGE | | | | | | | |
| Hamburgers, cheeseburgers, at home or in a restaurant | | | | | | | | | | How much 1 sm 1 lrg 2 |
| Hot dogs, or sausage like Polish, Italian or chorizo | | | | | | | | | | How many hotdogs 1 2 3 |

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| | NEVER | A FEW TIMES per YEAR | ONCE per MONTH | 2-3 TIMES per MONTH | ONCE per WEEK | 2 TIMES per WEEK | 3-4 TIMES per WEEK | 5-6 TIMES per WEEK | EVERY DAY | HOW MUCH ON THOSE DAYS (SEE PORTION SIZE PICTURES FOR A-B-C-D) |
|---|---|---|---|---|---|---|---|---|---|---|
| Lunch meat like bologna, sliced ham, turkey bologna, or any other lunch meat | | | | | | | | | | How many slices: 1 2 3 4 |
| Meat loaf, meat balls | | | | | | | | | | How much: B C D |
| Steak, roast beef, or beef in frozen dinners or sandwiches | | | | | | | | | | How much: A B C D |
| Tacos, burritos, enchiladas, tamales, with meat or chicken | | | | | | | | | | How much: A B C D |
| Ribs, spareribs | | | | | | | | | | How much: A B C D |
| Pork chops, pork roasts, cooked ham (including for breakfast) | | | | | | | | | | How much: A B C D |
| Veal, lamb, deer meat | | | | | | | | | | How much: A B C |
| Liver, including chicken livers or liverwurst | | | | | | | | | | How much: A B C |
| Pigs feet, neck bones, oxtails, tongue | | | | | | | | | | How much: A B C |
| Menudo, pozole, caldo de res, sancocho, ajiaco | | | | | | | | | | Which bowl: B C D |
| Any other beef or pork dish, like beef stew, beef pot pie, corned beef hash, Hamburger Helper | | | | | | | | | | How much: B C D |
| Fried chicken, including chicken nuggets, wings, chicken patty | | | | | | | | | | How many medium pieces: 1 2 [illegible] nugts 3 |
| Roasted or broiled chicken or turkey | | | | | | | | | | How much: B C |
| Any other chicken dish, like chicken stew, chicken with noodles, chicken salad, Chinese chicken dishes | | | | | | | | | | How much: B C D |
| Oysters | | | | | | | | | | How much: A B C |
| Shellfish like shrimp, scallops, crabs | | | | | | | | | | How much: A B C D |
| Tuna, tuna salad, tuna casserole | | | | | | | | | | How much of the tuna: A B C |
| Fried fish or fish sandwich | | | | | | | | | | How much: A B C |
| Other fish, not fried | | | | | | | | | | How much: A B C |
| **BREADS** | | | | | | | | | | |
| Biscuits, muffins, croissants (not counting breakfast sandwiches with eggs) | | | | | | | | | | How many: 1 sm 1 med 2 |
| Hamburger buns, hotdog buns, hoagie buns, submarines | | | | | | | | | | How many: 1 2 |
| Bagels, English muffins, dinner rolls | | | | | | | | | | How many: 1/2 1 |
| Tortillas (not counting those eaten in tacos or burritos) | | | | | | | | | | How many in a day: 1 2 3 4 |
| Corn bread, corn muffins, hush puppies | | | | | | | | | | How many pieces in a day: 1/2 1 2 |
| Any other bread or toast, including white, dark, whole wheat, and what you have in sandwiches | | | | | | | | | | How many slices in a day: 1 2 3 4 |
| Rice, or dishes made with rice | | | | | | | | | | How much in a day: B C D |

