THE EFFECT OF NON-SURGICAL PERIODONTAL INTERVENTION ON PULSE WAVE VELOCITY: A MARKER OF ARTERIAL STIFFNESS AND FUNCTION

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PREFACE

This thesis reports on research work related to the investigation of periodontal treatment on carotid-dorsalis pedis pulse wave velocity that was carried out during my PhD candidature at the school of Dentistry, the University of Adelaide and the Menzies School of Health Research, Darwin, Northern Territory from April 2010 until June 2014.

This thesis is structured in a publication format and consists of eight chapters. The literature review which develops the foundation for the present study aims and hypotheses is presented in Chapter 1. The general materials and methods for this study are presented in Chapter 2 while specific details of methods are further refined in the four manuscripts that are presented in Chapters 3-6 inclusive. Chapters 3-6 have been written as manuscripts for publication and have either been published or accepted for publication. Chapter 7 discusses the main findings of the four manuscripts and places them into context of the wider literature. Attempts to extrapolate the findings into making recommendations for future research are also included within Chapter 7. The appendices comprise the contents of Chapter 8.

Acknowledgements of key personnel and institutions that have assisted in one form or another in the work presented within this thesis have been recorded in pages xii-xiii. Additionally, to conform to journal requirements, acknowledgments of funding bodies and specific highlights to contributors have been made at the end of each manuscript chapter.

ABSTRACT

Cardiovascular disease and periodontitis are highly prevalent diseases that are thought to be associated by chronic inflammation. The aims of the present study were: 1) to determine whether a relationship between periodontal disease and arterial stiffness exists in a sample of Indigenous Australians with periodontal disease; and 2) using a randomised-trial design, determine whether treatment of periodontal disease will result in an improvement in arterial stiffness. Findings presented in this thesis indicate that a positive association between periodontal disease and arterial stiffness exists but that a single session of non-surgical periodontal therapy is insufficient to alter arterial stiffness.

DECLARATION

This work contains no material which has been accepted for the award of any other degree in any university or tertiary institution to Kostas Kapellas and, to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference has been made in the text.

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KOSTAS KAPELLAS

Dated this 20th day of June 2014

PUBLICATIONS ARISING FROM WORK CONTAINED WITHIN THIS THESIS

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Kapellas, K., Do, LG., Bartold, PM., Skilton, MR., Maple-Brown, LJ., O'Dea, K., Brown, A., Celermajer, DS., Slade, GD., Jamieson, LM. (2013) Effects of full-mouth scaling on the periodontal health of Indigenous Australians: a randomized controlled trial. Journal of Clinical Periodontology 40, 1016-1024. http://onlinelibrary.wiley.com/doi/10.1111/jcpe.12152/abstract

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http://hyper.ahajournals.org/content/64/4/702.short

CONFERENCE PRESENTATIONS FROM WORK ARISING IN THIS THESIS

K. Kapellas, LJ. Maple-Brown, PM. Bartold, A. Brown, LG. Do, K. O'Dea, GD. Slade, DS. Celermajer, LM. Jamieson, MR. Skilton. Oral presentation: Effect of a periodontal intervention on pulse wave velocity in Indigenous Australians with periodontal disease: the PerioCardio randomized controlled trial. World Congress of Cardiology, Scientific Session, Melbourne, Australia 6th May 2014

K. Kapellas, LM. Jamieson. Oral presentation: Is multiple imputation a correct method to assess missing data from an RCT with heavy loss to follow-up? 6th Dental Biostatistics Conference – Methodological Issues in Oral Health Research, Adelaide, Australia 2nd April 2014.

K. Kapellas, MR. Skilton, LJ. Maple-Brown, LG. Do, PM. Bartold, GD. Slade. Oral presentation: Periodontal Outcomes from Single-visit Non-surgical Periodontal Therapy among Indigenous Australians. International Association of Dental Research (IADR) 91st General Session, March 23rd Seattle, USA 2013.

CONFERENCE POSTER PRESENTATIONS

K. Kapellas, LJ. Maple-Brown, LM. Jamieson, LG. Do, K. O'Dea, A. Brown, DS. Celermajer, GD. Slade, MR. Skilton. The effect of periodontal therapy on carotid intima-media thickness among Indigenous Australians: A randomised controlled trial. World Congress of Epidemiology, Anchorage, USA 18th August 2014.

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1 INTRODUCTION

Periodontitis is a disease associated with infection of the supporting structures around teeth by a biofilm of commensal bacteria which is modified by a host inflammatory reaction. The oral cavity is home to hundreds of different species of bacteria, many of which are likely commensal and non-pathogenic (Socransky and Haffajee, 2002, Socransky et al., 1998). Several species not limited to *Porphyromonas gingivalis* and *Prevotella intermedia* have been associated with periodontal disease activity however, periodontal disease is a mixed infection given that the periodontal pocket is never colonised by a single species (Feng and Weinberg, 2006, Haffajee and Socransky, 1994). Bacteria thought to be pathogenic and associated with periodontal disease progression are often isolated from healthy periodontal pockets. Thus, although the presence of periodontal bacteria or dental plaque is necessary for disease to occur, oftentimes these same bacteria are not sufficient to cause periodontal disease. Instead, periodontitis should be considered the result of the immune and inflammatory responses to the presence of bacteria. Establishment of the bacterial complex initiates both innate and adaptive host-immune responses to combat this invasion. Despite an infectious aetiology, it is understood that periodontitis involves strong inflammatory components that contribute to the pathogenesis of the disease (Van Dyke and van Winkelhoff, 2013).

1.1 Concepts on atherogenesis

Atherosclerosis initially thought to develop from chronically elevated circulating lipids and oxidation of low-density lipoproteins (LDL) within arterial vessels, is now understood to also involve inflammatory and innate immune components. Endothelial dysfunction arising from the interaction of modified lipoproteins, free radicals from smoking, hypertension, hyperglycaemia, infectious organisms or a combination of the above, initiate a series of compensatory responses within the endothelium altering the homeostatic

properties of medium to large arteries (Ross, 1999). These changes commence with recruitment of monocytes from the circulation in response to adhesion molecule secretion by endothelial cells. Chemotactic factors promote monocyte migration within the intimal wall and subsequent maturation of macrophages, and following uptake of oxidised lipoproteins, the fatty streak forms (Pearson et al., 2003). The direct effect is to augment platelet and leukocyte adhesion leading to a pro-coagulant phenotype and the production of a host of growth factors, cytokines and vasoactive molecules inevitably influencing endothelial permeability and inflammation. Persistent inflammation stimulates the proliferation and migration of smooth-muscle cells into the site of injury creating the 'intermediate lesion'. Progression herein leads to thickening of the arterial wall which initially dilates to maintain blood flow, but over time the formation of the fibrous cap and the subsequent remodelling of the endothelium towards the 'advanced lesion' sees intrusion into the lumen and disruption of blood flow (Ross, 1999).

Inflammatory responses during the different stages of atherogenesis appear to be mediated by monocyte-derived macrophages, T lymphocytes (Jonasson et al., 1986, Shinmada, 2009) and dendritic cells (Yilmaz et al., 2007). One of the key protective roles macrophages play in the atheromatous lesion is to remove modified LDL from the circulation via phagocytosis. This process results in the accumulation of foam cells within the atheroma and continued recruitment of monocytes. Modified LDL is chemotactic for monocytes and promotes the up-regulation of monocyte-chemotactic protein and macrophage colony stimulating factor by endothelial cells. Through these mechanisms, the presence of LDL may sustain the inflammatory response by encouraging replication of monocyte-derived macrophages in addition to recruitment of new monocytes into inflammatory lesions.

1.2 Current understanding of aetiology - infection in vascular disease

An association between infection with the respiratory pathogen *Chlamydophila Pneumoniae* (formerly *Chlamydia Pneumoniae*) and atherogenesis has been established for some time (Kuo, 1999, Saikku, 1999). In 1988, the first serological association between antibodies to *C. Pneumoniae* and coronary heart disease was reported (Saikku et al., 1988). Reports since then have contributed to further understanding this relationship (Kuo et al., 1995, Siscovick et al., 2000). Given this purported infectious aetiology, several studies over the past decade have investigated the use of antibiotics for the secondary prevention of coronary events. The Weekly Intervention with Zithromax for Atherosclerosis and Related Disorders trial (O'Connor et al., 2003) enrolled 7,747 adults with a history of myocardial infarction but stable coronary artery disease and positive IgG titer for *C. Pneumoniae* from clinical practices in North America, Europe, Argentina and India, and followed participants for an average of 14 months. The intervention in this study was 600mg per day Azithromycin for one week followed by 600mg per week for weeks 2 through 12 inclusive. No significant risk reduction of any primary end-points including death, recurrent infarctions and revascularisation procedures were found at the conclusion of the trial (O'Connor et al., 2003). A similarly-designed trial on 4,012 North Americans also with stable coronary artery disease reported no significant risk reductions for cardiac events following a one-year course of weekly Azithromycin (Grayston et al., 2005). Furthermore, yet another study examined the benefits of antibiotic therapy in 4,162 patients from eight countries with acute coronary syndromes but also failed to find a beneficial effect after 10-day per month administration of the Fluroquinolone Gatifloxacin over two years (Cannon et al., 2005). To this end, there is little evidence in support of either short-medium or long-term antibiotic therapy against *C. Pneumoniae* providing a benefit for cardiovascular events and mortality. Confirmation of minimal evidence via two meta-analyses has since been published (Andraws et al., 2005, Song et al., 2008).

The low-grade but chronic inflammation seen in periodontitis may contribute to changes within arteries and consequently atherosclerosis. In periodontitis, bacterial infection is required for the inflammation to occur and persist. However, the most important factor from a periodontal perspective is the way in which the host responds to the presence of bacteria, determining the type and level of inflammation. In cardiovascular disease, inflammation can occur without primary infection, as oxidised LDL is known to elicit strong monocytic responses, foam cell maturation and expression of genes for macrophage colony stimulating factor (Ross, 1999). Under these conditions, a heightened inflammatory state may ensue and a subsequent superimposition of periodontal infection may exacerbate this process. This evokes an intriguing hypothesis that could be examined; whether periodontitis impacts arterial health.

Evidence exists in support of periodontal disease as a mode of infection being a risk factor for several systemic diseases including cardiovascular disease (Cullinan et al., 2009, Moutsopoulos and Madianos, 2006) and that infection by *P. gingivalis* may contribute to atherosclerosis in animals and humans (Brodala et al., 2005, Lalla et al., 2003, Pussinen et al., 2003). In this 'infection-mediated' pathway towards atherosclerosis, pathogen(s) must be chronically present and persistently active in generating a low-grade infection. Periodontopathogens fulfil these criteria making them plausible contributors in atherosclerosis.

Unlike the relatively infrequent occurrence of dental procedures, daily oral hygiene practices such as brushing and dental flossing (Crasta et al., 2009, Lockhart et al., 2008, Lockhart et al., 2009) and mastication (Geerts et al., 2002) have been reported to also lead to short-term bacteraemia. In terms of the potential magnitude for infection, dental procedures would be expected to elicit a greater bacterial load over a short period given their invasive nature compared to daily practices such as tooth brushing or mastication. However, the relative speed at which bacteria are cleared from systemic circulation may

indicate that bacteraemia is of little importance for otherwise healthy individuals. It is currently unclear what the long-term effects of chewing on a periodontally-compromised dentition are on systemic bacteraemia (Forner et al., 2006, Geerts et al., 2002, Murphy et al., 2006).

Transient bacteraemia commonly occurs following dental treatment procedures such as tooth extractions (Lockhart et al., 2008) and dental scaling (Forner et al., 2006, Reyes et al., 2013). Studies investigating bacteraemia following dental scaling show that in most instances, the immune response is sufficient to effectively clear bacteria soon after having entered the systemic circulation. In two reports, between 55-74% of study participants had periodontal bacteria isolated immediately following a 10-minute manual scaling procedure but only 14-19% were found to have circulating bacteria 30 minutes later (Castillo et al., 2011, Lafaurie et al., 2007). In another study where a single quadrant was scaled in patients with chronic periodontitis, 43% had bacteraemia 5 minutes after commencement, but only 7% had bacteraemia detected after 10 minutes (Zhang et al., 2013). The incidence of bacteraemia immediately following full-mouth scaling was 23% (7/23 participants) in one study (Kinane et al., 2005), while another reported an incidence of 75% 30 seconds after completion of scaling but only 10% after 30 minutes (Forner et al., 2006).

Chronic periodontitis has been simulated in animals in efforts to investigate biological evidence in support of the role of periodontal infection in atherosclerosis. Modelling chronic bacteraemia from periodontal infection, one study intravenously injected live *P. gingivalis* into heterozygous apolipoprotein $E^{+/-}$ mice weekly for between 10 to 24 weeks, after which time the mice were sacrificed and their aortic vessels examined for evidence of atherosclerosis progression (Li et al., 2002). Repeated systemic exposure to *P. gingivalis* led to accelerated atherosclerosis progression, however, external factors such as a genetic susceptibility and high-fat diet were also required since inoculated mice fed their regular diet also developed sclerotic lesions albeit smaller and at a slower rate. In support of an

inflammatory contribution to atheromas, systemic inflammatory levels of interleukin (IL)-1β and serum amyloid A were significantly up-regulated in mice fed a high fat diet. Similarly, ligature-induced periodontitis and subsequent oral inoculation of *P. gingivalis* in apolipoprotein E-null mice resulted in 40% larger atherosclerotic lesions compared to controls (Lalla et al., 2003). Furthermore, levels of plasma IL-6 and evidence of vascular activation in the form of significantly elevated vascular cell adhesion molecule-1 and tissue factor were significantly increased in infected mice relative to non-infected controls (Lalla et al., 2003). These findings do not unequivocally signify that *P. gingivalis* infection is the definitive cause of atherosclerosis in this model as apolipoprotein E-null mice are known to spontaneously develop rapid atherosclerosis (Plump et al., 1992, Zhang et al., 1994), but it is supportive of the concept.

More recently, a porcine model reported opposing results (Brodala et al., 2005). Normocholesterolemic pigs injected with *P. gingivalis* developed significantly greater aortic and coronary atherosclerosis compared to control animals while there was no significant difference in atherosclerosis rates in hypercholesterolemic pigs exposed to *P. gingivalis* compared to controls (Brodala et al., 2005). Since atherosclerosis developed in both hypercholesterolemic conditions and with *P. gingivalis* in normocholesterolemic conditions, this suggests that *P. gingivalis* bacteraemia may act independently of lipid status to promote atherosclerosis.

Animal studies have provided some insight into potential mechanisms by which periodontal bacteria may interact with the endothelium and underlying tissues to promote atherosclerosis. It is important to acknowledge that periodontal disease is never caused by a single strain of bacteria. Within a given periodontal pocket hundreds of different species can be detected (Wade, 2011, Zarco et al., 2012), few of which may be pathogenic, some opportunistic while the vast majority compose the natural oral flora. Further, these same bacterial species interact and compete for the same ecological niche subgingivally and

likely enter the systemic circulation to much the same extent as those modelled in the aforementioned studies. Animal studies investigating a single species provide, at best, proof-of-principal evidence of causation.

It has been proposed that infection by multiple pathogens and therefore the resulting 'pathogen-burden' is more likely to be a stronger risk factor for atherosclerosis than a single pathogen infection (Epstein, 2002). To model chronic systemic exposure to multiple pathogens, repeated intra-peritoneal immunisations of either *P. gingivalis, C. pneumoniae,* a combination of the two or control vehicle into apolipoprotein E-null mice resulted in marked atherosclerotic lesion formation after 22 weekly exposures to *P. gingivalis* compared to *C. pneumoniae,* mixed infections or control animals (Ford et al., 2007). This study also reported that superimposing a *C. pneumoniae* infection on an existing *P. gingivalis* antibody response led to a reduced disease development while the converse occurred when *P. gingivalis* was followed by a *C. pneumoniae* inoculation. Immunohistological assessment of the lesions revealed a predominance of macrophage/foam cell infiltration with few T lymphocytes, which corresponds to atheromatous lesions found in other animal models and in humans. While this study model is not a good representation of the route of infection seen in periodontitis, its strengths lie in the notion that pathogens, once having entered the systemic circulation, can be burdensome and illicit activation of the immune responses and atherogenesis.

Multiple studies have reported the presence of periodontopathogens or bacterial products within atherosclerotic lesions in humans. Two pathogens commonly detected in dental disease - *P. gingivalis* and *Streptococcus sanguis* - along with other bacteria of non-dental origin were identified during histological examination of carotid endarterectomy specimens and more than half contained two or more bacteria (Chiu, 1999). Similarly, 44 percent of atherectomy specimens contained bacteria of periodontal origin including *P. gingivalis* in another study (Haraszthy et al., 2000). Along with aortic and carotid

atheromatous plaques (Cavrini et al., 2005, Padilla et al., 2006, Figuero et al., 2011), periodontal bacteria have been found in thrombi of coronary arteries (Ohki et al., 2012), abdominal aortic aneurisms (Kurihara et al., 2004) and in the occluded arteries of patients with Buerger's Disease (Iwai, 2009).

The presence of bacteria or bacterial DNA surrounding and within arterial plaques as reported in these studies above does not indicate that bacteria are alive and pathogenic. To date, a single study has demonstrated that live *P. gingivalis* and *Actinobacillus actinomycetemcomitans* extracted from atherosclerotic plaques can invade human epithelial ECV-304 cells in culture (Kozarov et al., 2005). At the time this study was conducted, ECV-304 cells used were thought to have arisen from human umbilical vein endothelial cells. Subsequent DNA fingerprinting of this cell line has revealed that ECV-304 cells were in-fact contaminated with cells arising from a bladder carcinoma and thus are of epithelial origin (Dirks et al., 1999). Despite this difference, the work by Kozarov and colleagues (Kozarov et al., 2005) showed that live periodontal bacteria present in atheromatous tissue may be present as a result of bacterial invasion of host cells rather than due to phagocytosis.

Due to the biofilm-centred aetiology, antibiotic monotherapies cannot effectively treat periodontal disease (Socransky and Haffajee, 2002). Instead, antimicrobials are effective when used as adjuncts to the physical disruption of the biofilm via subgingival debridement and there is little evidence supporting antibiotic monotherapy for the treatment of periodontitis (Herrera et al., 2008). Promising findings have been reported in respect to the macrolide Azithromycin (Hirsch et al., 2012). Commonly used to treat bacterial infections of the upper respiratory tract, otitis media (Arguedas et al., 2005), sexually transmitted diseases and malaria (Hoepelman and Schneider, 1995, Miller et al., 2006), treatment of periodontal disease with adjunctive Azithromycin has shown positive outcomes in a series of case reports and one randomised trial (Gomi et al., 2007, Hirsch,

2010). A more recent randomised trial however, reported that clinical and microbiological outcomes were equivalent when comparing non-surgical monotherapy to periodontal treatment and adjunctive Azithromycin after 6-months (Sampaio et al., 2011). Given our understanding that periodontal disease is an inflammatory condition with an infectious aetiology, this drug along to a lesser extent other macrolides in its class, possess both bacteriostatic and anti-inflammatory properties which make it a potentially useful tool in the treatment of periodontal disease (Bartold et al., 2013, Hirsch et al., 2012). That said, periodontal treatment including Azithromycin was found to yield comparable clinical outcomes compared to Metronidazole in a rare trial assessing the two (Haffajee et al., 2007). In spite of these findings, current periodontal treatment guidelines reserve the prescription of adjunct antibiotics for patients that do not respond to conventional periodontal treatment. Reasons for this are few, however the most important of which is that (generally) conventional periodontal treatment including regular periodontal maintenance is a relatively easy and effective method to eliminate periodontal pockets. In most patients, adjunctive antibiotics will be unnecessary.