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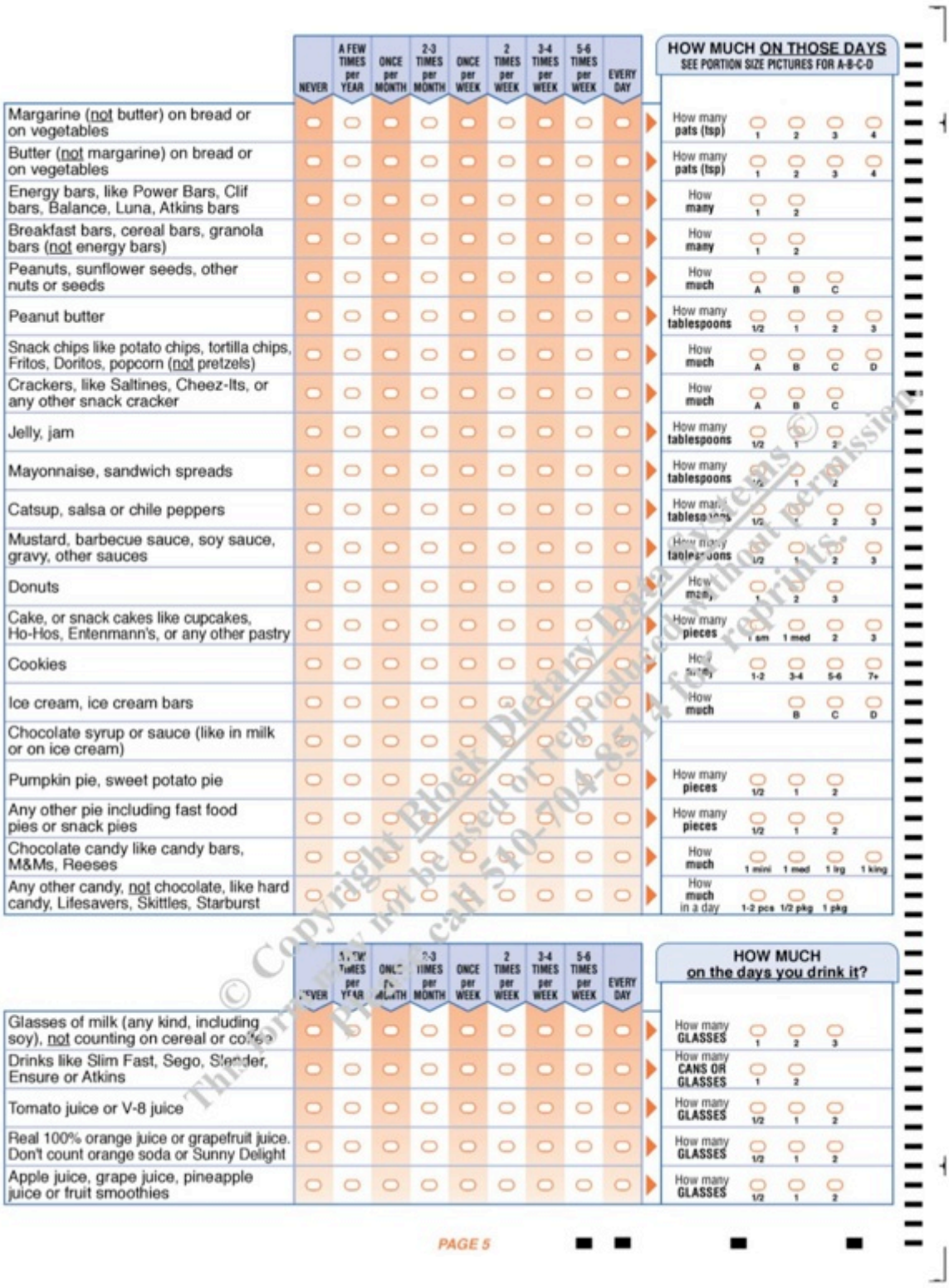


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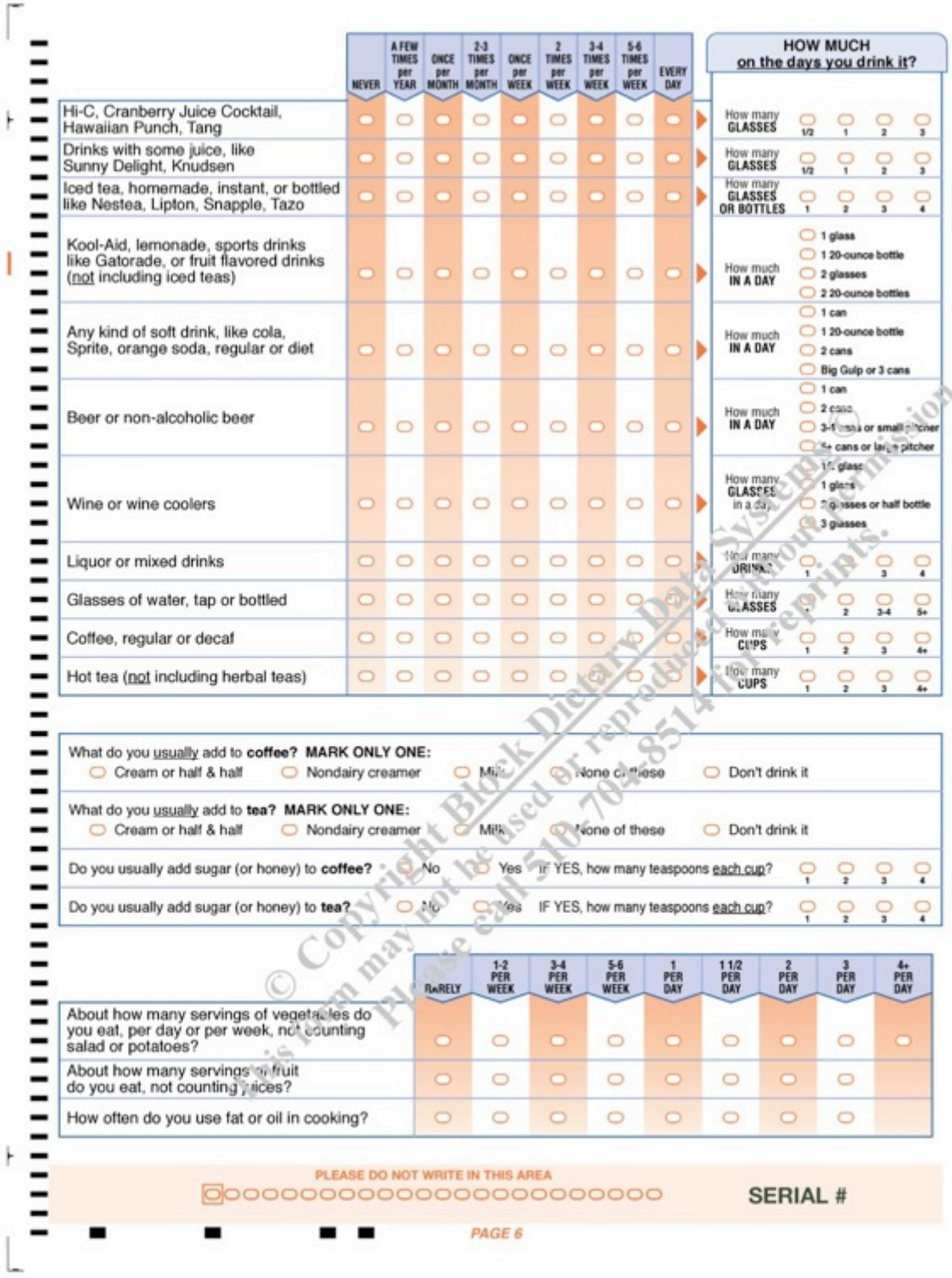

| | NEVER | A FEW TIMES per YEAR | ONCE per MONTH | 2-3 TIMES per MONTH | ONCE per WEEK | 2 TIMES per WEEK | 3-4 TIMES per WEEK | 5-6 TIMES per WEEK | EVERY DAY | HOW MUCH on the days you drink it? |
|---|---|---|---|---|---|---|---|---|---|---|
| Hi-C, Cranberry Juice Cocktail, Hawaiian Punch, Tang | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many GLASSES ○ 1/2 ○ 1 ○ 2 ○ 3 |
| Drinks with some juice, like Sunny Delight, Knudsen | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many GLASSES ○ 1/2 ○ 1 ○ 2 ○ 3 |
| Iced tea, homemade, instant, or bottled like Nestea, Lipton, Snapple, Tazo | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many GLASSES OR BOTTLES ○ 1 ○ 2 ○ 3 ○ 4 |
| Kool-Aid, lemonade, sports drinks like Gatorade, or fruit flavored drinks (not including iced teas) | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much IN A DAY ○ 1 glass ○ 1 20-ounce bottle ○ 2 glasses ○ 2 20-ounce bottles |
| Any kind of soft drink, like cola, Sprite, orange soda, regular or diet | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much IN A DAY ○ 1 can ○ 1 20-ounce bottle ○ 2 cans ○ Big Gulp or 3 cans |
| Beer or non-alcoholic beer | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How much IN A DAY ○ 1 can ○ 2 cans ○ 3-4 cans or small pitcher ○ 5+ cans or large pitcher |
| Wine or wine coolers | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many GLASSES in a day ○ 1 glass ○ 1 glass ○ 2 glasses or half bottle ○ 3 glasses |
| Liquor or mixed drinks | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many DRINKS ○ 1 ○ 2 ○ 3 ○ 4 |
| Glasses of water, tap or bottled | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many GLASSES ○ 1 ○ 2 ○ 3-4 ○ 5+ |
| Coffee, regular or decaf | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many CUPS ○ 1 ○ 2 ○ 3 ○ 4+ |
| Hot tea (not including herbal teas) | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | How many CUPS ○ 1 ○ 2 ○ 3 ○ 4+ |

What do you usually add to **coffee?** **MARK ONLY ONE:**
○ Cream or half & half ○ Nondairy creamer ○ Milk ○ None of these ○ Don't drink it