Periodontal and cardiovascular diseases are highly prevalent across the world and share common risk factors making it tempting to suggest periodontitis may increase the risk of developing cardiovascular disease. For example, both conditions occur more frequently in those with a history of smoking, people with diabetes and both are commonly associated with aging. It is possible that no causal relationship exists between periodontitis and cardiovascular diseases but that they both occur over the same timeframe. However, the traditional risk factors for coronary heart disease (high circulating cholesterol levels, hypertension, smoking, diabetes, level of exercise and obesity), only account for about half of the observed incidence of cardiovascular disease (Braunwald, 1997). It is currently unclear whether periodontitis constitutes an additional risk factor and if so, to what extent. The biochemical and physiological roles of infection and inflammation present in

periodontitis may work concurrently with the traditional cardiovascular disease risk factors to initiate the early stages of atheroma formation and promote the progression of the disease. Current evidence from the cardiology literature does not support generic prescription of antibiotics for the prevention of cardiovascular events. Similarly, antibiotics should be reserved for specific cases of periodontal disease that are unresponsive to traditional methods of treatment.

1.3 Surrogate markers of cardiovascular disease

Inflammatory markers including C-reactive protein (CRP), interleukin 6 (IL-6) and serum amyloid A are abnormally elevated several years prior to cardiovascular events (Pearson et al., 2003). Prospective studies conducted on individuals without a previous history of cardiovascular disease have shown that elevated plasma concentrations of CRP are strong independent predictors of future myocardial infarctions and stroke in men and women (Ridker et al., 1997, Ridker et al., 2000). Importantly, the level of CRP can be used to predict cardiovascular event risk among people with currently recommended levels of blood cholesterol (Ridker et al., 2000). A clinical trial conducted among healthy participants considered to be at low risk for cardiovascular events based on Framingham risk scores but at elevated risk based on CRP levels of ≥2mg/L provided strong evidence in support (Ridker et al., 2008). Study participants randomised to statin therapy halved their LDL concentrations and reduced CRP levels by 37% resulting in a 44% risk reduction for major cardiovascular events compared to placebo. The predictive validity of screening tests in terms of coronary risk are strengthened by adding CRP levels to the traditional fasting lipid assessment (Ridker, 2003, The Emerging Risk Factor Collaboration, 2010, Wilson et al., 2008). Although CRP evaluation is now used in risk assessments, CRP in and of itself is not a risk factor for cardiovascular events but rather an indicator of the existing inflammatory burden.

Numerous observational studies have confirmed that people with periodontal disease have elevated systemic levels of CRP and IL-6 when compared to healthy controls (Amar et al., 2003, Ebersole et al., 1997, Glurich et al., 2002, Loos et al., 2000, Noack et al., 2001, Fitzsimmons et al., 2009). Aggressive forms of periodontitis result in exceedingly high CRP and IL-6 concentrations (Salzberg et al., 2006, Sun et al., 2009), and evidence from the dental component of the 'Atherosclerosis Risk in Communities Study' indicates that participants with extensive periodontal pocketing (defined as 30% or more of sites with probing pocket depths of ≥4mm) had CRP levels that were a third higher than individuals exhibiting less than 10% pocketing eluding to a potential dose-dependent relationship (Slade et al., 2003).

Along with infection and inflammation, periodontitis has also been associated with lipid disturbances including increased levels of total cholesterol and low-density lipoprotein (LDL) (Cutler et al., 1999, Katz et al., 2002), reduced high-density lipoprotein (HDL) (Buhlin et al., 2003), and elevated levels of triglycerides (Cutler et al., 1999, Lösche et al., 2000). Periodontitis has also been implicated in reducing the anti-atherogenic influence of HDL, possibly due to how inflammation disturbs the reverse cholesterol transport process (Pussinen et al., 2004).

Evidence from a meta-analysis of five prospective cohort studies including data from over 86,000 patients revealed that people with periodontitis had 14% higher risk (95% CI 1.1-1.2) of developing coronary heart disease than controls (Bahekar et al., 2007). Within the same study, meta-analysis of five cross-sectional studies inclusive of some 17,000 patients indicated that the prevalence of coronary heart disease is 1.59 (95% CI 1.3-1.9) in people with periodontitis compared to those without (Bahekar et al., 2007).

Systematic and state-of-the-science reviews have recently summarised the effects of periodontal intervention trials on inflammatory markers (D'Aiuto et al., 2013, Demmer et al., 2013, Teeuw et al., 2014). Limiting to randomised trials, systemic concentrations of

CRP were significantly reduced in patients undergoing periodontal treatment compared to untreated controls (WMD -0.37mg/L [95% CI -0.63, -0.11]) and sub-group analysis revealed a greater effect for periodontal treatment with adjunct antibiotics versus no-treatment (WMD -0.75 mg/L [95% CI -1.17, -0.33]) (Demmer et al., 2013). More recently, another systematic review with meta-analysis combined data from randomised and clinical controlled trials to report that mean CRP reduction in the treatment versus no-treatment groups was comparable to the former (WMD -0.50 mg/L [95% CI -0.78, -0.22]) (Teeuw et al., 2014). Two meta-analyses considering change in systemic IL-6 have also been conducted but have reported conflicting findings (D'Aiuto et al., 2013, Teeuw et al., 2014). Restricting to seven randomised trials, periodontal treatment was not found to reduce serum IL-6 concentrations (standardised mean difference -0.13 ng/L, p=0.14) (D'Aiuto et al., 2013) however, a meta-analysis including clinical controlled trials reported a larger and significant reduction (WMD 0.48 ng/L [95% CI -0.90, -0.06]) (Teeuw et al., 2014).

Systematic reviews and meta-analyses evidence relating to the effects of periodontal treatment on lipids are similarly conflicted. No significant differences to any lipid markers were found when only assessing randomised trials leaving the authors to conclude that *"there was moderate evidence to not support any positive effects of periodontal treatment on lipid profiles"* (D'Aiuto et al., 2013). In contrast, after combining clinical controlled with randomised trials, total cholesterol (WMD -0.11 mmol/L [95% CI -0.21, -0.01]) and HDL (WMD 0.04 mmol/L [95% CI 0.03, 0.06]) were found to be reduced in favour of periodontal treatment compared to no-treatment (Teeuw et al., 2014).

Debate exists on whether systematic reviews should be limited to randomised trials (Shrier et al., 2007). By design, randomised trials are considered to be free from the effects of confounding and if conducted well, provide greater evidence when testing causal relationships than other study designs. Alternative arguments posed contest that randomised trials although considered free from bias and confounding at baseline are not

necessarily superior to a controlled clinical study if affected for example, by attrition bias. Additionally, meta-analysis of different study designs may be appropriate in instances where randomisation is unethical. An obvious example would be investigating the effects of tobacco smoking on health status (Shrier et al., 2007). Based on the combined evidence from randomised and non-randomised studies reviewed, there is sufficient evidence to indicate that periodontal treatment results in statistically significant reductions in biomarkers of cardiovascular disease. However, it is important to note that studies reviewed to date have provided evidence for short-medium term (≤6 months) changes in these markers and the long-term clinical significance of these changes is currently unknown.

1.4 Surrogate measures of cardiovascular status

1.4.1 Pulse Wave Velocity (PWV)

While the role of infection on atherosclerosis is still being clarified, long-term chronic inflammation and aging are known to be considerable contributors to the structural and functional changes of arterial walls leading to reduced elasticity and increased stiffness. The degree of arterial stiffening can be effectively measured non-invasively via pulse wave velocity (PWV) (Laurent et al., 2006). This method is thought to be the easiest, most reproducible and robust approach to determine arterial stiffness (Boutouyrie et al., 2009). Measured along the aortic and aorto-iliac arteries, carotid/femoral PWV has been widely used to demonstrate cardiovascular risk and predict future events in numerous studies (Blacher et al., 1999a, Blacher et al., 1999b, Boutouyrie et al., 2002, Cruickshank et al., 2002, Laurent et al., 2001, Laurent et al., 2003, Mattace-Raso et al., 2006, Meaume et al., 2001, Sutton-Tyrrell et al., 2005, Willum Hansen et al., 2006). Unlike traditional measures of CVD risk such as blood cholesterol and blood pressure, measurement of vascular

stiffening provides a representation of the cumulative impact of both known and unknown risk factors which may include periodontal disease.

Associations between periodontal disease and arterial stiffness have so far, been limited to observational studies. Using the Community Periodontal Index of Treatment Needs to define periodontal disease and the brachial-ankle PWV (baPWV) as a surrogate measure of atherosclerosis, the first study examined 291 Japanese men cross-sectionally but no relationship was found between the two conditions following adjustment for confounders including age, smoking and systolic blood pressure (Miyaki et al., 2006). Validation of the baPWV has shown it to correlate with other measures of central arterial stiffness (Sugawara et al., 2005, Yamashina et al., 2002) however the measure is yet to be assessed for its relationship to mortality and morbidity of cardiovascular diseases. A relationship between increasing periodontal pocketing but not clinical attachment loss and the cardio-ankle vascular index (CAVI) has been reported in a study of 1053 Japanese participants following adjustment for common confounders including age, sex, lipids, smoking status and glycaemic control (Hayashida et al., 2013). The CAVI simultaneously assesses arterial stiffness of the aorta, femoral and tibial arteries (Kadota et al., 2008) and is thought to be a superior measure to baPWV on account that it is not susceptible to blood pressure variability during measurement (Takaki et al., 2008). For each 1mm increase in mean periodontal pocket depth, CAVI increased by 0.13 units (p=0.040) in multiple linear regression analysis and, using the same periodontal pocket depth increment, a high CAVI defined as ≥8 units resulted in an odds-ratio of 1.32 (95% CI 1.00 to 1.74, p=0.047) following multiple logistic regression analysis (Hayashida et al., 2013). The CAVI is yet to be validated against the gold-standard carotid/femoral PWV making interpretation of these findings difficult. An association between peripheral (carotid-radial) PWV and periodontal inflammation has been reported in a cohort of patients with type 2 diabetes (Franek et al., 2012). In that study, 18 subjects with periodontitis from an overall sample of 121

participants presented with increased PWV when compared to those periodontally healthy or with gingivitis; the differences were non-significant.

Only two studies have examined associations between periodontal disease and carotid-femoral PWV (Shanker et al., 2013, Vieira et al., 2010). In a sample of 79 people with heterozygous familial hypercholesterolemia, carotid-femoral PWV was significantly higher among those with severe periodontitis compared to non-severe periodontitis (mean [SD] 9.7m/s [1.7] versus 9.0 m/s [1.2], p=0.03) respectively (Vieira et al., 2010). However, those with severe periodontitis were on average, 8 years older and had significantly higher diastolic blood pressure than those without meaning this finding is likely confounded by both age and blood pressure. Finally, carotid-femoral PWV was compared in 532 people with gingivitis against 282 people with periodontitis in a recent study from India (Shanker et al., 2013). The methods used to assess oral status are unclear however it appears that visual criteria were employed for both disease classifications. That aside, those with periodontitis presented with higher PWV than those with gingivitis (mean [SD] 9.8m/s [1.9] versus 8.5 m/s [1.0], (p<0.001) respectively (Shanker et al., 2013). Currently, there have not been any studies that have investigated whether treatment of periodontal disease can influence PWV.

In summary, there is conflicting evidence of an association between periodontal disease and arterial stiffness on the basis that notable heterogeneity between studies completed to date. Firstly, different anatomical sites have been used to measure arterial stiffness and not all locations are comparable to the gold standard carotid-femoral PWV (Tillin et al., 2007). Peripheral arteries supported by muscle are less susceptible to atherosclerosis than central arteries (Greenwald, 2007). Principally, this is attributed to differences in the physiology of central versus peripheral vasculature. For example, in large elastic vessels such as the aorta, pulmonary and carotid arteries, the elastin content is reduces with age while the collagen content of these arteries increases (Hosoda et al., 1984). A fundamental role of the

central arteries is to buffer the blood bolus during the cardiac cycle and use the stored energy within arterial walls to facilitate blood flow systemically. At these sites, the pressure imposed by the blood on the central vessels is high thus the requirement for high quantity of elastin. Progressive ageing (which may be thought of conceptually as *wear* and *tear*) of the arteries leads to fragmentation of elastin fibres and subsequent repair with collagen – leading to arterial stiffness. Conversely, peripheral arteries receive blood under markedly reduced pressures and therefore the requirement for distensibility (the requirement to stretch or dilate to facilitate blood flow) is low. Physiologically, peripheral arteries contain higher quantities of vascular smooth muscle cells and a lower content of elastin making peripheral arteries less susceptible to atherosclerosis (Boutouyrie et al., 1992, Greenwald, 2007, Bortolotto et al., 1999). The second point of difference is the use of various devices to measure PWV. Four commercial devices have currently been validated as accurate measures of arterial stiffness (Boutouyrie et al., 2009). Of the five studies reviewed, only two have used an accepted device; Compilor System® (Artech, France) (Vieira et al., 2010) and SphygmoCor® (AtCor Medical, Australia) (Franek et al., 2012).

1.4.2 Flow Mediated Dilatation

Early, preclinical events preceding atherosclerosis are characterised by an altered function of the endothelium (Deanfield et al., 2007). Traditional cardiovascular risk factors such as hyperlipidaemia, diabetes, smoking and hypertension are associated with intra-vascular oxidant stress (Bonetti et al., 2003). This leads to reduced bioavailability of nitric oxide (NO), a potent vasodilator (Joannides et al., 1995, Vallance and Chan, 2001), and impedes the ability of arteries to cope with sudden increases in blood volume. Nitric oxide also inhibits adhesion of inflammatory cells to vascular walls while also mediating proliferation of smooth muscle cells (Moncada and Higgs, 2006). Endothelial function can

be measured non-invasively via flow-mediated dilatation (FMD) which employs high resolution ultrasound to image and record blood flow following temporary occlusion of an artery (Alam et al., 2005, Corretti et al., 2002, Faulx et al., 2003). Commonly measured at the brachial artery, blood flow is increased following release of an occlusion cuff causing dilation of the vasculature distal to cuff placement. An acute increase in blood flow heightens arterial sheer stress resulting in dilation of the brachial artery. Until recently, it was thought that vasodilation is primarily controlled by endothelial synthesis of NO (Deanfield et al., 2007, Faulx et al., 2003) and that FMD provides an index of endothelium-derived NO function. A recent meta-analysis however, has shown that FMD has equal prognostic value for future cardiovascular events when measuring NO-dependent versus NO-independent mechanisms suggesting that FMD is not solely a proxy for NO (Green et al., 2011) and that other mechanisms contribute to endothelial function.

Endothelial dysfunction may be indirectly influenced by inflammation through its effects on suppressing arginine, a substrate for NO synthase. A dose-dependent negative association between hsCRP and arginine was reported in 746 elderly people participating in the Hoorn Screening Study of the Netherlands (van der Zwan et al., 2011). The authors of this study proposed that inflammation contributed to increased arginase (its effect is to degrade arginine) production and a concomitant stimulus for ADMA synthesis (inhibitor of NOS). Consistent with these findings, periodontal disease severity measured by CAL and maximum probing depth in 108 patients with hypertension was found to be positively associated with increased ADMA and that higher concentrations of hsCRP further contributed to this relationship (Tsioufis et al., 2010).

Understanding potential associations between periodontal inflammation and impaired endothelial function has arisen from several small observational and intervention studies. The first showed that brachial artery FMD was significantly lower in a sample of non-smoking healthy subjects with severe periodontitis compared to age and sex-matched

periodontally-healthy controls (Amar et al., 2003). Those with severe periodontitis were reported to show evidence of lower FMD (mean [SD] 7.8% [4.6] versus 11.7% [5.3], p=0.005) and twice the level of hsCRP (2.3 mg/L [2.3] versus 1.0 mg/L [1.0], p=0.03) compared to controls. Another study involving 30 patients with severe periodontitis and 31 periodontally healthy controls also reported reduced FMD among subjects with periodontitis (6.1% [4.4] versus 8.5% [3.4]) (Seinost et al., 2005). Following conventional non-surgical periodontal therapy with adjunct systemic antibiotics, FMD in patients with periodontitis improved to 9.8% [5.7%], p=<0.01 while hsCRP was also significantly reduced after three months. An earlier study reported a greater magnitude of improvement in FMD 6-weeks after conventional treatment was completed without adjunctive of antibiotics (Mercanoglu et al., 2004). Here, FMD increased from a baseline 8.4% [4.0] to 17.7% [5.7], p=<0.01. Similarly 22 people with periodontitis undergoing full-mouth disinfection showed that FMD improvement (baseline 8.6% [4.7] versus 10.2% [3.9], p=0.034) can be obtained after as little as one month following periodontal treatment if accompanied with periodontal surgery and extraction of teeth that cannot be saved (Elter et al., 2006).

Only one randomised trial has compared intensive periodontal treatment comprising of scaling and root planing, extraction of 'hopeless' teeth and localised administration of Minocycline microspheres into pockets versus supragingival scaling and polishing (Tonetti et al., 2007). The authors demonstrated that an improvement in periodontal status and a reduction of inflammation in the intensively-treated group was the primary driver for the concomitant improvement in FMD after 2 and 6 months (Tonetti et al., 2007). Results from this trial show promise for a new area of research however it may not be appropriate to extrapolate to a general population with periodontal disease for at least two reasons. Firstly, the classification of severe periodontitis used for inclusion into the study required PPD ≥6 mm and marginal bone loss of >30% affecting 50% or more of teeth. Recent

evidence from NHANES 2009-2010 data showed that only 8.5% of the US population, and up to 11% of those aged 65 years or older would be classified with 'severe' periodontitis using a lower threshold of the CDC-AAP periodontitis case definition (Eke et al., 2012). Secondly, the periodontal treatment provided to those randomised to the intensive intervention group may not be practicable in a general dental setting.

Of interest, in many of the studies reviewed above, FMD was impaired prior to periodontal treatment while nitroglycerin-mediated FMD did not significantly differ. This further supports the notion of inflammation impeding NO function within the vasculature since resolution of periodontal inflammation as evidenced by reduced hsCRP levels commonly leads to improved FMD. Though studies to date have provided evidence of the benefits treatment of periodontal disease can have on endothelial function, there is still insufficient evidence of its value in terms of long-term cardiovascular risk reduction (Bouchard et al., 2010, Lockhart et al., 2012).