What do you usually add to **tea?** **MARK ONLY ONE:**
○ Cream or half & half ○ Nondairy creamer ○ Milk ○ None of these ○ Don't drink it

Do you usually add sugar (or honey) to **coffee?** ○ No ○ Yes IF YES, how many teaspoons each cup? ○ 1 ○ 2 ○ 3 ○ 4

Do you usually add sugar (or honey) to **tea?** ○ No ○ Yes IF YES, how many teaspoons each cup? ○ 1 ○ 2 ○ 3 ○ 4

| | RARELY | 1-2 PER WEEK | 3-4 PER WEEK | 5-6 PER WEEK | 1 PER DAY | 1 1/2 PER DAY | 2 PER DAY | 3 PER DAY | 4+ PER DAY |
|---|---|---|---|---|---|---|---|---|---|
| About how many servings of vegetables do you eat, per day or per week, not counting salad or potatoes? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| About how many servings of fruit do you eat, not counting juices? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | |
| How often do you use fat or oil in cooking? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | |

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SERIAL #

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**If you eat the following foods, what type do you usually eat? MARK ONLY ONE ANSWER FOR EACH QUESTION**

| | | | | |
|---|---|---|---|---|
| **Milk** | ○ Whole milk<br>○ Reduced-fat 2% milk | ○ Low-fat 1% milk<br>○ Non-fat milk | ○ Soy milk<br>○ Rice milk | ○ Don't drink |
| **Slim Fast, Sego, Slender or Ensure** | | ○ Low-Carb like Atkins | ○ Regular | ○ Don't drink |
| **Orange juice** | ○ Calcium-fortified | ○ Not calcium-fortified | ○ I don't know | ○ Don't drink |
| **Soda or pop** | ○ Diet soda, low-calorie | ○ Regular | ○ Don't drink | |
| **Iced tea** | ○ Homemade, no sugar | ○ Homemade, w/sugar | ○ Bottled, no sugar | ○ Bottled, regular ○ Don't drink |
| **Beer** | ○ Regular beer ○ Light beer | ○ Low-Carb beer | ○ Non-alcoholic beer | ○ Don't drink |
| **Hamburgers or cheeseburgers** | | ○ Hamburgers | ○ Cheeseburgers | ○ Don't eat |
| **Hot dogs** | ○ Low fat or turkey dogs | ○ Regular hot dogs | ○ Don't eat | |
| **Lunch meats** | ○ Low-fat or turkey lunch meats | ○ Regular lunch meats | ○ Don't eat | |
| **Spaghetti or lasagna** | ○ Meatless | ○ With meat sauce or meatballs | | ○ Don't eat |
| **Cheese** | ○ Low Fat | ○ Not Low Fat | ○ Don't eat | |
| **Salad dressing** | ○ Low-Carb | ○ Low-fat | ○ Regular | ○ Don't use |
| **Energy bars like Power Bar, Clif, Atkins** | ○ Low-Carb, low sugar | ○ Low-fat | ○ Regular | ○ Don't eat |
| **Breakfast bars, cereal bars, or granola bars** | ○ Low-Carb, low sugar | ○ Low-fat | ○ Regular | ○ Don't eat |
| **Bread** | ○ 100% whole wheat | ○ Low-Carb | ○ Regular | ○ Don't eat |
| **Tortillas** | ○ Corn | ○ Flour | ○ Don't know or don't eat | |
| **Chocolate candy or chocolate candy bars** | ○ Low-Carb, low sugar | ○ Low-fat | ○ Regular | ○ Don't eat |
| **Cookies** | ○ Low-Carb, low sugar | ○ Low-fat | ○ Regular | ○ Don't eat |
| **Cake, snack cakes, and other pastries** | ○ Low-Carb, low sugar | ○ Low-fat | ○ Regular | ○ Don't eat |
| **Ice cream** | ○ Low-Carb, low sugar | ○ Low-fat or ice milk | ○ Regular | ○ Don't eat |
| **Jelly or jam** | ○ Low-Carb, low sugar | ○ Regular | ○ Don't use | |
| **Beef or pork** | ○ Avoid eating the fat | ○ Sometimes eat the fat | ○ Often eat the fat | ○ Don't eat |
| **Chicken or Turkey** | ○ Avoid eating the skin | ○ Sometimes eat the skin | ○ Often eat the skin | ○ Don't eat |