1.4.3 Carotid Intima Media Thickness

Thickening of the intima and media of the carotid artery (IMT) is strongly associated with the prevalence of cardiovascular disease, the incidence of myocardial infarctions and stroke (Bots et al., 1997, Yamashina et al., 2002, Lorenz et al., 2006). Often, decades of arterial wall disturbances precede the clinical signs and symptoms of cardiovascular disease. The very early changes to the endothelium of arteries can be measured using high resolution B-mode ultrasound to determine sub-clinical atherosclerosis (Persson et al., 1994, Touboul et al., 2004, Bots et al., 2003). This non-invasive measure can be easily obtained with minimal training and is highly reproducible making it a valuable clinical and diagnostic tool in medical and epidemiological research. Thickening of the intima-media complex and arterial plaques are commonly detected distal to the carotid bulb where volumetric turbulence is high. As cIMT thickens with age, the absolute value of cIMT in

relation to risk for future events is age-dependant. Data from the Carotid Atherosclerosis Progression Study indicate that for each 0.1 mm increase in cIMT above 0.8 mm, the hazard rate ratio for future myocardial infarctions in people aged under 50 years was 1.34 (95% CI 1.16-1.55) (Lorenz et al., 2006). Recently, a meta-analysis of 16 studies comprising almost 37,000 individuals which contributed to some 257,000 person-years of data reported the hazard ratio for future myocardial infarction was 1.22 (95% CI 1.14-1.30) for each standard deviation increase in cIMT after adjustment for age, sex and mean cIMT progression (Lorenz et al., 2012).

A number of observational studies have investigated potential associations between carotid arterial wall thickness and periodontitis. Using case-control study designs, three investigations all with relatively small samples (less than 100 in each), reported that severe periodontitis is associated with sub-clinical atherosclerosis of either the common or internal carotid arteries (Cairo et al., 2008, Leivadaros et al., 2005, Soder et al., 2005). Stronger evidence was provided from the dental component of the Atherosclerosis Risk in Communities (ARIC) study (Beck et al., 2001) where cross-sectional data from 6,017 participants aged 52-75 years showed severe periodontitis defined as ≥30% extent in CAL of 3mm or more was associated with 1.31 (95% CI 1.03-1.66) times the odds of having IMT ≥1.0 mm compared to non-periodontitis cases after adjustment for confounders in multivariate logistic regression modelling. Consistent findings were more recently reported among 1,053 Japanese participants aged 40 years or older whereby a 1mm increase in mean PPD corresponded with an increased risk of carotid IMT ≥1mm (OR=1.43 [95% CI 1.07-1.92], p=0.017) (Hayashida et al., 2013). Conversely in a sample of 5,359 elderly South Koreans, unadjusted estimates of PPD and CAL were significantly associated with carotid IMT but not following adjustment for some 15 potential confounders including age, sex, medications and anthropometric variables although bleeding on probing was found to be significantly associated with IMT in women only (Jung et al., 2014).

Mechanisms unifying the association between periodontitis and IMT have focused on the aggregate bacterial burden as evidenced by the direct relationship between sub-clinical atherosclerosis and periodontal bacterial species (Desvarieux et al., 2005). The Oral Investigations and Vascular Disease Epidemiology Study (INVEST) reported that increased cIMT was associated with the presence of high counts of etiologic bacteria commonly thought to represent active periodontitis when compared to commensal flora predominantly found in health (Desvarieux et al., 2005). Prospective analysis of this cohort of participants over a median 3 years (range 2-7 years) has recently reported that natural progression of IMT is significantly lower when either the number of periodontal pockets reduce or where a decrease in the amount of etiological bacteria occurs compared to baseline (Desvarieux et al., 2013). Reasons to explain the attenuation of IMT progression was beyond the aims of the oral INVEST study. It is highly probable that an improvement in periodontal status and reduction in causative bacteria could have occurred in response to periodontal treatment. Although the INVEST study did not stratify their prospective findings by those whom had received periodontal therapy, the study nevertheless, provides additional support to the earlier observational findings and further suggests that the association between periodontal disease and IMT may be dose-dependent. Extrapolation of these findings would advocate that successful periodontal therapy could be beneficial to the vasculature.

A single non-randomised pilot study comprising 35 adults with a mean age of 46.2 years [range 38-57] and moderate to severe periodontitis has reported the effects of periodontal treatment on carotid IMT (Piconi et al., 2009). Following conventional non-surgical periodontal treatment conducted over a 4-week period, carotid IMT reduced from mean [SD] 0.49 mm [0.02] at baseline to 0.38 mm [0.04] 6-months later, p=0.003 and 0.37 mm [0.03] after one year, p<0.001. It is difficult to draw any meaningful conclusions from a single study which did not include randomisation and had a small sample size. Therefore

at the present time, it is only possible to surmise that evidence periodontal treatment has any effect on vascular structure is limited.

1.5 Periodontal intervention studies and cardiovascular events

There have not been any intervention trials that directly explore the effect of periodontal treatment on the incidence of new CVD events. However, the Periodontitis and Vascular Events (PAVE) study investigated, using a multi-centre randomised trial design, whether periodontal treatment reduced the risk of future cardiovascular events in patients with periodontitis and coronary artery disease (Beck et al., 2008, Offenbacher et al., 2009). This pilot study was somewhat compromised by the fact that one third of people randomised to the control (community care group whereby participants were free to access dental services on their own volition) received some degree of dental prophylaxis and 20% of the control group received scaling or periodontal surgery within 6-months from recruitment. Despite the number of cardiovascular events reported being twice as high for control versus treatment (6.6% versus 3.3%, P=0.19), findings from this trial revealed no differences in cardiovascular events between the two randomised groups (Beck et al., 2008).

The PAVE study highlighted potential ethical issues that may arise when conducting intervention studies on patients considered at risk for future cardiovascular events. In line with current understanding of the association between periodontal and cardiovascular disease, the hypothesis of the PAVE study suggested that periodontal disease, as a chronic source of infection and inflammation may increase the risk of future events. It may be considered unethical to deny periodontal treatment to people deemed “at risk”. There are few alternatives available for a control-arm in a periodontal intervention other than the community care approach utilized in the PAVE. A larger sample size recruited and followed-up for a longer period may have yielded different results. Being the first trial,

there was insufficient evidence to warrant funding and conducting a study of greater magnitude than the PAVE.

1.6 Indigenous Australian health

Indigenous Australians are considered to have the oldest living culture in the world and they represent 2.5% of Australia's population (ABS, 2009). Compared to non-Indigenous Australians, Indigenous Australians exhibit social and economic disadvantage resultant from lower educational attainment, higher unemployment rates and overcrowded housing (AIHW, 2005). Life expectancy is estimated to be some 10-12 years lower than non-Indigenous Australians primarily due to high rates of infection and chronic diseases (ABS, 2009, AIHW, 2005, AIHW, 2010). Factors driving observed differences in life expectancy are related to social disadvantage (Marmot, 2011) just as much as some lifestyle factors. For example, almost half (46%) of Indigenous adults aged 15 years of older are current smokers compared to 21% of Australians overall (AIHW, 2010). Chronic alcohol consumption at risky levels has been steady at 15-16%, marginally higher than non-Indigenous Australians (AIHW, 2008, AIHW, 2005) while the prevalence of type 2 diabetes among Indigenous Australians is almost 3.5 times higher than in non-Indigenous Australians (ABS, 2007, AIHW, 2010). The incidence of type 2 diabetes is also known to occur earlier among Indigenous Australians (AIHW, 2008).

Diseases of the cardiovascular system are estimated to account for 30% of deaths annually (World Health Organisation, 2005). Equally, one third of all deaths in Australia are attributed to a cardiovascular disease aetiology (AIHW, 2010). In a recent national health survey, 17 percent of Australian adults reported at least one existing disease of the circulatory system (AIHW, 2010). The incidence of coronary heart disease however, occurs much earlier among Indigenous Australians compared to their non-Indigenous counterparts (Wang and Hoy, 2013) and more often leads to adverse outcomes (Brown,

2009, Coory and Walsh, 2005). Accounting for differences in life expectancy, the age-standardised mortality by cardiovascular disease was 3 to 3.7 times higher for men and women respectively compared to non-Indigenous Australians (AIHW, 2008).

Few studies have examined the oral health status of Indigenous Australian adults. A survey in western New South Wales identified extensive treatment need for dental restorations, a high prevalence of periodontal disease and poor oral hygiene practices (Schamschula et al., 1980). Among young adults aged 16-23 years, the mean DMFT was reported to be 17.1 with the majority (87%) untreated caries. Furthermore, dental caries and periodontal disease was attributed to at least 75% of teeth lost in those aged 40 years or older (Schamschula et al., 1980).

Research from northern Western Australia revealed surprisingly consistent findings. DMFT ranged from 4.6 through to 15.5 in a sample aged between 18 to 88 years while 60% of study participants reported infrequent tooth brushing habits (Kruger et al., 2008). Periodontal disease defined as the presence of periodontal pocketing of ≥4 mm was diagnosed in 39% of participants and additionally, two-thirds were deemed as needing dental restorations while 50% required extraction of at least one tooth (Kruger et al., 2008). A similar cross-sectional investigation of five other rural and remote Western Australian communities also reported problem-associated visiting patterns, infrequent brushing, a high proportions of people with untreated caries and periodontal pocketing of ≥4 mm in 999 Indigenous Australian adults aged between 18 to 85 years (Smith et al., 2007).

More recently, the prevalence of severe periodontitis was reported to be high in an urban Indigenous Australian population (Roberts-Thomson et al., 2014). Findings in this convenience sample of 251 people were consistent with the wider literature. For example, almost 60% were current smokers and a quarter had been diagnosed with diabetes (Roberts-Thomson et al., 2014). Cigarette smoking is known to increase the risk of periodontitis (Do et al., 2008, Bergström, 2006), and the relationship between periodontitis

and diabetes is thought to be bi-directional (Lalla and Papapanou, 2011, Preshaw et al., 2012).

The National Survey of Adult Oral Health (NSAOH) provided representative data for the Australian population. There were no significant differences in the prevalence of DMFT among Indigenous Australians of all ages; 14.8 [95% CI= 12.2-17.3] compared to 12.8 [95% CI= 12.4-13.3] for non-Indigenous Australians (Roberts-Thomson and Do, 2007). Similarly, although mean untreated caries was more than threefold higher among Indigenous Australians (2.7 [95% CI= 0.6-4.8] compared to 0.8 [95% CI= 0.7-0.9]), there was no significant difference when precision of the estimates were compared. Estimates for the prevalence of moderate to severe periodontal disease were 29% [95% C= 16.7-45.5] compared to 23% [95% C= 21.2-24.6] for Indigenous and non-Indigenous Australians respectively (Roberts-Thomson and Do, 2007). Oral assessments were conducted in only 87 Indigenous Australians in the NSAOH out of a total sample of 5,505 participants.

Questions have been raised concerning the true representativeness of Indigenous Australian estimates (Australian Research Centre for Population Oral Health, 2009). That aside, there is presently limited information on the true oral health status of Indigenous Australians at a population level. Given that stark general health disparities exist between non-Indigenous and Indigenous Australians, it is possible that estimates from the NSAOH are in-fact underestimations of the extent and severity of dental disease.

1.7 Study aims

Presently, it is uncertain whether periodontopathogens are sufficient to initiate the atherogenic process following bacteraemia. Such a cause will require an inflammatory response or direct specific host immune effect on the bacteria following displacement from the periodontal tissues. To elaborate, assuming vascular health with no evidence of

atherosclerosis, bacteraemia arising from periodontitis would have to adhere to or invade the endothelium prior to initiation of atherogenesis. Both *in-vitro* and *in-vivo* studies suggest this may plausible (Choi et al., 2005, Deshpande et al., 1998, Honda et al., 2005). Alternatively, periodontal bacteria may encounter already-formed atheromas and either invade or become incorporated within such lesions as a result of the perturbed endothelium housing high concentrations of immune cells. Therefore, the presence of bacteria usually associated with periodontitis within atheromas thought to arise from bacteraemia may represent a secondary phenomenon within the vessels.

Extending these two concepts to include periodontal disease, which we currently understand to have strong infection and inflammatory aetiologies, poses the following question: What will happen to atherosclerosis if we effectively treat periodontitis? If periodontal disease can be treated and more importantly stabilized long-term, then periodontal infection and the corollary inflammation can be eliminated from the potential causal pathway of atherogenesis. Promising results from recent periodontal intervention trials have shown that modification of oral inflammation can positively influence endothelial dysfunction (Elter et al., 2006, Tonetti et al., 2007) however treatment of periodontal disease cannot, as yet, be promoted as a preventative regimen for cardiovascular disease. Similarly, current studies to date have been unable to determine whether direct infection of the arterial wall by periodontal bacteria or whether a pro-inflammatory state in response to periodontitis initiates or exacerbates atherosclerosis.

The aim of this study was to quantify the effect of non-surgical periodontal therapy on arterial stiffness measured via pulse-wave velocity. It is proposed that following the intervention, periodontal inflammation will be reduced and that this will lead to an improvement in PWV. Findings from this study will contribute towards the limited literature in this area and to the wider field of periodontal medicine. It is intended at the completion of this research that our understanding of the mechanisms involved in early

atherosclerosis, and how periodontitis may contribute to this process is progressed. Therefore, in the first instance, the study will aim to determine associations between periodontal disease and pulse-wave velocity, a measure of central arterial stiffness among Indigenous Australian adults with periodontitis. Secondly, using a randomised study design, the present study intends to describe changes in pulse-wave velocity following non-surgical periodontal treatment of moderate/severe periodontitis in an Indigenous Australian population.

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2 GENERAL MATERIALS AND METHODS

This chapter presents a description of the materials and methods used in this study. Specific details relating to statistical procedures and analytical methods have been presented in Chapters 3, 4, 5 and 6 respectively.

2.1 Project outline

The effect of non-surgical periodontal therapy on carotid-dorsalis pedis pulse wave velocity (PWV), a surrogate measurement of arterial stiffness was investigated using a parallel-group randomised controlled trial design. Details of the data collection procedures and statistical methods are presented herein.

2.2 Eligibility criteria

Eligibility for inclusion was determined via the combination of an interview, medical history and oral assessment. Participants were aged 18 years or older without a previous history of cardiovascular disease, had a minimum of five natural teeth, and at least moderate periodontitis based on the US Centres for Disease Control and Prevention and American Academy of Periodontology classification (Page and Eke, 2007). Moderate periodontitis using this classification is defined as having at least two or more interproximal sites with ≥ 4mm clinical attachment loss (CAL), or two or more sites with probing pocket depth (PPD) of ≥ 5mm provided that they are on alternate teeth. The periodontal status of Indigenous Australian adults residing in the Northern Territory was examined by two dental clinicians to verify periodontitis case status.

Individuals receiving periodontal treatment within the preceding six months, those with a history of any cardiovascular condition (with the exception of angina pectoris), rheumatic fever or any other cardiac or medical conditions which require preventive antibiotic

prophylaxis, pregnant women, or people with clinically visible endodontic or orofacial infections were excluded from participation.

2.3 Study design

The present study was nested within a larger parallel-group randomised controlled trial known as the PerioCardio study (Skilton et al., 2011a). Participants were recruited and followed-up two additional times within one year to assess changes in periodontal status and investigate arterial response to periodontal therapy. The first recall assessment was 3-months from baseline based on evidence from previous trials investigating the effects of treating periodontal disease on surrogate cardiovascular disease and inflammatory markers which indicated that the effects of periodontal treatment can be sustained for up to three months (Buhlin et al., 2009, Montebugnoli et al., 2005, Piconi et al., 2009, Seinost et al., 2005, Taylor et al., 2006). Additionally, three months is required for the periodontal tissues to complete the healing response post-treatment. The final recall appointment approximately one-year from recruitment was set to primarily measure medium-term changes in PWV and secondarily to assess periodontal status; whether periodontal disease (progressed/regressed) over time. Those eligible for this study were invited to participate in the following.

2.3.1 Visit 1: Baseline

All participants were required to provide signed, informed consent prior to enrolment, completion of a medical history via face-to-face interview and oral assessment. Following periodontal screening participants without periodontitis were asked to complete a questionnaire prior to dismissal. Participants with moderate or severe periodontitis were subsequently randomised prior to PWV measurement, anthropometric parameters, blood biochemistry and questionnaire. Subjects randomised into the ‘treatment’ group received

the intervention on the day of baseline measures or were rescheduled at a time convenient for the participant within two weeks from recruitment to have it completed. Details of the periodontal intervention are included [Section 2.9 Periodontal Intervention below].

2.3.2 Visit 2: 3-month follow-up

Participants were recalled between 2½ to four months from baseline and after medical history was updated to note any changes; oral assessment, PWV measurement, anthropometric parameters and blood biochemistry were repeated.

2.3.3 Visit 4: 12-month follow-up

Participants were recalled between 11-months and 15-months from baseline, medical history updated, oral assessment, PWV measurement, anthropometric parameters, blood biochemistry repeated. Individuals randomised into the 'control' group were subsequently invited to receive the periodontal intervention in the same manner as the 'treatment' group.

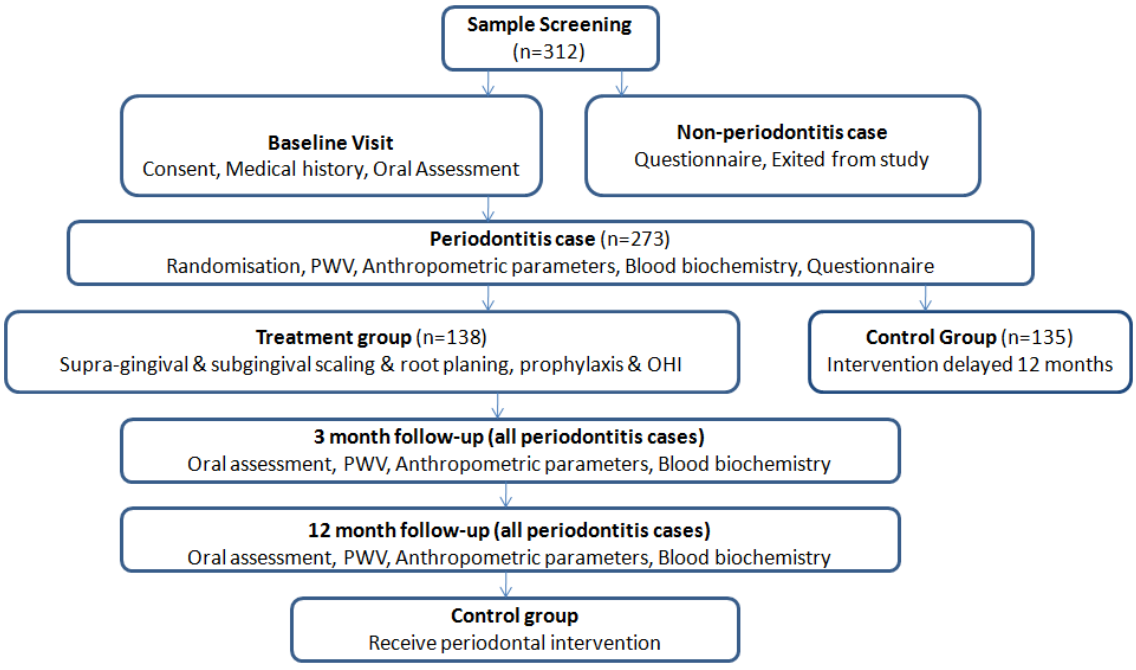

Figure 2.1: Study plan flow-diagram.