What kinds of **fat or oil** do you usually use in cooking? **MARK ONLY ONE OR TWO**

| | | | |
|---|---|---|---|
| ○ Don't know, or Pam | ○ Stick margarine | ○ Corn oil, vegetable oil | ○ Lard, fatback, bacon fat |
| ○ Butter | ○ Soft tub margarine | ○ Olive oil or canola oil | ○ Crisco |
| ○ Butter/margarine blend | ○ Low-fat margarine | | |

If you eat **cold cereals**, what do you eat? Choose one or two that you eat most often. (If you usually just eat one kind, just choose one.)

| | | |
|---|---|---|
| ○ Low-carb cereals like Atkins, Low-Carb Special K | ○ Total | ○ Other fiber cereals like Raisin Bran, Fruit-n-Fiber |
| ○ Cheerios, Grape Nuts, Shredded Wheat, Wheaties, Wheat Chex | ○ Fiber One | ○ Sweetened cereals like Frosted Flakes, Froot Loops |
| | ○ Product 19, Complete | ○ Other cold cereals, like Corn Flakes, Rice Krispies, Special K |
| | ○ All Bran, Bran Buds | |

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**What vitamin supplements do you take fairly regularly?**

| **Multiple Vitamins.** Did you take... | HOW OFTEN: DIDN'T TAKE | A FEW DAYS per MONTH | 1-3 DAYS per WEEK | 4-6 DAYS per WEEK | EVERY DAY | FOR HOW MANY YEARS?: LESS THAN 1 YEAR | 1 YEAR | 2 YEARS | 3-4 YEARS | 5-9 YEARS | 10+ YEARS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Prenatal vitamins | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Regular Once-A-Day, Centrum, Theragran, "senior" vitamins or house brands of multiple vitamins | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Stress-tabs or B-Complex type | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| **Single Vitamins,** not part of multiple vitamins | | | | | | | | | | | |
| Vitamin A (not beta-carotene) | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Beta-carotene | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Vitamin C | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Vitamin E | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Folic Acid, Folate | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Calcium or Tums | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Vitamin D, alone or combined with calcium | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Zinc | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Iron | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Selenium | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Omega-3, fish oil, flax seed oil | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |

**If you took Once-a-day, Centrum or Thera-type multiple vitamins, did you usually take types that**
○ contain minerals, iron, zinc, etc. ○ do not contain minerals ○ Don't know

**If you took vitamin C,** how many milligrams of **vitamin C** did you usually take, on the days you took it?
○ 100 ○ 250 ○ 500 ○ 750 ○ 1000 ○ 1500 ○ 2000 ○ 3000+ ○ Don't know

**If you took vitamin E,** how many IUs of **vitamin E** did you usually take, on the days you took it?
○ 100 ○ 200 ○ 300 ○ 400 ○ 600 ○ 800 ○ 1000 ○ 2000+ ○ Don't know

**Did you take any of these supplements at least once a week?**
○ Ginkgo ○ St. John's Wort ○ Echinacea ○ DHEA ○ Didn't take these
○ Ginseng ○ Kava Kava ○ Melatonin ○ Glucosamine/Chondroitin

***SOME LAST QUESTIONS ABOUT YOU***

Would you say your health is ○ Excellent ○ Very good ○ Good ○ Fair ○ Poor

Are you currently trying to lose weight? ○ Yes ○ No

Was there ever a time in your life when you often drank more beer, wine or liquor than you do now? ○ Yes ○ No

Do you smoke cigarettes now? ○ Yes ○ No
**IF YES,** On average about how many cigarettes a day do you smoke now? ○ 1-5 ○ 6-14 ○ 15-24 ○ 25-34 ○ 35+

Are you ○ Hispanic or Latino ○ Not Hispanic or Latino

What race do you consider yourself to be? (MARK ALL THAT APPLY)
○ White ○ Asian ○ Native Hawaiian or Other Pacific Islander
○ Black or African American ○ American Indian or Alaska Native ○ Do not wish to provide this information

***Thank you very much for filling out this questionnaire.***
*Please take a minute to go back and fill in anything you may have skipped.*

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #



MM050470-2 321

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## Appendix 8.5 Dietary Supplement Questionnaire

**Supplement Questionnaire**

*(Please print)*

**Patient Name:** _____

**Date:** _____

| Supplement | Dose | Brand | Frequency | Duration |
|---|---|---|---|---|
| ☐ B vitamin complex | | | | |
| ☐ β - carotene | | | | |
| ☐ Calcium | | | | |
| ☐ Chondroitin | | | | |
| ☐ Copper | | | | |
| ☐ Dong Quai | | | | |
| ☐ Echinacea | | | | |
| ☐ Fish oil (DHA or EPA) | | | | |
| ☐ Omega 3, 6, 9 | | | | |
| ☐ Cod Liver oil | | | | |
| ☐ Flaxseed (ground) | | | | |
| ☐ Flaxseed (unground) | | | | |
| ☐ Flaxseed oil | | | | |
| ☐ Folic Acid (Folate) | | | | |
| ☐ Garlic | | | | |
| ☐ Ginko | | | | |
| ☐ Glucosamine | | | | |
| ☐ Goldenseal | | | | |
| ☐ Green tea | | | | |
| ☐ Iron | | | | |
| ☐ Kava | | | | |
| ☐ Lycopene | | | | |
| ☐ Magnesium | | | | |
| ☐ Multivitamin/multimineral | | | | |

**(Continued on reverse)**

| Supplement | Dose | Brand | Frequency | Duration |
|---|---|---|---|---|
| ☐ Selenium | | | | |
| ☐ St. John's wort | | | | |
| ☐ Valerian root | | | | |
| ☐ Vitamin B6 | | | | |
| ☐ Vitamin B12 (oral or injection) | | | | |
| ☐ Vitamin C | | | | |
| ☐ Vitamin D | | | | |
| ☐ Vitalux (for eye health) | | | | |
| ☐ Zinc | | | | |
| ☐ Other(s): _____ | | | | |
| ☐ _____ | | | | |
| ☐ _____ | | | | |
| ☐ _____ | | | | |
| ☐ _____ | | | | |

Dr. Fritz is committed to providing his patients with evidence-based care. In doing so, there may be research questions that he would like to answer using data collected during your visit to improve future patient care. Please know that in using such information your personal identity would not be revealed.

☐ Please check this box if you **DO NOT** want your information included in a future research study. Your decision will in no way impact the care you receive.

## Appendix 8.6 Patient Medical History Form

### Confidential Patient Information and Medical History

*(Please print)*

Patient Name: _____ Date of Birth (dd/mm/yyyy): _____

Home Address: _____

City: _____ Postal Code: _____

Home Telephone: _____ Cell Telephone: _____

Best Phone Number to Contact You: _____

E-Mail Address: _____ Marital Status: _____

Employer: _____ Occupation: _____

Business Address: _____

Business Telephone: _____

Name of your general dentist: _____ Referred by: _____

Do you have dental insurance? ☐ Yes ☐ No

In case of an emergency, contact:

Name: _____ Relationship: _____ Phone Number: _____

Family Physician's Name: _____ Phone Number: _____

Pharmacy: _____ Phone Number: _____

My last physical examination was: _____ Are you in good health? ☐ Yes ☐ No

Has there been any change in your health in the last year? ☐ Yes ☐ No

If so, please elaborate. _____

Has it ever been recommended that you routinely have antibiotic coverage before surgery or dental treatment? ☐ Yes ☐ No

**PLEASE LIST ALL MEDICATIONS YOU ARE CURRENTLY TAKING.**

_____

_____

_____

_____

**ARE YOU CURRENTLY TAKING ANY MINERAL, VITAMIN AND/OR HERBAL SUPPLEMENTS? IF SO, PLEASE SEE BLUE SHEET.**

**ALLERGIES - Are you allergic or have you reacted adversely to any of the following?**

| | Yes | No | | Yes | No |
|---|---|---|---|---|---|
| Aspirin | | | Codeine or other narcotics | | |
| Penicillin | | | Sedatives or sleeping pills | | |
| Tetracycline | | | Local Anesthetics | | |
| Other antibiotics | | | Latex gloves | | |
| Other allergies (please list): | | | | | |

**Do you smoke? ☐ Yes ☐ No**

If so, how many cigarettes/day? _____ How many years have you smoked? _____

**Are you a former smoker? ☐ Yes ☐ No**

If so, how many cigarettes/day? _____ How many years did you smoke? _____

How long ago did you quit? _____

**Do you have or have you had any of the following conditions?**

| | Yes | No | | Yes | No |
|---|---|---|---|---|---|
| High Blood Pressure/Heart Trouble | | | Sleep Apnea | | |
| Angina Pectoris | | | Artificial Joint | | |
| Mitral Valve Prolapse (MVP) | | | Kidney Trouble | | |
| Heart Murmur | | | Stomach Ulcer | | |
| Artificial Heart Valve | | | Glaucoma | | |
| Heart Surgery or Heart Attack | | | Liver Disease | | |
| Anemia | | | Hepatitis A/B/C | | |
| Excessive Bruising | | | Yellow Jaundice | | |
| Thyroid Disease | | | Leukemia | | |
| Hemophilia or Blood Transfusion | | | HIV (AIDS) | | |
| Persistent Cough | | | Venereal Disease | | |
| Emphysema/Bronchitis | | | Cold Sores | | |
| Asthma | | | Drug Addiction | | |
| Tuberculosis | | | Alcohol Dependency | | |
| Hayfever | | | Fainting | | |
| Sinus Troubles | | | Eating Disorders | | |
| Diabetes or Excessive Thirst | | | Psychiatric Treatment | | |
| Arthritis | | | Epilepsy/Seizures | | |
| Cancer | | | Stroke | | |
| Osteoporosis | | | Rheumatic or Scarlet Fever | | |