2.4 Oral assessment

Two dental clinicians calibrated against a gold-standard examiner from the Australian National Survey of Adult Oral Health (NSAOH) completed all assessments and interventions. Both dental clinicians involved held different qualifications. The primary clinician was an oral health therapist with three years of clinical experience treating periodontal disease. Dental clinician two was an overseas-trained dentist who, at the time of the study, was undergoing Australian Dental Council examinations for Australian registration.

The oral assessment followed standard epidemiological methods by obtaining information on tooth presence, caries experience, in addition to soft tissue assessment of gingival bleeding, plaque scores, calculus and periodontal destruction. The kappa and intra-class correlation statistics for both examiners are detailed in (Section 2.11.1) of this chapter. The dental armamentarium included disposable mirrors, Mirrorlite^{TM} Defend, Hauppauge, New York, U.S.A. and periodontal probes with 2 millimetre markings (Hu-Friedy, Chicago, USA, product number PCP2) for identification of periodontal destruction.

The following clinical parameters were evaluated:

1. Each tooth was allocated a status as sound (S), decayed (D), missing (M), filled (F) for subsequent calculation of the DMFT index.
2. PPD: Distance in millimetres from the gingival margin to the deepest aspect of the periodontal pocket.
3. Gingival recession: Distance in millimetres from the cemento-enamel junction to the gingival margin.
4. Bleeding on probing (BOP): The presence of BOP was recorded for each tooth examined using the following criteria:

   0 = no BOP, 1 = mild BOP following probing, 2 = moderate BOP characterised by constant light oozing from probed site, or 3 = severe bleeding characterised by

heavy flow following probing. The extent and severity of BOP was recorded as the percentage of sites with a given score.

5. Plaque and calculus scores using the Silness and Loe indices (Silness and Löe, 1964).
6. Gingival recession and PPD were measured at four sites of all teeth (excluding third molars). The sites assessed for periodontal status were: mesio-buccal - adjacent to the actual or imaginary contact point of the tooth's mesial surface, mid-buccal - the mid-point of buccal surface in single-rooted teeth and mid-point of the mesio-buccal root in multi-rooted teeth, disto-buccal - adjacent to the actual or imaginary contact point of the tooth's distal surface, and, disto-lingual - adjacent to the actual or imaginary contact point of the tooth's disto-lingual/palatal surface.

At the completion of the oral assessment, participants were advised of clinical findings and referrals to the public dental service (where required), were made.

2.5 Location of recruitment/intervention

A convenience sample of Indigenous Australian adults residing in Darwin, Katherine and Alice Springs in Australia's Northern Territory were recruited from community medical and dental health clinics, Aboriginal medical services and correctional facilities in the first instance. Employers with high concentrations of Indigenous staff were contacted and asked for their support. Printed advertisements were placed in newspapers and community notice boards at major shopping centres throughout metropolitan Darwin and Palmerston. Additionally, a "snowballing method" was utilised by seeking to recruit friends and family of those already recruited as a secondary approach.

Data collection for cardiovascular outcomes and anthropometric measures were principally conducted at the Menzies School of Health Research, Darwin. Other locations included Danila Dilba, Palmertson; Palmerston GP-Plus clinic; Wurli Wulinjiang Health

Service, Katherine; Darwin Correctional Facility, Berrimah; Alice Springs Correctional Facility, Alice Springs and Baker IDI, Alice Springs. The periodontal interventions were principally conducted in Oral Health Services – Northern Territory (NT) clinics of Casuarina, Royal Darwin Hospital and Katherine in addition to the dental clinics of Danila Dilba, Palmertson, Darwin Correctional Facility, and Alice Springs Correctional Facility.

2.6 Randomisation method

Following confirmation of moderate or severe periodontitis case status, participants were randomised into one of two groups: 1) treatment group (non-surgical periodontal therapy) and 2) control group (delayed treatment for 12 months). Randomisation sequence was generated using a common computer database program (Microsoft Office, 2002) via the permuted block method using varying block sizes by an investigator not involved in the recruitment, collection of clinical variables nor intervention of participants involved in this study. The allocation ratio was 1:1 for treatment and control groups respectively and randomisation was conducted on individual study participants.

A total of four randomisation databases were created to accommodate recruitment at different locations in the Northern Territory. The first randomised a total of 181 people from Darwin/Palmerston. The second randomised 24 participants from Katherine while randomisation databases three and four were developed for the correctional facilities in Darwin (Berrimah) and Alice Springs whereby 58 and 46 were randomised respectively.

2.7 Assessment of arterial stiffness

All assessments of arterial stiffness were undertaken with participants in the supine position for at least 10 minutes prior to commencement of measures. The two anatomical sites of interest for this measure are the common carotid and dorsalis pedis arteries. Location of the common carotid artery is via lateral palpation along the thyroid cartilage

while the dorsalis pedis artery is located between the first and second metatarsals on the dorsal surface of the foot. Measurement of PWV is via applanation tonometry using SphygmoCor-PVMx device, AtCor Medical, Sydney, Australia. The pressure tonometer was placed transcutaneously above the corresponding artery to record the pulse pressure waveforms while simultaneously recording the electrocardiography signal (ECG), which provided an R-wave timing reference. The resultant PWV score was calculated via computer algorithm as the mean time difference between the R-wave and the pressure wave per heart/pulse beat and the arterial path length between the two recording sites. To correct against measurement error, the distance from the common carotid location and the sternal notch was subtracted from the distance between the sternal notch and the dorsalis pedis site. Data quality control for these measurements required that standard deviation not exceed 10%. In the instances that this occurred, PWV was remeasured.

As an additional validation, carotid-femoral PWV was compared to carotid-dorsalis pedis on a sample of age and sex-matched non-indigenous controls.

2.8 Blood draw and urine sampling

Venous blood samples were collected via the antecubital vein under non-fasting conditions into four vacutainer tubes, two 8.5 mL serum separator tubes (SST), and two containing ethylenediaminetetraaceic acid (EDTA) (one 6 mL and one 4 mL). Upon collection, one tube containing SST and the 4 mL tube of EDTA were transported to a local pathology for analysis of lipid profile (total cholesterol, LDL, HDL), and HbA1c. The remaining two tubes were subsequently centrifuged at 3000rpm for 10 minutes to extract blood serum and plasma. Urine samples were collected to examine renal function measured via the albumin to creatinine ratio. Aliquots of plasma and serum were transferred into three 1.5 mL Eppendorf vials while urine was aliquotted into two and

frozen at -80 °C until the end of the study for the analysis of hsCRP, interleukin-6 (IL-6), asymmetric dimethyl-arginine (ADMA), apolipoprotein alpha and beta.

2.9 Anthropometric measurements

All physical and anthropometric measurements were collected at baseline and during both 3 and 12-month follow-up sessions. Participants were advised to wear light clothing to the appointments and remove their shoes.

Height: Participant height was measured using a metric stadiometer with maximum height of two metres that was placed against a wall. Subjects were required to stand with their backs and heels against the wall with their head in the Frankfort plane. Height was recorded to the nearest single decimal.

Weight: A portable weight scale (Tanita model HD-351, Arlington Heights, USA) was used to measure weight in kilograms (kg). Participants were requested to stand on the scales with their head in the Frankfort plane and eyes focusing forward and ensure that weight distribution was evenly spread to both feet. Weight was recorded to the nearest single decimal.

Body Mass Index (BMI): BMI was calculated from the weight (kg) divided by the square of height (meters).

Waist: The circumference of the abdomen at its narrowest point was measured using a metric tape (Model W606PM Lufkin, USA). After locating via palpation the iliac crest and vertebro-costal margin, the tape was wrapped horizontally around the centre of the two points to obtain the measurement.

Hip: The circumference of the hips was measured at its widest point of the buttocks by wrapping the tape horizontally around to obtain the measurement.

Blood pressure (BP): Three BP measurements were obtained using an automated device (Spot Vital Signs 4200B-E1, Welch Allen, Skaneateles Falls, USA) while the participant

was sitting upright in a chair. The mean values of the final two sitting recordings were used as the mean sitting BP measurement. A further three BPs were obtained while the participant was in the supine position; the first after 5 minutes, the second after 10 minutes of being supine and the final following completion of PWV measurement.

Central arterial pulse pressure (Brachial PP): Brachial PP was calculated post-hoc by subtracting the diastolic from the systolic blood pressure values using the final measurement.

Heart rate: The heart rate was recorded *post hoc* for both carotid and DP arteries.

2.10 Periodontal intervention

All dental procedures were performed by two dental clinicians. Participants randomised into the 'control group' were advised that their treatment would be delayed by 12 months and received oral hygiene instruction prior to being rescheduled for the first follow-up appointment three months from baseline. Individuals randomised into the treatment group underwent an untimed appointment for the intensive removal of subgingival and supragingival calculus and plaque biofilm via scaling, root planing and coronal polishing following administration of local anaesthesia if requested by the participant. The intervention was carried out with the use of Gracey hand scalers (Hu-Friedy, Chicago, USA), and piezoelectric ultrasonic scaler (Kyungwon Ferrite, Gyeonggi-Do, South Korea) using universal tips while the coronal polishing was conducted with medium coarseness prophy paste. Oral hygiene instructions were provided to all participants at the completion of the first treatment appointment along with an oral hygiene pack containing a toothbrush and tooth paste.

As the intervention did not provide any dental services beyond non-surgical periodontal therapy, participants in both treatment arms were informed that they are free to seek dental treatment elsewhere if desired or required. In the event where participants reported having

dental extractions or periodontal treatment beyond that provided in the study during the period of enrolment, this information was recorded for future sensitivity analysis.

Treatment was usually commenced and completed on the day of randomisation in most instances, or alternatively within two weeks of recruitment. For those participants randomised into the 'treatment' group who refused to undergo the intervention or who failed to attend for scheduled periodontal treatment appointments, respective coding for per-protocol analysis at trial completion was recorded. It was not possible to blind participants or clinicians of intervention group.

2.11 Data quality control measures

The reliability of the two clinicians was tested for both the oral assessment and PWV measurements. A total of six participants were used to assess inter-examiner reliability for periodontal status. Intra-examiner reliability of periodontal measures could not be conducted due to difficulties recalling study participants for subsequent assessment. A total of six participants were used to assess inter-examiner reliability and nine were used for intra-examiner reliability for PWV.

2.11.1 Oral Assessment method

Inter-examiner reliability: On occasions where few participants were scheduled to attend for a given day, participants were asked if they would consent to being re-examined on that day by the other clinician. Following consent, the first examiner completed all measures and recordings based of the criteria listed in [2.4 Oral assessment] with a research assistant (who was not the second clinician) recording the findings on the MS Access database. Following completion, the second clinician (blinded to the assessment of the first), subsequently re-examined the patient using the same oral assessment criteria with the following modifications:

Gingival index was not assessed as this measure is likely to be influenced by the previous examination, and;

Two of the four quadrants were examined; one quadrant in the maxilla and the contra-lateral quadrant in the mandible. Quadrant selection for the maxilla was chosen via a coin toss with 'heads' indicating quadrant 1 and 'tails' indicating quadrant 2.

2.11.2 PWV method

Inter-examiner reliability: Subsequent measurements of PWV were conducted on the same day on participants who provided consent and who were not involved in the oral assessment reliability assessments. Participants were requested to maintain supine positioning for the period of measurement, with a timed 5 minute break between recordings. Methods for PWV measurement followed those listed in [2.7 Assessment of arterial stiffness]. Inter-examiner reliability was rated as 'moderate' (intra-class correlation = 0.72).

Intra-examiner reliability: Participants were used for intra-examiner reliability assessment and followed the methods listed above. The reliability of the three clinicians was measured by calculating the intra-class correlation coefficient based on the criteria described by (Landis and Koch, 1977). Intra-examiner reliability between the two examiners was rated 'good'; examiner 1: ICC = 0.86, examiner 2: ICC = 0.83.

2.12 Sample size

Participants were a convenience sample of Indigenous adults aged 18 years or older who resided in Darwin, Katherine, or correctional facilities in Darwin or Alice Springs, Northern Territory. There have not been any published studies that have reported the change in pulse-wave velocity of the central aortic vessels in response to periodontal treatment. In place of information on PWV, several periodontal intervention trials

measured FMD - a surrogate marker for endothelial dysfunction of the brachial artery (Elter et al., 2006, Seinost et al., 2005, Tonetti et al., 2007). The mean change of flow-mediated dilatation (FMD) in these studies approximated 10% post-therapy.

Increased PWV of the brachial artery was also reported in patients with periodontitis compared to controls cross-sectionally (Miyaki et al., 2006). Further, two non-dental studies reported follow-up data on aortic PWV (carotid/femoral) in patients with rheumatoid arthritis undergoing anti-inflammatory or statin therapies (Mäki-Petäjä et al., 2007, Mäki-Petäjä et al., 2006). An eight percent change on follow-up was detected after 12 weeks of statin therapy (Mäki-Petäjä et al., 2007), while a change of 14% was found in rheumatoid arthritis patients on tumor necrosis factor alpha blocker medications. Using the ankle-brachial PWV as a measure of arterial stiffness, a 13% change upon follow-up was reported in hypercholesterolemic patients on statin therapy (Matsuo et al., 2005).

There is limited data available on measures of arterial stiffness in Indigenous Australians. The most relevant draws on cross-sectional data collected from two remote communities in the Northern Territory (Maple-Brown et al., 2007b). The mean carotid/femoral PWV in this population was 8.0 m/s ± 1.7. The primary outcome of PWV was selected as the variable from which to calculate sample size. Based on the above data and findings, an *a priori* conservative change (reduction) of 10% in carotid/dorsalis pedis PWV score was proposed post-periodontal treatment yielding a sample size of 72 people in each group (144 in total) to achieve 80% power. A conservative assumption that 20% of the sample in treatment group and 15% in the control group would be lost to follow-up in addition to five percent of the control group receiving treatment elsewhere, the total sample was multiplied by 140% to obtain the final projected sample size of 201 people. The following calculation was derived with the use of SAS 9.1.3 for Windows, Cary, N.C. USA, 2003:

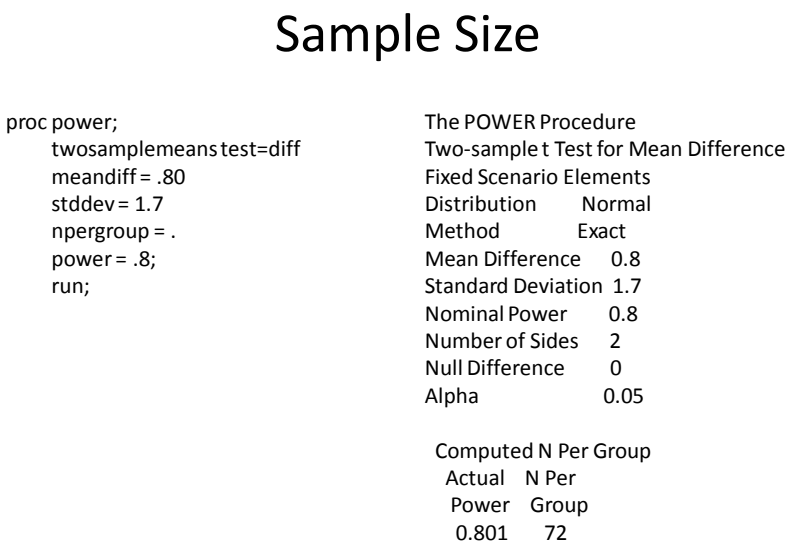

Figure 2.2: Sample size calculation.

2.13 Statistical analysis

Specific details of the statistical methods are included within Chapters 3-6 inclusive. Briefly, data where appropriate, were presented as means ± standard deviation. All primary analysis of randomisation results were based on the complete-case analysis method in the first instance using analysis of covariance (ANCOVA) to examine repeated measures that account for the correlation between baseline and follow-up values. Frequencies were measured using χ^2 test while variables with skewed distributions were examined via non-parametric methods or log-transformed prior to assessment using parametric methods as appropriate.

2.14 Data collection

2.14.1 Self-Reported

Information relating to socio-demographic characteristics, lifestyle factors (such as smoking, alcohol consumption, level of physical activity) and oral health behaviours

including oral hygiene habits and dental attendance history were collected via self-reported questionnaire. These questions have been widely used and validated by previous surveys from the Australian Research Centre for Population Oral Health and their use in this study allowed for direct comparisons with previous research. The questionnaire was administered as part of a face-face interview at the baseline appointment which allowed for research staff to directly clarify any queries by participants when the need arose.

2.15 Key variables

2.15.1 Dependent variable

The dependent variable will be change in carotid-dorsalis pedis PWV following periodontal intervention.

2.15.2 Independent variables

Dental variables: include tooth count, decayed, missing and filled teeth, periodontal status (probing pocket depth and clinical attachment loss), plaque and calculus scores;

Self-reported variables: socio-demographic, general health and lifestyle questions. The latter two types of questions were used to adjust for confounding factors known to influence cardiovascular disease during baseline analysis;

Biochemical variables: hsCRP, IL6, HbA1c, lipid profile, apolipoprotein alpha & beta,

Anthropometric variables: systolic/diastolic blood pressure, and height and weight to calculate body mass index.

2.16 Study aims and hypotheses

Aim 1: To determine baseline associations between periodontitis and arterial stiffness measured by PWV among Indigenous Australian adults with and without periodontitis.

Hypothesis 1: Study participants with periodontitis will have higher PWV values than individuals without periodontal disease following adjustment for cross-sectional confounders.

Aim 2: To describe change in arterial stiffness (PWV) following non-surgical periodontal treatment of moderate/severe periodontitis in Indigenous adults.

Hypothesis 2: PWV values in study participants randomised in the treatment group will reduce by 10% compared to the control group.

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3 PERIODONTAL DISEASE AND DENTAL CARIES AMONG INDIGENOUS AUSTRALIANS LIVING IN THE NORTHERN TERRITORY, AUSTRALIA

The following manuscript was accepted for publication in the *Australian Dental Journal* on 16 June 2013.

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Contribution to the Paper	Collected the data, performed statistical analysis and interpretation, wrote manuscript and acted as corresponding author.		
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Contribution to the Paper	Drove the design of the study protocol for funding and ethics applications, coordinated data collection, reviewed and revised manuscript prior to submission.		
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Periodontal disease and dental caries among Indigenous Australians living in the Northern Territory, Australia

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ABSTRACT

Background: The aim of this study was to describe the caries experience and severity of periodontal disease in a convenience sample of Indigenous Australians living in the Northern Territory.
Methods: Data were gathered via self-reported questionnaire and dental examination by calibrated examiners. Socio-demographic characteristics were compared with data from the 2011 Australian census while prevalence of periodontal disease and dental caries was compared against weighted estimates from the National Survey of Adult Oral Health 2004–2006. In each comparison, non-overlapping 95% confidence intervals inferred a significant difference. Within-study comparisons were assessed via chi-square, *t*-tests and analysis of variance for differences among study participants.
Results: A total of 312 Indigenous Australian participants provided completed data (average age 39.5 ± 10.5 years, 174 males). Of these, 87.5% were confirmed periodontitis cases; 3.5 times that of national-level estimates. The experience of untreated caries was five times that of national estimates (mean decayed 3.0 versus 0.6). Periodontitis case status was positively associated with older age, male gender and presence of diabetes.
Conclusions: Periodontal disease and untreated caries were significantly more prevalent in this sample of Indigenous Australians compared to the general Australian population. The prevalence of periodontal disease was markedly higher than that previously described for Indigenous Australians.

Keywords: Cigarette smoking, dental caries, diabetes mellitus, type 2, Indigenous Australian, periodontitis.

Abbreviations and acronyms: CAL = clinical attachment loss; DMFT = decayed, missing and filled teeth; NSAOH = National Survey of Adult Oral Health; PD = probing depth.

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INTRODUCTION

Indigenous Australians represent 2.5% of Australia's 22 million population[1] and it is estimated that the Northern Territory (NT) has the largest proportion of Indigenous Australians per capita in the country.[2] The life expectancy of Indigenous Australians is estimated to be some 10–12 years lower than non-Indigenous Australians owing to high rates of infection and chronic diseases.[3–5] Indigenous Australians exhibit social and economic disadvantage resultant from lower educational attainment, higher unemployment rates and overcrowded housing.[3]

Periodontitis is an inflammation of the periodontal tissues resulting in loss of connective tissue attachment, destruction of alveolar bone and formation of pathological pockets around diseased teeth.[6] In severe cases it may lead to tooth loss. Dental caries arises from a complex interplay between the tooth surface, gram positive bacteria, and a regular source of carbohydrate.[7] Treatment for caries can range from remineralizing the tooth structure to restorations. In the event where a tooth has decayed beyond repair, extraction is required.

The National Survey of Adult Oral Health 2004–2006 (NSAOH) estimated the mean decayed, missing

and filled teeth (DMFT) among Indigenous Australian adults was 14.8 compared to 12.8 for non-Indigenous Australians, while the respective estimates for the prevalence of periodontal disease was 29% compared to 23%.[8] These national estimates may under-estimate the true prevalence of caries and periodontal disease among Indigenous Australians due to methodological constraints.[8]

Few studies have examined the oral health status of Indigenous Australian adults. One survey of two towns in western New South Wales identified extensive treatment need for restorations, a high prevalence of periodontal disease and little evidence of oral hygiene practices.[9] More recent research from Western Australian communities have reported consistent findings of problem-associated visiting patterns, infrequent brushing, combined with high proportions of untreated caries and periodontal pocketing.[10,11] Diabetes has impacted the oral health status of an Indigenous community located in north-western South Australia. Among those with diabetes, almost 80% were reported to have periodontitis compared to 30% without diabetes and the number of missing teeth was 3.5 times higher among persons with diabetes versus those without.[12] A series of dental surveillance studies have also shown differences in caries experience exist between Indigenous Australians living in metropolitan locations compared to remote areas of Australia.[13]

We aimed to determine associations between key risk indicators and oral health status of a convenience sample of Indigenous Australian adults residing in the NT. We also aimed to compare these findings against national level estimates.

MATERIALS AND METHODS

A convenience sample of Indigenous Australian adults residing in Darwin, Katherine and correctional facilities in Darwin and Alice Springs in the NT, Australia were recruited. All study participants were otherwise healthy and had no contraindications to undergoing a dental examination. This study reports findings from all participants who underwent an oral health assessment and completed a self-reported questionnaire.

Oral assessment

Dental assessments replicated the methods used in the NSAOH.[8] The oral assessment obtained information on tooth presence, caries experience, periodontal destruction, gingival bleeding, dental plaque, and calculus scores. Examiners distinguished missing teeth due to dental caries or periodontal disease as opposed to teeth missing for other reasons which included orthodontic, dental trauma or congenitally missing. Root fragments due to caries were coded separately from fragments arising from dental trauma and were incorporated into the 'decayed' component of the index. Caries experience was recorded for each tooth as: decayed, recurrent caries (new decay around existing filling), filled unsatisfactorily (defective restorations without decay), filled, and sound. Oral plaque score was recorded for six index teeth (if present) based on published criteria.[14] Light plaque was defined as present only after scraping tooth surface; moderate as an accumulation visible to naked eye; and heavy, defined when plaque was abundant around the tooth surface. The selected teeth were the most anterior molar in each quadrant, in addition to the maxillary right central and mandibular left central incisors. The same six teeth were assessed for dental calculus.

Periodontal probing and gingival recession was measured at four sites at every tooth excluding third molars. The sites assessed included the mesio-buccal, mid-buccal, disto-buccal and disto-lingual. Periodontitis was based on the US Centers for Disease Control and Prevention and American Academy of Periodontology classification.[15] This case definition was selected to correspond with that used in the NSAOH. Periodontitis was ascertained by algorithm via a combination of probing depth (PD), the distance from the base of the periodontal pocket to the gingival margin, and clinical attachment loss (CAL) determined as the sum of gingival recession and PD. Moderate periodontitis was diagnosed if participants exhibited at least two interproximal sites with CAL $\geq$4 mm, or at least two interproximal sites with PD $\geq$5 mm; and severe periodontitis as $\geq$2 interproximal sites with CAL $\geq$6 mm and at least one site with PD $\geq$5 mm. A gingival bleeding score was collected for each tooth periodontally assessed.[16] The extent of periodontal disease was calculated as the average number of sites per person with periodontal destruction relative to the number of teeth using published methods.[17] The extent of PD $\geq$4 mm in the Australian population has previously been reported in the NSAOH (Table 5.21, p. 131).[8] However, comparisons between NSAOH and the present study participants were made by comparing the number of sites that exhibited both CAL $\geq$3 mm and PD $\geq$4 mm. This measure reduces the risk of bias caused by pseudopocketing due to inflammation, and facilitates comparisons between populations that differ in oral hygiene practices.

Dental armamentarium included disposable mirrors (MirrorliteTM Defend, Hauppauge, USA) and a periodontal probe with 2 mm markings (Hu-Friedy, Chicago, USA, product number PCP2).

Socio-demographic, lifestyle and medical history

A questionnaire to obtain information regarding socio-demographic characteristics, including education, employment, and household income, in addition to lifestyle factors and medical history, including diabetes status, was administered to all participants. Lifetime exposure to smoking was quantified as the number of 'pack years' using a published formula:[18]

$$\text{pack years} = \frac{\text{number of cigarette smoked per day} \times \text{number of years smoked}}{20}$$

Statistical analysis

NSAOH contains weighted representative estimates of the Australian population. To facilitate comparisons of periodontal disease between NSAOH and the present study, the disto-lingual measurements were dropped prior to analysis and reinstated for within-sample analyses. Similarly, only data from NSAOH participants aged between 22 to 73 years were analysed to match with the age range of the current study sample. Statistical significance for comparisons of frequencies and means between our study and NSAOH participants and between the present study and the 2011 Australian Census was determined on the basis of non-overlapping 95% confidence intervals. Age and socio-economic data pertaining to the 2011 Australian Census findings for Indigenous Australians residing in Darwin was obtained from the Australian Bureau of Statistics using data from the Darwin local government area (http://www.censusdata.abs.gov.au; accessed 23 May 2013).

Statistical significance was inferred at $P < 0.05$ for comparisons of frequencies (chi-square test), independent samples *t*-test for comparisons between moderate and severe periodontitis cases and ANOVA using Student-Neuman-Keuls *post hoc* tests for means among all participants. Continuous, normally distributed variables are reported as mean ± standard deviation and 95% confidence interval. Data were analysed using SAS Version 9.3 (Cary, North Carolina, USA).

Ethical approval

Approval for the present study was obtained from the Human Research Ethics Committee of the NT Department of Health and Menzies School of Health Research, the Central Australian Human Research Ethics Committee, the NT Department of Correctional Services Research Committee, the University of Adelaide Human Research Ethics Committee and the Aboriginal Health Council of South Australia. Study participants gave informed consent before participating. Research was conducted in accordance with the World Medical Association Declaration of Helsinki (Version VII, 2008).

RESULTS

Recruitment commenced in June 2010 and concluded in January 2012. Some 312 participants completed both an oral assessment and self-reported questionnaire.

Compared with Australian Census data of Darwin residents, participants in our study were similarly aged and a comparable number had completed secondary schooling. There were significant differences in mean weekly household income. For example, over half of the participants in the present study reported an income of ≥$1000 per week compared to 21% of Darwin residents in the Census (Table 1). Compared with the NSAOH, participants were younger, had a higher prevalence of self-reported diabetes, and were almost three times more likely to be current smokers (Table 2). Although five times as many participants in the present study had teeth with untreated caries, NSAOH participants had a 50% higher DMFT owing to more teeth missing or filled as a result of oral disease.

Both indicators of periodontal disease experience (prevalence and extent), were significantly higher in our study sample than in the NSAOH. Of the 312 persons assessed, 273 had moderate or severe periodontitis representing a prevalence of 87.5%; 3.5 times that of national-level estimates of the same age (Table 2). The extent of periodontal sites with attachment loss and periodontal pocketing was nine times higher in the current study compared to the national-level estimates. There were also significant differences in oral hygiene measured in the form of dental calculus, plaque and gingival bleeding.

Tables 3 and 4 are limited to participants of the present study. Periodontal case status did not differ by lifetime exposure to smoking nor location of recruitment. Participants with severe periodontal disease were older, more likely to be male and previously diagnosed with diabetes (Table 3).

Marked differences in oral assessment findings were apparent by location of recruitment, with study participants from Darwin exhibiting significantly higher filled teeth and overall DMFT but significantly fewer sites with dental calculus compared to the three other study locations (Table 4). The average number of teeth with untreated caries was almost twice as high in the Darwin correctional facility compared to the Alice Springs correctional centre. Both correctional facility samples were more likely to present with moderate or heavy plaque levels and more sites with bleeding on probing compared to the non-institutionalized samples.

Table 1. Socio-demographic characteristics of NT sample participants compared to the Indigenous Australians residing in Darwin from the 2011 Australian Census

	Variable	NT sample % (95% CI)	NT Indigenous Australian 2011 Census % (95% CI)	Ratio (NT sample/Darwin Census)
Age (n = 181)	20–34 years	29.8 (23.1–36.6)	34.5 (32.9–36.2)	0.9
	35–54 years	54.1 (46.8–61.5)	46.2 (44.5–47.9)	1.2
	55+ years	16.0 (10.6–21.4)	19.3 (18.0–20.7)	0.8
Education (n = 172)	Completed Yr 12	29.7 (22.8–36.5)	22.9 (21.0–25.0)	1.3
Housing (n = 172)	Own	4.7 (1.5–7.8)	8.2 (7.1–9.4)	0.6
	Buying	30.2 (23.3–37.2)	25.5 (23.8–27.3)	1.2
	Renting	53.5 (46.0–61.0)	56.7 (54.7–58.7)	0.9
	Rent free	11.6 (6.8–16.5)	6.6 (5.9–7.4)	1.8
Weekly h-hold income (n = 130)	$0–299	7.7 (3.1–12.3)*	31.7 (30.3–33.2)	0.2
	$300–599	13.1 (7.2–19.0)*	20.4 (19.2–21.7)	0.6
	$600–999	23.1 (15.7–30.4)	17.1 (16.0–18.3)	1.4
	≥$1000	56.2 (47.5–64.8)*	21.0 (19.8–22.2)	2.7

*Non-overlapping 95% CIs for NT sample and NT Indigenous Australian 2011 Census.

Table 2. Characteristics of NT sample participants versus weighted national estimates of similarly-aged Australians in NSAOH

	NT sample (n = 312)	NSAOH (n = 4967)	Ratio (NT sample/ NSAOH)
Mean age yrs (95% CI)	39.5 (38.4–40.7)*	45.5 (44.9–46.0)	0.9
No. male	174	1959	
% (95% CI)	55.8 (50.2–61.3)	49.5 (47.7–51.4)	1.1
Self-reported diabetes – Yes	41	258	
% (95% CI)	13.2 (9.4–17.0)+	4.7 (3.9–5.4)	2.8
Current smoker – Yes	162	726	
% (95% CI)	51.9 (46.4–57.4)+	17.5 (15.9–19.1)	3.0
Former smoker – Yes	41	1380	
% (95% CI)	13.1 (9.8–17.3)+	30.2 (28.6–31.9)	0.4
Never smoker	109	2298	
% (95% CI)	34.9 (29.9–40.4)+	52.3 (50.3–54.2)	0.7
Indigenous Australian	312	75	
% (95% CI)	100‡	1.2 (0.8–1.6)	
CDC periodontitis case	273	1477	
% (95% CI)	87.5 (83.8–91.2)+	25.2 (23.5–26.9)	3.5
Mean no. teeth (95% CI)	26.5 (25.9–27.1)	25.7 (25.5–25.9)	1.0
Mean no. decayed (95% CI)	3.0 (2.6–3.4)+	0.6 (0.5–0.6)	5
Mean no. filled (95% CI)	2.9 (2.5–3.3)*	8.9 (8.7–9.2)	0.3
Mean no. missing (95% CI)	3.8 (3.2–4.4)*	4.9 (4.7–5.1)	0.8
Mean no. DMFT (95% CI)	9.7 (8.9–10.6)*	14.4 (14.1–14.8)	0.7
Mean no. root fragments	0.6	-	
Mean no. sites CAL ≥3 mm and ≥PPD 4 mm (95% CI)	11.3 (9.8–12.7)+	0.8 (0.7–0.9)	14.1
Extent CAL ≥3 mm and PPD ≥4 mm (% sites) (95% CI)	12.1 (10.6–13.6)+	1.3 (1.1–1.4)	9.3
Mean index teeth with calculus (95% CI)	4.1 (3.9–4.2)+	1.1 (1.1–1.2)	3.7
Gingival bleeding ≥2	226	929	
% (95% CI)	72.4 (67.5–77.4)+	20.3 (18.5–22.1)	3.6
Plaque score ≥2	147	1412	
% (95% CI)	47.3 (41.7–52.8)+	28.7 (26.9–30.5)	1.6

NSAOH: National Survey of Adult Oral Health; DMFT: decayed, missing and filled teeth; CDC: Centers for Disease Control and Prevention; PD: periodontal pocket depth; CAL: clinical attachment loss.
All percentages are of the total: NT sample n = 312, NSAOH n = 4967.
*Non-overlapping 95% CI (NSAOH > NT sample).
+Non-overlapping 95% CI (NT sample > NSAOH).
‡All study participants Indigenous Australian.

DISCUSSION

This study describes the dental caries experience and severity of periodontal disease from a convenience sample of Indigenous Australians living in the NT. Information regarding adult Indigenous Australian oral health is scarce.[9,10,12,13,19] Despite this, the high prevalence of periodontal disease in the current study (87.5%) was unexpected; previous estimates using the same periodontitis case definition had a prevalence at

Table 3. Characteristics of NT sample participants stratified by periodontitis case status

	Non-case (n = 39)	Moderate (n = 188)	Severe (n = 83)	P value
Mean age (years)*	35.5 ± 9.8	39.1 ± 10.1*	42.6 ± 10.1*	0.01
No. male (%)	16 (41.0)	99 (52.4)	57 (68.7)‡	0.01
[a]Location (%)				
Darwin	29 (74.4)	104 (55.6)	44 (53.0)	0.38
Katherine	2 (5.1)	17 (9.1)	9 (10.8)	
Darwin correctional centre	4 (10.3)	39 (20.9)	15 (18.1)	
Alice Springs correctional centre	4 (10.3)	27 (14.4)	15 (18.1)	
[b]Smoker (pack years) (%)				
None	15 (42.9)	68 (40.0)	18 (26.1)	0.10
>0–5	11 (31.4)	36 (21.2)	17 (24.6)	
>5	9 (25.7)	66 (38.8)	34 (49.3)	
[c]No. self-reported diabetes – Yes	0	21 (11.2)	20 (24.4)	<0.01
Mean sites CAL ≥3 mm and PPD ≥4 mm	0.5 ± 0.6	8.7 ± 8.2*	22.1 ± 18.1*	<0.01
Mean extent CAL ≥3 mm and PPD ≥4 mm	0.5 ± 0.6	8.8 ± 8.0*	24.9 ± 17.1*	<0.01

Results not available for all participants.
a–c (n = no case, moderate, severe if n <312): a: 39, 187, 83; b: 35, 170, 69; c: 39, 187, 82; *Significant difference in means versus 'non-periodontitis case' (ref) via Student-Neuman-Keuels (SNK) *post hoc* ANOVA test; ‡significant difference in frequencies versus 'non-periodontitis case' (ref) via chi-square.
Categorical variables: frequency (%); means reported as: mean ± SD.

Table 4. Oral assessment findings of NT sample participants stratified by location of recruitment

	Darwin (n = 180)	Katherine (n = 28)	Alice Springs correctional facility (n = 46)	Darwin correctional facility (n = 58)
Age	42.1 ± 11.1	39.5 ± 8.9¶	34.5 ± 5.7*¶	35.7 ± 8.5*
Mean no. teeth	25.2 ± 6.6	28.9 ± 2.5*	27.6 ± 4.1*	28.4 ± 4.1*
Mean no. decayed teeth	3.1 ± 3.6	2.5 ± 2.6	2.0 ± 2.3*+	3.8 ± 3.7+
Mean no. filled teeth	3.8 ± 4.2	1.7 ± 2.4*	1.9 ± 2.4*	1.4 ± 2.3*
Mean no. missing teeth	4.8 ± 6.3	1.2 ± 1.5*	3.2 ± 4.1	2.7 ± 4.1
Mean no. DMFT	11.6 ± 8.0	5.4 ± 4.2*	7.1 ± 5.8*	8.0 ± 6.4*
Mean no. sites with calculus	3.4 ± 1.8	4.3 ± 1.2*	4.9 ± 1.0*	5.2 ± 1.0*
Freq mod/heavy plaque (%)	54 (30.2)	10 (35.7)	35 (76.1)‡	48 (82.8)‡
Freq mod/heavy gingival bleeding (%)	112 (62.2)	20 (71.4)	43 (93.5)‡	51 (87.9)‡

*Significant difference in means versus Darwin (ref) via Student-Neuman-Keuels (SNK) *post hoc* ANOVA test.
+Significant difference in means between correctional facilities via SNK *post hoc* ANOVA test.
¶Significant difference in means between Alice Springs correctional facility and Katherine via SNK *post hoc* ANOVA test.
‡Significant difference in frequencies versus Darwin (ref) via chi-square.
Categorical variables: frequency (%); means reported as: mean ± sd.

approximately 30%.[8] Although prevalence of periodontal disease in our study differed by gender, age, and employment status, it remained above 80% in all sub-groups assessed.

Most studies that measured periodontitis in Indigenous Australian adults have used indices to describe the various disease states; with reports of between 30% to 61% of participants showed evidence of periodontal pockets.[9–12] The major drawback of these measures is that there are no provisions to account for CAL. By measuring only gingival inflammation and PD, both the CPI and Russell's PI risk underestimating the true prevalence of periodontal disease in older age groups, while potentially overestimating periodontitis in younger people. This bias may be exacerbated if plaque-associated inflammation leads to misclassification of periodontitis due to pseudo-pocketing when oral hygiene is poor. The use of the CDC/AAP periodontitis case definition is thus recommended.

Indigenous participants in NSAOH had 2.3 times the prevalence of untreated dental caries compared with their non-Indigenous counterparts.[8] Our findings are consistent with this, with the mean number of untreated carious teeth being five times that of the national estimate. Conversely, our study participants were less likely to have had teeth filled or extracted due to disease compared to NSAOH estimates. This may suggest our participants were less likely to utilize dental services than those in the NSAOH as both restorations and extractions require clinical intervention. Conversely, the cost of dental treatment has been identified as a barrier[10,20] and access to timely and culturally-appropriate dental services is known to be problematic for some Indigenous Australians.[21] Our findings clearly reflect an important priority for service providers and policy makers interested in improving the oral health status of vulnerable populations such as Indigenous Australians.