| | Yes | No |
|---|---|---|
| Have you ever been hospitalized? If so, what was the illness or operation? _____ | | |
| Are there any medical conditions that run in your family? (ie. High blood pressure, diabetes, cancer) | | |
| Have you ever had abnormal bruising or bleeding associated with previous extractions, surgery or injuries? | | |
| Have you had any serious trouble with any previous dental treatment? | | |
| Do you have any disease, condition or problem not listed above you think we should know about? | | |
| Women only: Are you pregnant? If so, what month are you due? _____ Are you nursing? | | |

Are you willing to spend 15 minutes a day to keep your teeth a lifetime? ☐ Yes ☐ No

On a scale of 1 to 5, how nervous are you about dental treatment? *(Please circle)*

(Not nervous at all) 1.....2.....3.....4.....5 (very nervous)

---

I understand the above information is necessary to provide me with dental care in a safe manner. I have answered all questions truthfully and to the best of my knowledge. I consent to your obtaining, from other practitioners who are currently treating me or have treated me, such further information as may be necessary for providing me with proper dental treatment and care.

Signature: _____ Date: _____

D.D.S.: _____

**Please be advised that our office policy is not to accept assignment of benefits as payment for accounts.**

## Appendix 8.7 Godin Leisure-Time Exercise Questionnaire

### Godin Leisure-Time Exercise Questionnaire

**INSTRUCTIONS**

In this excerpt from the Godin Leisure-Time Exercise Questionnaire, the individual is asked to complete a self-explanatory, brief four-item query of usual leisure-time exercise habits.

**CALCULATIONS**

For the first question, weekly frequencies of strenuous, moderate, and light activities are multiplied by nine, five, and three, respectively. Total weekly leisure activity is calculated in arbitrary units by summing the products of the separate components, as shown in the following formula:

Weekly leisure activity score = (9 × Strenuous) + (5 × Moderate) + (3 × Light)

The second question is used to calculate the frequency of weekly leisure-time activities pursued "long enough to work up a sweat" (see questionnaire).

**EXAMPLE**

Strenuous = 3 times/wk

Moderate = 6 times/wk

Light = 14 times/wk

Total leisure activity score = (9 × 3) + (5 × 6) + (3 × 14) = 27 + 30 + 42 = 99

Godin, G., Shephard, R. J.. (1997) Godin Leisure-Time Exercise Questionnaire. Medicine and Science in Sports and Exercise. 29 June Supplement: S36-S38.

## Godin Leisure-Time Exercise Questionnaire

1. During a typical **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number).

| | Times Per Week |
|---|---|
| a) **STRENUOUS EXERCISE** **(HEART BEATS RAPIDLY)** (e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling) | _____ |
| b) **MODERATE EXERCISE** **(NOT EXHAUSTING)** (e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing) | _____ |
| c) **MILD EXERCISE** **(MINIMAL EFFORT)** (e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking) | _____ |

2. During a typical **7-Day period** (a week), in your leisure time, how often do you engage in any regular activity **long enough to work up a sweat** (heart beats rapidly)?

| OFTEN | SOMETIMES | NEVER/RARELY |
|---|---|---|
| 1. ☐ | 2. ☐ | 3. ☐ |

## Appendix 8.8 Patient Report

Faculty of Applied Health Sciences
Department of Kinesiology &
Department of Health Sciences

Brock University
Niagara Region
500 Glenridge Ave.
St. Catharines, ON
L2S 3A1 Canada
T 688-5550 X3024
F 688-8364

wward@brocku.ca

Dear (Patient Name),

Thank you very much for participating in our research study, "Dietary Intakes & Periodontal Outcomes After Sanative therapy". As a token of our appreciation, please find your dietary intake report and your serum vitamin D results enclosed. If you have any questions about these reports do not hesitate to contact us or enquire at your next visit with Dr. Fritz.

The study is currently still in progress. Once final results of the completed study are available, you will receive a summary of outcome of the study.