Both caries and periodontal disease arise as a result of a complex interplay between individual factors (including diet and lifestyle habits) and are complicated further by external environmental influences such as water fluoridation, location of primary residence and availability and accessibility of dental services. Rural and remote areas of Australia where one-quarter of the Indigenous Australian population reside[22] have the lowest proportional ratio of practising dentists per capita.[23] These factors may be one part of the explanation why rates of untreated caries, periodontal disease and tooth loss remain high among certain sub-groups of the Australian population.

Differences in overall DMFT between locations of recruitment in the present study could partially be explained by the older age distribution of participants from Darwin as caries experience is cumulative. However, the observational nature of this investigation makes it difficult to identify definitive causes. The variation in untreated caries between correctional facilities may be the result of differences in access to dental services within prisons. It was not possible to ascertain the length of sentence for those incarcerated. Caries may also be influenced by geography. Darwin has low naturally occurring fluoride in the water supply and is fluoridated by water authorities. In contrast, Alice Springs and many of the surrounding communities have naturally occurring levels of fluoride of at least 1 parts per million or higher.[24] Lifetime exposure to fluoride sources was not collected as part of this study. Dental fluorosis has been frequently reported in children living in remote areas of Australia including central Australia.[25] Although recording fluorosis was beyond the aims of the current study, it was often observed by examining clinicians in Alice Springs.

A recent report indicated that almost half (46%) of Indigenous adults were current smokers compared to 21% of Australians overall.[4] This estimate is lower than that recorded in the current study. We have reported smoking in the form of 'pack years' as this measure provides information on the lifetime exposure to tobacco products. The effects of smoking as a risk factor for periodontal disease and its dose-dependent association with both the progression and severity of periodontitis is well understood.[26] In light of this, the high proportion of periodontitis cases diagnosed in the present study may be the result of long-term exposure to smoking products. Once again, however, the cross-sectional nature of this investigation prevents any examination of causal pathways.

Type 2 diabetes prevalence among Indigenous Australians is considerably higher than among non-Indigenous Australians.[27] In the current study, 13% of participants reported being previously diagnosed with diabetes, which is lower than estimates for Indigenous Australians published elsewhere.[4] The relationship between periodontal disease and diabetes mellitus is well established.[28] Long-term control of blood glucose levels is paramount in preventing diabetic complications, one of which is periodontal disease.[29] Among participants with severe periodontitis, the extent of periodontal pocketing with 4 mm or more was 25%. This degree of periodontal disease could be a significant source of systemic inflammation which may have metabolic consequences.

Limitations of this study should be noted. One-third of our sample was recruited from correctional facilities in Darwin and Alice Springs. Prison inmates are likely to differ from the general population in terms of oral disease status and risk factors influencing these. Shortcomings aside, our findings provide further evidence that Indigenous Australians appear to have markedly worse clinical oral health status in comparison to their non-Indigenous counterparts. Given the link between periodontal disease and other chronic conditions, and the impact of quality of life resulting from untreated caries, our findings reinforce the importance of making and keeping oral health a priority area in national Aboriginal and Torres Strait Islander health plans.

CONCLUSIONS

In conclusion, untreated decay and periodontal disease were significantly more prevalent among a convenience sample of Indigenous Australians in the NT compared to national-level estimates. The prevalence of periodontal disease was markedly higher than previous reports among Indigenous Australians.

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4 ASSOCIATIONS BETWEEN PERIODONTAL DISEASE AND CARDIOVASCULAR SURROGATE MEASURES AMONG INDIGENOUS AUSTRALIANS

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Associations between periodontal disease and cardiovascular surrogate measures among Indigenous Australians ☆

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ABSTRACT

Background/objectives: Inflammation is a key pathogenetic factor in atherogenesis. Periodontitis is a chronic inflammatory source which can have systemic impacts. Indigenous Australians have a higher prevalence of periodontal disease and experience cardiovascular disease earlier than non-Indigenous Australians. The aim was to describe the association between severity of periodontal inflammatory disease and measures of arterial structure and function.

Methods: Periodontal disease in a convenience sample of Indigenous Australians was assessed clinically; for those with periodontal disease, the extent of periodontal pockets ≥4 mm was stratified into quartiles. Vascular health was measured non-invasively via carotid-dorsalis pedis pulse-wave velocity (PWV), and via B-mode ultrasound of the common carotid intima-media (IMT). Non-fasting blood samples were collected for lipid and inflammatory marker evaluation. Linear regression models were constructed to determine the associations between extent of periodontal pocketing and vascular health, adjusting for traditional cardiovascular common risk factors.

Results: 273 Indigenous Australian adults were recruited and complete data was available for 269 participants (154 males), median age 39 years. Arterial stiffness (PWV) significantly increased with increasing extent of periodontal pocketing (p trend = 0.001). By contrast, carotid IMT did not differ across quartiles.

Conclusions: Periodontal pocketing was associated with central arterial stiffness, a marker of presymptomatic arterial dysfunction, in Indigenous Australian adults with periodontal disease.

1. Introduction

Periodontitis is characterised by chronic low-grade inflammation of the periodontal tissues which results in the destruction of the alveolar bone and formation of pathological pockets around diseased teeth that can lead to tooth loss [1].

Inflammation is a core component of the arterial wall disturbances leading to atherosclerosis [2]. The source of inflammation, however, does not necessarily have to arise from within the artery [3]. Long-term systemic inflammatory events such as those observed in periodontitis may contribute to atherosclerosis. The various mechanisms in which this may occur have recently been reviewed [4].

Periodontitis and cardiovascular disease (CVD) share common risk factors. For example, both occur more frequently among those with a history of cigarette smoking, people with diabetes and both are also associated with ageing. It is currently unclear whether periodontitis contributes to the initiation and/or progression of atherosclerosis, and if so, to what extent. It remains possible that no causal relationship

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exists, but rather, that periodontal and cardiovascular diseases are associated simply due to both developing over the same timeframe [5].

Periodontal disease has been linked with increased pulse wave velocity (PWV) of peripheral arteries [6–8]. Associations of PWV at different sites and atherosclerosis have suggested that peripheral arterial PWV may not be comparable to the assessment of central arterial stiffness made when measuring carotid-femoral PWV to determine CVD risk [9]. Little is known about the relationship between periodontitis and central arterial stiffness [7].

Life expectancy of Indigenous Australians is estimated to be 10–12 years lower than non-Indigenous Australians due to high rates of chronic diseases including CVD [10,11]. The occurrence of coronary heart disease is much earlier among Indigenous Australians compared to their non-Indigenous counterparts [12].

We hypothesised that increased severity of periodontal disease would correspond with increased levels of CVD risk markers and surrogate measures of vascular health. Accordingly, we sought to: 1) determine the associations between periodontal pocketing, measures of inflammation and vascular health in Indigenous Australian adults with periodontitis; and 2) determine if the association corresponds to a dose–response relationship between periodontal disease, IMT and PWV.

2. Materials and methods

A convenience sample of Indigenous Australian adults residing in Darwin, Katherine and Alice Springs in Australia's Northern Territory was recruited from community medical and dental health clinics, Aboriginal medical services and correctional facilities in the first instance. The "snowballing method" was also utilised by seeking to recruit friends and family of those already recruited as a secondary approach. The resultant sample was recruited for the purposes of a randomised controlled trial investigating the effect of periodontal therapy on surrogate markers of cardiovascular disease [13]. This study uses baseline data from the over-arching trial to investigate the present study aims. Study participants were examined by two dental clinicians to verify periodontitis case status defined as the presence of at least 2 interproximal sites with clinical attachment loss (CAL) ≥4 mm, or at least two interproximal sites with probing depth (PD) ≥5 mm [14]. Eligibility for inclusion was further determined via combination of oral interview and medical history. To be eligible, participants had to be aged 18 years or older and have a minimum of five natural teeth. Individuals having received periodontal treatment in the preceding six months, those with a history of any cardiovascular condition (with the exception of angina pectoris), rheumatic fever or any other cardiac or medical conditions which require preventive antibiotic prophylaxis, pregnant women, or people with clinically visible endodontic or orofacial infections were excluded from participation.

2.1. Oral assessment

Clinical oral assessments were conducted to obtain information on tooth presence, gingival bleeding and periodontal destruction. Probing depth and gingival recession were measured at 4 sites at every tooth excluding third molars. The sites included mesio-buccal, mid-buccal, disto-buccal and disto-lingual. A single gingival bleeding value was recorded for each tooth periodontally assessed. The scoring system of the Gingival Index (values 2 & 3) [15] was used to indicate the number of teeth with positive gingival bleeding. Dental armamentarium included disposable mirrors (Mirrorlite™ Defend, Hauppauge, New York, U.S.A.) and a periodontal probe with 2 mm markings (Hu-Friedy, Chicago, USA, product number PCP2).

2.2. Common carotid intima-media thickness imaging

Participants rested in a supine position for 10 min prior to bilateral ultrasound of the carotid arteries with a portable ultrasound and L38e 10–5 MHz linear transducer (Sonosite Micromaxx, Bothell, USA). Carotid intima-media thickness (IMT) was assessed as previously described [13]. In brief, the carotid artery was imaged in longitudinal section, focusing on the distal wall of the common carotid artery (CCA) just proximal to the bulb. Images were stored for later analysis by an observer blinded to participant characteristics using semi-automated and validated software (Carotid Analyzer, Medical Imaging Applications, USA). The average IMT over a 10 mm long segment of distal wall of the carotid artery was measured twice from each carotid artery. A third measure was obtained if the first two measures differed by more than 10%. Inter-observer variability for mean IMT was excellent (ICC = 0.993), as derived from a second independent observer in a random sample of 20 participants.

2.3. Arterial stiffness

Carotid-dorsalis pedis (DP) pulse wave velocity (PWV) was measured via applanation tonometry using a Millar transducer and SphygmoCor-PVMx device (AtCor Medical, Sydney, Australia) [16] after participants had rested in a supine position for at least 10 min. The distance from each site to the suprasternal notch was measured, and the path length determined using the subtraction method [17]. The PWV score was calculated using the 'foot-to-foot' method. Two trained examiners were calibrated prior to commencement of the study. Inter-observer repeatability was tested throughout the study and was rated as 'good' (intra-class correlation = 0.72). Intra-observer repeatability was equally comparable between the two examiners; examiner 1 = 0.86 and examiner 2 = 0.83.

2.4. Blood and urine sampling

Non-fasting venous blood samples were collected via the antecubital vein. Samples were transported to a local commercial pathology clinic for analysis of lipid profile: total cholesterol (TC) and high-density lipoprotein (HDL). Serum and plasma were stored at −80 °C until batch analysis for high-sensitivity C-reactive protein (hsCRP) and apolipoproteins A1 and B from serum, and interleukin-6 (IL-6), arginine and asymmetric dimethyl-arginine (ADMA) from plasma. Urine samples were collected to examine renal function measured via the albumin to creatinine ratio (ACR) at the same pathology laboratory.

Direct methods were used to determine lipid profile and ACR using an ADVIA 2400 Chemistry System (Siemens, Tarrytown, USA). Apolipoproteins A1 and B were measured on an auto-analyser using an immunoturibidimetric assay. Plasma asymetric dimethylarginine was assessed using high-performance liquid chromatography with simultaneous UV and fluorescence detection as previously described [18]. Serum high sensitivity CRP was measured via particle-enhanced immunonephelometry using the BN II system. Plasma IL-6 was measured via commercial ELISA assay (Human IL-6 Quantikine kit, R&D Systems Inc., Minneapolis, USA).

2.5. Anthropometric measurements

Participant height was recorded to the nearest 0.1 cm using a metric stadiometer. A portable weight scale (Tanita model HD-351, Arlington Heights, USA) was used to measure weight to the nearest 0.1 kg. Body Mass Index (BMI) was calculated as weight (kg) divided by the square of height (metres).Waist circumference of the abdomen was horizontally measured at the centre-point between the iliac crest and vertebro-costal margin while hip circumference was measured at its widest point of the buttocks using a metric tape (Model W606PM Lufkin, USA). Three blood pressure (BP) measurements were obtained using an automated device (Welch Allen, Medical Products, Skaneateles Falls, USA) at three-minute intervals while the participant was sitting upright in a chair. The final two sitting recordings were used to calculate the mean BP measurements.

2.6. Self-reported questionnaire

A questionnaire to obtain information relating to socio-demographic characteristics, tobacco smoking status and self-reported health was administered to study participants. Lifetime exposure to smoking was quantified as the number of 'pack years' [19]. Diabetes status was determined via self-report where study participants responded 'yes' to the question: "Has a doctor told you that you have diabetes". To account for potential undiagnosed diabetes, those with HbA1c ≥47.5 mmol/mol were included as having diabetes for this study.

2.7. Ethical approval

All participants provided informed consent and completed a medical history via interview prior to commencement. The PerioCardio study was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research, the Central Australian Human Research Ethics Committee, Northern Territory Correctional Services Research Committee, University of Adelaide Human Research Ethics Committee, and the Aboriginal Health Council of South Australia. Study participants gave informed consent before participating. Research was conducted in accordance with the World Medical Association Declaration of Helsinki (version VII, 2008).

2.8. Statistical methods

Extent of PD and CAL was calculated as the percentage of sites examined based on the methods of Carlos and colleagues [20]. Quartiles of extent PD ≥4 mm were calculated to stratify participants as a proxy for periodontitis exposure on cardiovascular risk factors. Mean carotid IMT, calculated as the average from the left and right carotid arteries, was used for analysis. Categorical variables were assessed using Chi-squared test. Continuous variables were assessed using various methods. For heavily skewed data, medians across quartiles were assessed using the Kruskal–Wallis test. For age and extent PD ≥4 mm, ANOVA using Scheffe's post-hoc tests for means was conducted (supplementary table). Least squares means across quartiles and respective standard errors were derived from ordinary least squares regression models after centering median age (39 years) and including modified Gingival Index and pack-years as covariates. A trend analysis was conducted to describe the type of relationship between extent PD ≥4 mm and PWV. The relationship between periodontal disease and IMT was assessed in two ways: firstly, comparisons of least squares means of extent PD ≥4 mm across quartiles, and secondly as a post-hoc

analysis using the stratifications of periodontal disease as described in the analysis from the dental component of the Atherosclerosis Risk in Communities (ARIC) study [21].

Three MANOVA models were constructed. The first quantified the extent to which periodontal disease acts as an exposure to the vasculature and potentially alters PWV after adjustment for age, gender, diabetes status, smoking and Gingival Index. These five covariates were chosen based on their strong individual associations to periodontal disease. The second model examined whether systolic blood pressure and BMI act as mediators in the periodontitis/PWV relationship. Finally, the third model additionally adjusted for periodontal and traditional Framingham risk factors which included systolic BP, HDL cholesterol, non-HDL cholesterol and equivalised household income which was derived by dividing the total household income by the number of occupants. This final model aimed to quantify how individual periodontal and cardiovascular factors collectively influence PWV. Statistical significance was inferred at $p < 0.05$ for all tests. Data were analysed using SAS version 9.3 (Cary, North Carolina, USA).

3. Results

A total of 312 participants were screened between June 2010 and January 2012. Of these, 273 (87.5%) were confirmed periodontitis cases. Complete data were available for 269 study participants with periodontitis, mean age 40.3 ± 10.2 years and age range 22–73 years. Males had a higher extent of PD ≥4 mm than females (Table 1). A higher proportion of those with a school-level education had periodontitis compared to those with a university-level qualification. Over 55% of people who self-reported a history of tobacco smoking exhibited the two highest quartiles of periodontal pocketing. In contrast, more than 65% of non-smokers were in the two lowest quartiles.

Measures of blood pressure and non-fasting cholesterol were not significantly different across quartiles of periodontal disease severity (Table 2). However, HDL cholesterol decreased with increasing extent of PD which, in turn, influenced the ratio of total versus HDL cholesterol. There were no significant differences to mean serum IL-6 and median hsCRP levels across quartiles, however, a wide range of hsCRP levels was detected within our sample (assay lower limit 0.16 mg/L, highest measured 31.9 mg/L) (data not shown). Supplementary Fig. 1 shows the proportions and respective frequencies of hsCRP across American Heart Association risk categories. Using these criteria, half of the present sample would be classified as high-risk for future events. A significant inverse relationship between increasing extent of periodontal pocketing and BMI was noted. In contrast, the ratio of waist to hip measurements significantly increased with increasing extent of PD ≥4 mm. The arginine/ADMA ratio and ADMA also did not significantly differ among quartiles of extent PD ≥4 mm (Table 2).

The two surrogate measures of vascular status yielded conflicting findings. While PWV increased monotonically with increasing extent of PD ≥4 mm, no consistent relationship was found concerning common carotid IMT at the univariate analysis level (Table 2). As a result, the remaining multivariable analysis focused on PWV data only. The relationship between PWV and extent PD ≥4 mm was mildly curvilinear, with the slope between the lowest and second lowest significantly steeper compared to the remaining slopes (Fig. 1).

Periodontal pocketing and age were significant indicators of PWV following MANOVA modelling that adjusted for age, gender, lifetime exposure to cigarettes, diabetes status and gingival inflammation ($r^2 = 0.24$) (Table 3). Model 2 shows that systolic blood pressure largely attenuates the association between periodontal severity and PWV ($r^2 = 0.46$) while the third MANOVA model which additionally adjusted for other Framingham risk factors contributed little supplementary information (Table 3).

To facilitate direct comparisons with the dental ARIC score, we calculated the extent CAL ≥3 mm in the present study as a post-hoc analysis. Seventy-five percent of participants had an extent CAL ≥3 mm of 30% or more (corresponding to 'severe' periodontal disease classification according to dental ARIC study criteria [21]). There was no significant difference in IMT after adjusting for age and sex in models of periodontal extent categorised as tertiles (p linear trend = 0.199), or quintiles (p linear trend = 0.274). The same models additionally adjusting for gingival bleeding and smoking were also not significant; tertiles (p linear trend = 0.068) and quintiles (p linear trend = 0.177). The entire post-hoc analytical process was repeated with extent PD ≥4 mm as the independent variable and similar findings were obtained (results not shown).

4. Discussion

This study evaluated the associations between extent of periodontal pocketing and surrogate measures of vascular health status, metabolic control and inflammation in Indigenous Australian adults with periodontal disease. We found that arterial stiffness increased with increasing extent of periodontal pocketing in this sample.

The tissue destruction arising from periodontitis can be extensive if numerous teeth are involved. The epithelial surface area exposed due to periodontitis has been estimated to be in the order of 8–20 cm^2 [22], although higher values up to 72 cm^2 have been proposed [23].

Table 1
Demographic characteristics stratified by quartile extent PD ≥4 mm.

	Group %	Lowest quartile	Second lowest	Second highest	Highest quartile	p-Value
Gender – male (n = 154)	57.9	29 (18.8)	34 (22.1)	41 (26.6)	50 (32.5)	<0.01
Female (n = 115)	42.1	39 (33.9)	33 (28.7)	25 (21.7)	18 (15.7)	
Location – Darwin (n = 150)	55.3	49 (32.7)	41 (27.3)	33 (22.0)	27 (18.0)	0.02
Katherine (n = 26)	9.5	5 (19.2)	7 (26.9)	7 (26.9)	7 (26.9)	
Darwin corrections (n = 52)	19.8	8 (15.4)	12 (23.1)	16 (30.8)	16 (30.8)	
Alice Springs corrections (n = 41)	15.4	6 (14.6)	7 (17.1)	10 (24.4)	18 (43.9)	
[a]Education – school level (136)	53.8	32 (23.5)	37 (27.2)	30 (22.1)	37 (27.2)	<0.01
Trade/certificate/diploma (93)	36.8	17 (18.3)	27 (29.0)	27 (29.0)	22 (23.7)	
University degree/higher (24)	9.5	16 (66.7)	1 (4.2)	4 (16.7)	3 (12.5)	
[b]Employment – employed (110)	45.3	42 (38.2)	28 (25.5)	21 (19.1)	19 (17.3)	<0.01
Unemployed (33)	13.6	1 (3.0)	8 (24.2)	12 (36.4)	12 (36.4)	
Not seeking/retired/other (100)	41.2	21 (21.0)	26 (26.0)	24 (24.0)	29 (29.0)	
[c]Diabetes – no (207)	80.5	54 (26.1)	57 (27.5)	52 (25.1)	44 (21.26)	0.03
Yes (n = 62)	19.5	12 (24.0)	7 (14.0)	11 (22.0)	20 (40.0)	
[d]Non-smoker (n = 87)	36.0	32 (36.8)	25 (28.7)	17 (19.5)	13 (14.9)	0.01
Smoker (n = 155)	64.0	30 (19.4)	39 (25.2)	43 (27.7)	43 (27.7)	

Group proportions (column %).
Quartile proportions presented as frequency (row %).
p values are Chi-square for differences between quartiles.
[a] Education status: missing data on 16 participants.
[b] Employment status: missing data on 26 participants.
[c] Diabetes status: self-reported 'yes' (N = 41) response or HbA1c ≥6.5% (N = 21).
[d] Smoking status: missing data on 27 participants.

Table 2
Cardiovascular risk factors stratified by quartile extent PD ≥4 mm.

	Group means ± SD	Least squares means (SE)			
		Low quartile	Second lowest	Second highest	Highest quartile
Age ± SD[a]	40.26 ± 10.24	41.91 ± 11.37	39.72 ± 9.76	39.72 ± 9.43	39.65 ± 10.33
Body Mass Index	29.12 ± 7.18	31.51 (1.00)	28.16 (0.92)*	28.65 (0.93)*	27.96 (1.09)*
Waist circumference (cm)	99.57 ± 14.75	102.42 (2.04)	96.63 (1.86)*	98.73 (1.98)	99.76 (2.22)
Waist-hip ratio	0.94 ± 0.07	0.92 (0.01)	0.92 (0.01)^	0.95 (0.01)^	0.96 (0.01)*
Total cholesterol (mmol/L)	4.98 ± 1.03	5.04 (0.15)	4.90 (0.14)^^	5.15 (0.15)	4.95 (0.16)
HDL cholesterol (mmol/L)	1.03 ± 0.32	1.09 (0.04)	1.07 (0.04)	0.99 (0.04)	0.96 (0.05)
TC/HDL ratio (mmol/L)	5.22 ± 1.85	5.05 (0.26)	5.05 (0.24)	5.51 (0.25)	5.54 (0.28)
Apolipoprotein-A1 (g/L)	1.25 ± 0.29	1.28 (0.04)	1.24 (0.04)	1.23 (0.04)	1.22 (0.05)
Apolipoprotein B (g/L)	0.97 ± 0.25	0.96 (0.04)	0.95 (0.04)	1.00 (0.04)	0.97 (0.04)
Interleukin 6 (pg/mL)	2.79 ± 2.27	2.82 (0.34)	2.50 (0.30)	2.76 (0.32)	2.80 (0.36)
hsCRP (mg/L)[b]	2.99 [1.42–6.09]	3.00 [1.29–6.97]	2.95 [1.25–8.80]	2.73 [1.43–6.37]	3.02 [1.83–5.58]
Urine ACR[b]	1.1 [0.50–2.50]	0.80 [0.30–1.80]	1.00 [0.50–1.75]	1.50 [0.50–3.50]	1.50 [0.50–5.20]#
ADMA (μM/L)	0.43 ± 0.12	0.44 (0.02)	0.43 (0.02)	0.42 (0.02)	0.42 (0.02)
Arginine/ADMA ratio	199.57 ± 64.88	186.88 (9.23)	198.97 (8.35)	212.03 (8.88)	198.02 (9.77)
Carotid IMT (mm)	0.63 ± 0.12	0.62 (0.01)	0.60 (0.01)	0.64 (0.01)	0.62 (0.02)
PWV (m/s)	8.34 ± 1.25	7.81 (0.16)	8.22 (0.14)*	8.51 (0.15)**	8.62 (0.17)**
Systolic BP (mm Hg)	118.36 ± 16.00	115.35 (2.14)	116.99 (2.00)	118.33 (2.05)	119.84 (2.37)
Diastolic BP (mm Hg)	75.30 ± 9.43	73.45 (1.25)	74.20 (1.16)	75.32 (1.19)	75.83 (1.37)

Age range [lowest Q1–highest Q4 quartile, minimum/maximum]: Q1 = 24/73; Q2 = 23/57; Q3 = 26/61; and Q4 = 22/62.
Range extent PD ≥4 mm [lowest Q1–highest Q4 quartile]: whole sample 8.97 [IQR = 15.18]; Q1 = 0–4.17; Q2 = 4.18–8.97; Q3 = 8.98–19.74; and Q4 = 19.75–72.32.
PWV: pulse wave velocity; BP: blood pressure; PP: pulse pressure; LDL: low-density lipoprotein; HDL: high-density lipoprotein; hsCRP: high-sensitivity C-reactive protein; urine ACR: urine albumin to creatinine ratio; ADMA: asymetric dimethylarginine; IMT: common carotid intima-media thickness.
[a] Age presented as mean ± SD. Comparison of quartile differences in extent PD ≥4 mm via ANOVA with Scheffe's post-hoc tests for means (non-significant quartile comparisons). Least squares means centred at median age = 39 years, additionally adjusted for severity of gingival bleeding and smoking exposure (pack-years).
[b] hsCRP & ACR presented as median [IQR].
* Significant difference versus lowest quartile (p-value <0.05).
** p-value <0.01.
^ Significant difference versus highest quartile (p-value <0.05).
^^ p-value <0.01.
# Significant difference versus lowest quartile (p-value <0.05) based on Kruskal–Wallis test.

The relative size of the 'periodontal wound' is important if the periodontium is considered a chronic source of infection and inflammation that has systemic implications. A dose–response relationship has been reported between the amount of periodontal inflamed surface area and glycaemic control in people with diabetes independent of sex, BMI and tobacco smoking [24].

Unlike traditional measures of CVD risk such as lipids and blood pressure, measurement of vascular stiffening provides a representation of the cumulative impact of both known and unknown risk factors

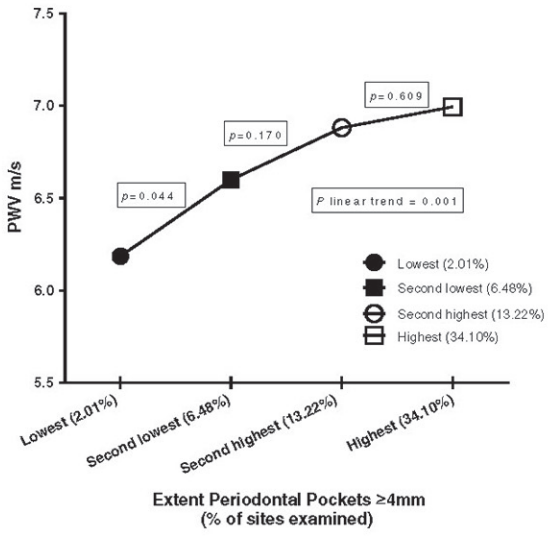

Fig. 1. Maximum likelihood estimates displaying the relationship between extent PD ≥4 mm and carotid/dorsalis pedis PWV. Probability values relate to between-quartile slope; p linear trend for overall relationship.

which may include periodontal disease. Our findings indicate that the severity of periodontal disease correlates with central arterial stiffening in a dose-dependent manner in a comparatively young sample. One study has reported that carotid–femoral PWV was significantly associated with severe periodontitis in a sample of people with heterozygous familial hypercholesterolemia [25]. An association between peripheral (carotid–radial) PWV and periodontal inflammation has been reported in a separate cohort of patients with type 2 diabetes [8]. In that study which included 18 subjects with periodontitis in a sample of 121 participants, there was a non-significant trend towards increased PWV when comparisons of those periodontally healthy were made against those with gingivitis and against those with periodontitis. Findings from Franek and colleagues [8] are difficult to compare with participants in the present study as all had periodontal disease, are some 20 years younger, and only 20% have diabetes. Similarly, results from the present study cannot be easily compared to people with genetic disorders of the lipid transport system [25]. Despite these differences, PWV between studies was similar suggesting that periodontal disease may contribute to premature vascular ageing, although how the mechanisms may interact has yet to be determined.

Carotid intima media thickness is known to increase with age [26]. Studies investigating the association between chronic periodontal disease and carotid IMT have focused on middle to older age groups [21,27,28] but two have studied aggressive forms among younger samples [29,30]. The most convincing evidence of a relationship between carotid intima-media thickening and periodontitis has arisen from the dental component of the Atherosclerosis Risk in Communities (ARIC) study [21]. In that study, data from IMT and periodontal assessments were available from 6017 participants aged 52 to 75. Following adjustment for age, gender and race, only participants with extent of CAL ≥3 mm of 17% or more corresponding to fourth and fifth highest quintiles had an IMT significantly thicker than the lowest quintile. Similarly, those classified with 'severe' periodontal disease using an alternate threshold defined as ≥30% extent CAL ≥3 mm had significantly higher IMT than moderate (10–30% extent) or non-periodontitis case (≤10%

Table 3
Multivariate ANOVA of periodontal disease effects on PWV.

	Model 1[a]				Model 2[b]				Model 3[c]			
	PWV	95% CI for β		p	PWV	95% CI for β		p	PWV	95% CI for β		p
		Low	High			Low	High			Low	High	
R^2	0.24				0.46				0.53			
Intercept	6.31	5.47	7.15	<0.001	2.77	1.68	3.85	<0.001	3.05	1.43	4.67	<0.001
Lowest quartile	Ref.				Ref.				Ref.			
Second lowest	0.32	−0.09	0.73	0.128	0.24	−0.12	0.60	0.187	0.08	−0.42	0.58	0.745
Second highest	0.53	0.09	0.96	0.018	0.40	0.03	0.77	0.037	0.36	−0.14	0.85	0.158
Highest quartile	0.57	0.07	1.06	0.026	0.32	−0.10	0.75	0.137	0.35	−0.25	0.96	0.249
Age	0.05	0.03	0.06	<0.001	0.03	0.01	0.04	<0.001	0.02	0.004	0.04	0.111
Gender	−0.32	−0.63	−0.01	0.044	−0.29	−0.56	−0.02	0.039	−0.34	−0.73	0.06	0.096
Diabetes	0.23	−0.16	0.63	0.243	0.32	−0.02	0.65	0.065	0.47	−0.04	0.99	0.071
Smoking	0.01	−0.003	0.02	0.172	0.01	−0.003	0.02	0.173	0.01	−0.01	0.02	0.304
Systolic BP	–	–	–	–	0.04	0.03	0.05	<0.001	0.04	0.03	0.05	<0.001
BMI	–	–	–	–	−0.005	−0.02	0.01	0.642	−0.01	−0.03	0.02	0.544
HDL cholesterol	–	–	–	–	–	–	–	–	0.39	−0.19	0.98	0.187
Non-HDL cholesterol	–	–	–	–	–	–	–	–	−0.10	−0.28	0.09	0.288
Income	–	–	–	–	–	–	–	–	−0.00	−0.001	0.000	0.279

PWV: pulse wave velocity; BP: blood pressure; BMI: Body Mass Index.
[a] MANOVA model 1) adjusted for age (median = 39), gender, smoking exposure (pack-years), diabetes status and severity of gingival bleeding.
[b] MANOVA model 2) adjusted for model 1 and BMI + systolic BP.
[c] MANOVA model 3) adjusted for model 2 and additionally for HDL cholesterol, non-HDL cholesterol and equivalised household income.

extent) [21]. By far the strongest contributor to thickening of the intima-media complex is the natural age-related physiological progression [26,31]. The age distributions of participants involved in the dental ARIC and the present study may explain the discrepancy between studies. Periodontal disease as a model of exposure, measured via the extent of PD or CAL may impart minimal effects on IMT in young to middle-aged adults. Further research will need to examine carotid IMT and periodontal disease associations in various age groups to clarify this.

Contrary to the wider periodontal literature, hsCRP was not associated with increasing extent of PD ≥4 mm after controlling for age, smoking and gingival bleeding severity in univariate analysis. A meta-analysis of cross-sectional studies showed that periodontitis increases systemic CRP levels with the mean in the order of 3.40 mg/L versus 1.76 mg/L for periodontitis cases and controls respectively and a weighted mean difference (95% CI) of 1.56 mg/L (1.21–1.90) [32].

Indigenous Australians have substantially higher baseline CRP levels when compared to other populations [33]. The present study includes a large proportion of participants who were overweight or obese, with the cohort having a mean BMI of 29 ± 7. Body fat distribution has been reported to differ among Indigenous Australians to that of Caucasians [34]. The correlations between the waist-to-hip ratio and BMI were weak (Pearson's $r = 0.09$ data not presented) in the present sample. Central abdominal obesity has been shown to be a strong indicator for elevated CRP levels among Indigenous Australians [35]. Both current smoking status and BMI were strong correlates for high concentrations of CRP and explained almost 60% of the variation in hsCRP values in a sample of some 10,000 Japanese men and women [36].

Almost half (46%) of Indigenous Australian adults are current smokers [11] which is comparable with our findings. Of the 155 participants in our sample who reported a history of smoking, 80% were current smokers [37]. The effect of smoking as a risk factor and its association with progression of periodontal disease has been thoroughly described [38–40]. Exposure to tobacco smoking contributes to premature ageing of the vasculature as evidenced by the risk of CVD among smokers clearly being higher than non-smokers [41]. Smoking and adiposity may be confounding the association between the extent of periodontal pocketing and hsCRP in the present sample. With this considered the degree to which periodontal disease is contributing to additional hsCRP levels in the present sample may be minimal. Accordingly, it is likely that the high overall levels of inflammatory markers, putatively related to periodontal disease and other risk factors, have reduced our ability to detect a specific association with the severity of periodontal disease.

Nevertheless, we propose that periodontal disease as a source of inflammation is influencing arterial function. Arterial stiffness is known to be related to the level of CRP in healthy individuals [42,43] and in people with hypertension [44], but evidence does not support this relationship among individuals with established coronary heart disease [45]. An increase in oxidative stress, the result of inflammation, may indirectly influence the bioavailability of nitric oxide a natural vasodilator, altering endothelial regulation and function [46]. The present study did not measure nitric oxide levels and therefore we cannot substantiate or conversely refute this notion. On the other hand, concentrations of ADMA, an inhibitor of nitric oxide synthase, did not differ among study participants; however other components of the nitric oxide synthesis pathway may be involved.

Results from our multivariate models suggest that the relationship between periodontal disease and PWV may be mediated by systolic blood pressure. Nonetheless, we did not find a significant association between periodontal disease severity and systolic blood pressure. This may be due to systolic blood pressure being measured at a single time-point which may not be representative or accurately reflect long-term blood pressure. Blood pressure at any given time-point does not influence PWV as it has been shown that rapid reduction of blood pressure in patients with hypertension when administered nitroglycerin fails to alter PWV [47]. In contrast, adaptive remodelling of the arterial wall does occur in response to prolonged exposure to hypertensive conditions. This results in the gradual fragmentation of elastin and repair with collagen, leading to arteriosclerosis.

4.1. Limitations

The cross-sectional nature of this study limits our ability to conclusively ascribe the severity of periodontal disease as the cause of increased PWV. While efforts were made to control for the effects of risk factors known to influence both periodontal disease and CVD (a notable example being age), it is not possible to completely eliminate both known and unknown residual confounders. For example one third of individuals with diabetes in this study were previously undiagnosed. Among those participants, long-term hyperglycaemic conditions may

have negatively impacted both their periodontal state and arterial vasculature. Additionally other inflammatory mediators produced during active phases of periodontal disease aside from hsCRP and IL-6 may be related to PWV. As our sample consists of Indigenous Australian adults, the results may not be directly generalisable to other ethnic groups which may have different body compositions and potentially fewer combined risk factors. Indigenous Australians frequently exhibit risk factors for both periodontal disease and CVD such as high rates of tobacco smoking and diabetes. We have reported that a large proportion of the present sample has levels of inflammation that if sustained, would place them at a high risk for future cardiovascular events. Other factors thought to impact on the overall health of Indigenous Australians such as socioeconomic pressures, housing and living conditions, infectious diseases and stress, have not been investigated in this present study. Despite a high proportion of those screened having moderate or severe periodontal disease, we do not believe that this is likely to have influenced our findings concerning associations of periodontal disease severity with measures of vascular health.

5. Conclusions

The severity of periodontal disease is associated with increased arterial stiffness, in a dose-dependent manner, in Indigenous Australian adults. These findings suggest a potential mechanism through which periodontal disease may influence cardiovascular disease.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2014.02.015.

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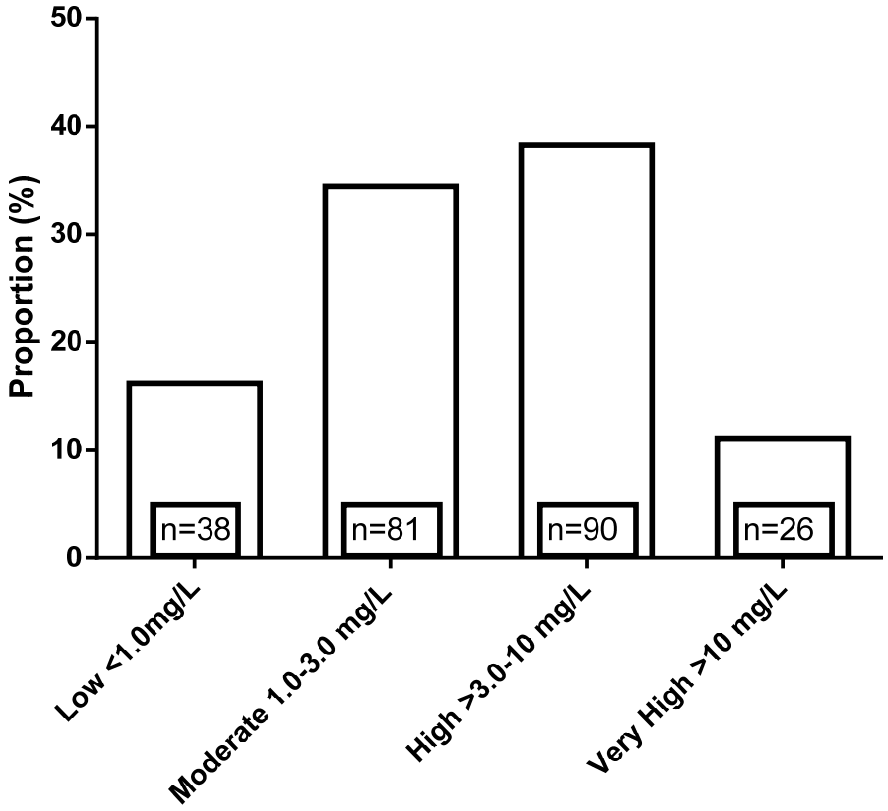
4.1 Online Supplement

Supplement Table 1: Oral Assessment Means Stratified by Quartile Extent PPD≥4mm.