Ultimately, your participation is making a valuable contribution to our understanding of the relationship between diet, supplement use and recovery after sanative therapy. This information will be available to periodontists and scientists worldwide, and as such, will likely help future patients undergoing a similar procedure to attain optimal periodontal health.

Sincerely,

Wendy Ward

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905-382-3308
dd12vw@brocku.ca

## Vitamin D Report

**Name:** Patient Name
**Sample Date:** Month 01, 2013

**Your Serum 25-Hydroxyvitamin D Level:** 75 nmol/L

**What does my serum 25-Hydroxyvitamin D level mean?**

| 25-Hydroxyvitamin D Level | Vitamin D status | What does this mean? |
|---|---|---|
| ≥ 75 nmol/L | Vitamin D Sufficient | Vitamin D levels are sufficient |
| 25 – 74 nmol/L | Vitamin D Insufficient | May benefit from additional Vitamin D |
| < 25 nmol/L | Vitamin D Deficient | At risk for rickets or osteomalacia |

(These categories are those used by LifeLabs Medical Laboratory Services)

**Information about Vitamin D[1]**

- It is important to get enough vitamin D from your diet because it helps our bodies absorb and use calcium and phosphorus for strong bones and teeth. Vitamin D can help protect older adults against osteoporosis.
- Vitamin D can also protect against infections by keeping your immune system healthy.
- It may help reduce the risk of developing chronic diseases such as multiple sclerosis and certain types of cancer, such as colorectal cancer but this is still being studied.

**How Much Vitamin D Do I Need?**

Health Canada currently recommends that men and women age 19-70 get 600 IU of vitamin D per day and that men and women 71 years and older get 800 IU of vitamin D per day[2].

However, some organizations such as the Canadian Cancer Society[3] and Osteoporosis Canada[4] recommend 1000 IU of vitamin D per day.

**Improving Vitamin D Status**

Food Sources of Vitamin D include[1]:

- Fatty Fish (250 IU/serving)
- Eggs (30 IU/egg)
- Milk Fortified with Vitamin D (100 IU/serving)

Since food provides relatively small amounts of vitamin D, vitamin D supplements are often needed to ensure an individual is meeting their vitamin D requirement.

***Sources of Information***
*1. Dieticians of Canada (www.dietitians.ca/Nutrition-Resources-A-Z/Factsheets/Vitamins)*
*2. Health Canada Dietary Reference Intakes (www.hc-sc.gc.ca/fn-an/nutrition /reference/table/index-eng.php).*
*3. Canadian Cancer Society (www.cancer.ca/en/prevention-and-screening/live-well/vitamin-d/?region=on).*
*4. Osteoporosis Canada (http://www.osteoporosis.ca/osteoporosis-and-you/nutrition/vitamin-d)*

**Dietary Intake Report**

**Name:** Patient Name

| | Your Average Daily Intake from Food | Health Canada Recommended Intake (Female 51-70) |
|---|---|---|
| **Macronutrients** | | |
| Calories (Kcal) | | Depends on Activity |
| Protein (g) | | 46 |
| Carbohydrates (g) | | 130 |
| Total Fat (g) | | Not yet determined |
| Saturated Fat (g) | | As low as possible |
| Monounsaturated Fat (g) | | Not yet determined |
| Linoleic Acid – Omega 6 (g) | | 11 |
| α-Linolenic Acid – Omega 3 (g) | | 1.1 |
| Dietary Fiber (g) | | 21 |
| Cholesterol (mg) | | As low as possible |
| **Minerals** | | |
| Calcium (mg) | | 1200 |
| Zinc (mg) | | 8 |
| Iron (mg) | | 8 |
| Potassium (mg) | | 4700 |
| Sodium (mg) | | 1200 |
| Magnesium (mg) | | 320 |
| **Vitamins** | | |
| Vitamin A (mcg RAE) | | 700 |
| Vitamin B1 – Thiamine (mg) | | 1.1 |
| Vitamin B2 – Riboflavin (mg) | | 1.1 |
| Vitamin B3 – Niacin (mg) | | 14 |
| Vitamin B6 (mg) | | 1.5 |
| Vitamin B9 – Folic Acid (DFE) | | 400 |
| Vitamin B12 (mcg) | | 2.4 |
| Vitamin C (mg) | | 75 |
| Vitamin E (mg) | | 15 |

Note: These values are general estimates of daily intake based on your responses to the food frequencies questionnaire and may not represent your actual intake on any given day. These values do not include supplements.

For more information and advice on how to meet your daily requirements, please visit Dietitians of Canada (www.dietitians.ca) or Canada 's Food Guide for Health Eating (www.hc-sc.gc.ca/fn-an/food-guide-aliment/index-eng.php).