	Group mean ± SD	**Lowest quartile**	**Second lowest**	**Second highest**	**Highest quartile**
Age ± SD	40.26 ± 10.24	41.91 ± 11.37	39.72 ± 9.76	39.72 ± 9.43	39.65 ± 10.33
Extent PPD ≥4mm	14.00 ± 13.95	2.01 ± 1.27	6.48 ± 1.31$^{**\wedge\wedge\#\#}$	13.22 ± 2.90$^{**\wedge\wedge\#\#}$	34.10 ± 12.46**
		Least Squares Means (SE)			
N. teeth	26.34 ± 5.96	26.17 (0.66)	27.75 (0.64)	26.77 (0.63)	26.19 (0.70)
Gingival Index$^{\alpha}$	1.45 ± 0.67	1.15 (0.06)	1.47 (0.06)$^{**\wedge}$	1.55 (0.06)**	1.70 (0.07)**
Index teeth with plaque	5.31 ± 1.19	5.34 (0.15)	5.45 (0.14)	5.50 (0.14)	5.23 (0.17)
Index teeth with calculus	4.17 ± 1.65	3.12 (0.20)	4.14 (0.18)$^{**\#\#}$	4.81 (0.19)$^{**\#\#}$	4.71 (0.22)**

Age and Extent PPD ≥4mm presented as mean ± SD. Comparison of quartile differences in extent PD ≥4mm via ANOVA with Scheffe's post-hoc tests for means.
Least squares means centred at median age (39 years) & adjusted for severity of gingival bleeding.
$^{\alpha}$Least squares means for Gingival Index centred at median age only.
**Significant difference versus lowest quartile (*p*-value <0.01); ^Significant difference versus highest quartile (*p*-value <0.05); ^^=*p*-value <0.01; $^{\#\#}$Significant difference versus middle quartiles (*p*-value <0.01)
Gingival Index: modified from Löe scoring system(Löe, 1967) (number of teeth with gingival bleeding / number of teeth periodontally assessed);
Maximum scores for plaque and calculus = 6.

There were no significant differences in terms of the mean number of teeth between quartiles. Overall, study participants were classified as having moderate gingival inflammation based on the modified Gingival Index (grouped mean). The severity of gingival inflammation increased with increasing extent of PPD ≥4mm despite no significant differences to plaque scores. Quartiles 2-4 had more index teeth with calculus compared to the lowest quartile.



Supplement Figure 1: Proportion and frequency of American Heart Association Risk Categories for hsCRP.

5 EFFECTS OF FULL-MOUTH SCALING ON THE PERIODONTAL HEALTH OF INDIGENOUS AUSTRALIANS: A RANDOMISED CONTROLLED TRIAL

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Journal of Clinical Periodontology

Effects of full-mouth scaling on the periodontal health of Indigenous Australians: a randomized controlled trial

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Abstract

Background: Simplified periodontal therapy might be a pragmatic strategy for public health programmes targeting Indigenous Australian adults. The objective of this randomized controlled trial was to evaluate oral health effects of single-visit, non-surgical periodontal therapy compared to no treatment.

Methods: This parallel-group, randomized, open label clinical trial enrolled 273 Indigenous Australians aged ≥18 years with periodontitis. Intervention participants received full-mouth periodontal scaling and root planing during a single visit while the control group received no treatment. Endpoints were summary variables derived from clinical assessments of probing depth, clinical attachment loss, plaque, calculus and gingival bleeding before treatment and 3 months later.

Results: Endpoints could be calculated for 169 participants with follow-up data. Compared to the control group, there were statistically significant reductions in extent of shallow pockets: PD ≥4 mm (mean difference −2.86, [95% CI −5.01 to −0.71], $p = 0.009$) and gingival bleeding (mean difference −0.25, [95% CI −0.43 to −0.08], $p = 0.005$) but not deeper pockets PD ≥5 mm (mean difference −0.48, [95% CI −1.78 to 0.82], $p = 0.468$) or plaque scores.

Conclusions: Periodontal therapy produced improvements in shallow periodontal pockets and measures of gingival bleeding in these Indigenous Australians.

Key words: full-mouth scaling; Indigenous Australian; non-surgical periodontal therapy; randomized controlled trial; smoking; subgingival scaling

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Conflict of interest and source of funding statement

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Indigenous Australians comprised Aboriginal and Torres Strait Islander people which collectively represent 2.5% of Australia's 22 million population (ABS 2009a). Thirty per cent of the Northern Territory (NT) population identify as Indigenous Australians (ABS 2008). Life expectancy among Indigenous Australians is up to 12 years lower that non-Indigenous Australians due to high rates of infectious and chronic diseases (AIHW 2005, 2010, ABS 2009b). Diabetes mellitus is 3.4 times as prevalent among Indigenous Australians relative to non-Indigenous Australians (ABS 2007). In 2002, just over half the Indigenous Australian population aged 15 years or older reported smoking cigarettes, with similar rates among men and women (AIHW 2005).

Few studies have investigated the periodontal status of Indigenous Australians. Observational studies from rural and remote communities of Australia have reported rates of periodontal disease ranging from

30% to 61% using the Community Periodontal Index (Endean et al. 2004, Smith et al. 2007, Kruger et al. 2008). The most recent national prevalence estimate of periodontal disease among Indigenous Australians is 29% compared to 23% for non-Indigenous Australians (Roberts-Thomson & Do 2007). Indigenous Australians are more likely to attend a dentist due to a problem, and have difficulty funding their dental care compared to non-Indigenous Australians, (AIHW DSRU 2000). Among publically funded patients, Indigenous Australians were also more likely to receive extractions than non-Indigenous Australians (Brennan et al. 1997, AIHW DSRU 2000).

Access to timely dental services is problematic for some Indigenous Australians (Harford et al. 2003). One quarter of the Indigenous Australian population live in locations considered remote or very remote (AIHW 2008); these areas of Australia are sparsely populated and have the lowest proportion of dental practitioners relative to the resident population (Balasubramanian & Teusner 2011).

Adult dental services in Australia are predominantly private. Government-funded dental care is provided to all school-aged children and adults whom meet means-tested eligibility criteria. Provision of dental services to remote communities of the NT is based on population size. For example, dentists visit communities with populations of 2000 or more people 1 week per month while smaller communities are serviced for 1 week quarterly (C. Handbury 2013, pers. comm., 13 Feb.). Anecdotal accounts from dental clinicians working in remote communities of the NT report that Indigenous patients attend appointments with expectations that presenting complaints are addressed and may not return for subsequent appointments. In light of this, oral health staff members attempt to complete as much treatment as possible in the single appointment. Similar accounts have been reported by oral health staff working in northern Queensland, a state to the east of the NT with similar Indigenous Australian demographic characteristics (Slater 2001).

Conventional periodontal treatment involves the removal of supra-subgingival calculus and plaque to disturb the microbial biofilm that initiate inflammatory processes. Both manual scaling and powered ultrasonic devices yield comparable outcomes (Ioannou et al. 2009). Successful treatment of periodontal disease not only results in resolution of periodontal inflammation as evidenced by reduction in periodontal pocketing and the potential for gain in attachment level, but also significantly reduces systemic markers of inflammation (D'Aiuto et al. 2005, López et al. 2011). A systematic review has recently shown that full-mouth scaling is equivalent to quadrant-wise scaling in improving periodontal clinical parameters including pocket depth (PD), clinical attachment loss (CAL) and bleeding on probing (Eberhard et al. 2008). The response to periodontal therapy is, however, not consistent among every person. Cigarette smoking, for example, has been found to impede the healing process following periodontal treatment, with non-smokers exhibiting greater signs of resolution compared to smokers (Heasman et al. 2006).

Indigenous Australians are a population that could be considered at increased risk for periodontal disease. There have been no studies to date that have investigated the effectiveness of periodontal treatment on this high risk population in a community setting. Given (a) Indigenous Australians have a higher reported prevalence of periodontal disease, (b) there are insufficient dental clinician resources available to perform comprehensive periodontal care and regular maintenance therapy and (c) there is a possibility that patients will not attend for multiple appointments; the purpose of the current intervention trial is to assess the effectiveness of single-visit full mouth non-surgical periodontal therapy in reducing periodontal pocketing, attachment loss and gingival bleeding among a sample of Indigenous Australians with periodontitis.

Materials and Methods

A detailed description of the methods has been published previously (Skilton et al. 2011). The PerioCardio study is a parallel-group, randomized, open label clinical trial investigating the effect of non-surgical periodontal therapy on surrogate markers of cardiovascular disease among Indigenous Australians. Recruitment commenced in June 2010 and was completed in January 2012. Subsequent manuscripts will report on the treatment effects on surrogate markers at different time-points over the 12 months study participants were monitored. This study examines the effectiveness of periodontal treatment on the periodontal health only.

The periodontal status of a convenience sample of Indigenous Australians residing in the NT, Australia was examined by two dental clinicians to verify periodontitis case. Eligibility for inclusion was determined via the combination of oral interview, medical history and oral assessment. To be eligible, participants had to be aged 18 years or older without a previous history of cardiovascular disease, have a minimum of five natural teeth, and a minimum of moderate periodontitis based on the US Centres for Disease Control and Prevention and American Academy of Periodontology (CDC-AAP) which is defined as at least two interproximal sites with CAL $\geq$4 mm, or at least two interproximal sites with PD $\geq$5 mm. (Page & Eke 2007).

Two changes to the original protocol were made during the study period. First, the inclusion criteria for participant age had listed the lower cut-point of 25 years. It was not expected that persons younger than 25 years would present with chronic periodontitis given it manifests much later in non-Indigenous Australians. A total of eight study participants aged 22 through 24 years were included in the PerioCardio study. Finally, the intervention was altered from two-time half-mouth mechanical debridement sessions to a single, full-mouth session due to operational issues relating to the availability of dental clinical facilities. A single session intervention was also chosen in accordance to study participant preference.

Individuals whom received periodontal treatment in the preceding 6 months, those with a history of any cardiovascular condition (with the exception of angina pectoris), rheumatic fever or any other cardiac or medical conditions which required

preventive antibiotic prophylaxis, pregnant women, or people with clinically visible endodontic or orofacial infections were excluded from participation.

Socio-demographic, lifestyle and medical history

A questionnaire to obtain information regarding socio-demographic characteristics including education level and household income, lifestyle factors such as smoking and medical history, including self-reported diabetes status, was administered to all participants. Lifetime exposure to smoking was quantified as the number of "pack years" equivalent to smoking 20 cigarettes per day for 1 year (Grossi et al. 1994).

Sample size

The sample size for the study was determined to address the study's primary endpoints of cardiovascular measures (Skilton et al. 2011). This study reports findings for periodontal parameters that are secondary endpoints. Briefly, power calculations indicated that a sample size of 144 participants equally randomized would be required to detect a 10% a priori change in carotid intima-media thickness and carotid-dorsalis pedis pulse wave velocity based on a power of 80% and $\alpha = 0.05$. To account for an a priori participant attrition of 25%, the original baseline sample size was set at 200. After 1 year of recruitment, almost 40% of the initial sample was lost to follow-up. A final sample size of 273 persons was subsequently recruited to ensure that the required sample size for the main study would be attained.

For this study, post hoc calculations using PROC POWER for all five outcomes showed that, with a sample of 169 subjects for whom oral health endpoints were available at the 3-month recall, the study had 99% power to detect each of the five between-group differences in means, assuming equal sized study groups and type I error of 0.05.

Oral assessment

Periodontal assessments were conducted at baseline and repeated at 3 months to obtain information on tooth presence, soft tissue assessment of gingival bleeding, plaque scores, calculus and periodontal destruction based on the methods used in the National Survey of Adult Oral Health (NSAOH) 2004–2006 (Slade et al. 2007). Dental plaque scores were recorded for six index teeth (if present) based on published criteria (Silness & Löe 1964). The selected teeth were the most anterior molar in each quadrant, upper right and lower left central incisors. The same six index teeth were assessed for calculus presence (0 = no, 1 = yes). Probing depth and gingival recession to generate CAL via algorithm were measured at four sites at every tooth excluding third molars. The sites included the mesio-buccal, mid-buccal, disto-buccal and disto-lingual. A single gingival bleeding value was recorded for each tooth periodontally assessed. The scoring system of the Gingival Index (values 2 and 3) (Löe 1967) were used to indicate the number of teeth with positive gingival bleeding. All periodontal recordings were entered into a conventional database (MS Access, 2007) where a computer algorithm was programmed to calculate the number of diseased sites to confirm periodontitis case status according to the CDC-AAP criteria. Dental assessment armamentarium included disposable mirrors (Mirrorlite™ Defend, New York, NY, USA) and a periodontal probe with 2 mm markings (product number PCP2; Hu-Friedy, Chicago, IL, USA).

Reproducibility of periodontal screening

Prior to study commencement, both dental clinicians involved in this study concurrently underwent training and calibration with the principal survey examiner of the NSAOH 2004–2006 (Slade et al. 2007). Five healthy volunteers were subsequently examined by the three clinicians each blinded to the assessment of the others. Periodontal measurements were standardized to within 1 mm among the three clinicians. Areas of difference arising from the assessments were discussed and agreement attained. Inter-examiner reliability between the two study clinicians during the study was assessed using volunteers not involved with the trail and precise agreement on the number of sites with PD ≥2 mm was the used as a cut-point. The weighted kappa statistic was 0.75 (95% CI 0.70–0.80) representing substantial agreement (Landis & Koch 1977).

Periodontal intervention

The periodontal intervention was provided upon completion of baseline assessments to those randomized into the treatment group and consisted of a single-visit full-mouth intensive non-surgical removal of sub and supragingival calculus and plaque biofilm ranging from 45 min. to 3 h following administration of local anaesthesia if requested. The intervention was provided at dental clinics located within government facilities, Indigenous Australian health services or within medical complexes of correctional facilities. Two clinicians, one oral health therapist (provider 1) and one dentist (provider 2) (see Fig. 1) conducted the intervention with the use of Gracey hand scalers (Hu-Friedy), and piezoelectric ultrasonic device (Kyungwon Ferrite, Gyeonggi-Do, Korea) using universal tips. The same intervention was provided to those randomized to the control group following completion of annual assessments. Both study arms received oral hygiene instruction along with toothbrush and toothpaste upon completion of their respective baseline assessments.

In the cases where oral assessments revealed a need for restorative services, study participants were provided a detailed report of clinical findings and were subsequently referred to either their local public dental service or their usual dental practitioner if not eligible for publically funded care.

Randomization

After screening for periodontal disease, those with moderate or severe periodontal disease were randomized on a 1:1 basis to either the treatment or control group. A computer generated permuted block randomization sequence with variable block sizes to control for the potential of selection bias, stratified by recruitment site (Darwin, Katherine, Darwin and Alice Springs correctional facilities), was created by a statistician not involved in the study. Enrolment of study participants was conducted by

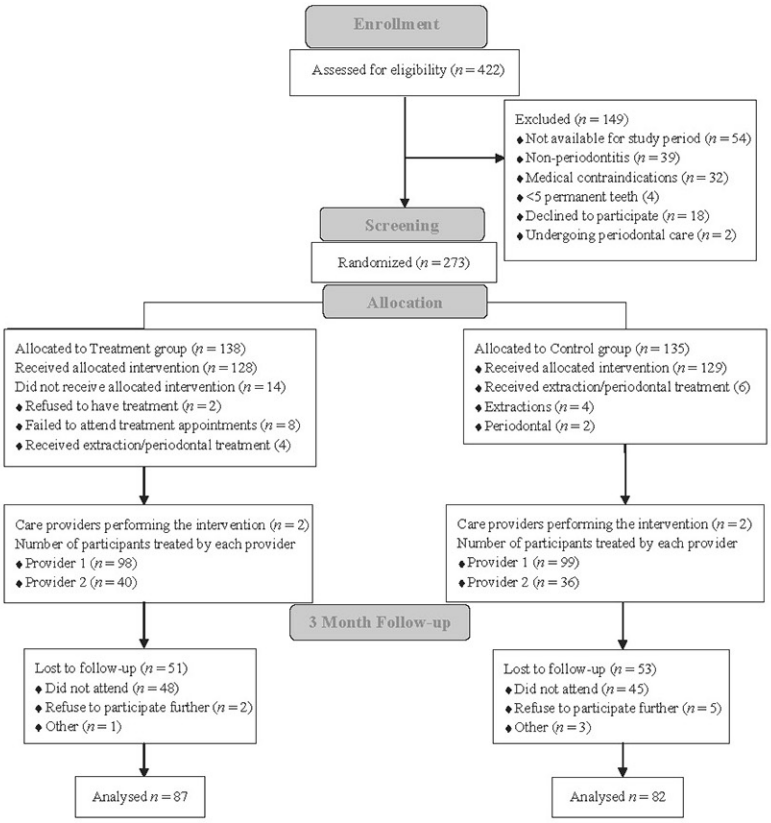

Fig. 1. PerioCardio study flow diagram

non-dental research staff. Randomization was allocated by the study clinicians unaware of block sizes following baseline measures by entering study participant's name and date of recruitment into the database. It was not possible to blind study participants from the periodontal intervention nor clinicians from allocation grouping. However, the clinician conducting the recall appointment was not informed of study participant group allocation in an attempt to minimize detection bias. One of the dental clinicians (KK) was responsible for assessing outcomes using de-identified data.

Statistical analysis

Primary analysis was based on the complete-case approach. Continuous variables are presented as mean ± standard deviation. Differences in means and proportions between completed and lost to follow-up cases were analysed using independent samples *t*-test and chi-square test respectively. Analysis of covariance (PROC GLM) was used to measure between-group comparisons on change in mean values with randomization as the factor and baseline values as covariates after being grand mean centred. Extent of PD and CAL were calculated as the percentage of sites examined based on the methods of Carlos and colleagues (Carlos et al. 1986). For ordinal measures of calculus and plaque assessed on six index teeth, the mean number of teeth with calculus or plaque was calculated. The extent of visible plaque [equivalent to Plaque Index (PI) values ≥2 (Löe 1967)] was determined to highlight degree of accumulation relative to total plaque. A modified gingival index score was calculated by dividing the number of teeth with gingival bleeding by the number of teeth periodontally assessed. An explanatory analysis was additionally conducted to evaluate the effect of factors known to be associated with periodontitis. Using a general linear model, age and lifetime exposure to cigarette smoking were entered as continuous variables along with self-reported diabetes and gender as binary variables.

Twelve study participants received either dental extractions or periodontal therapy beyond that of the study and 10 people from the treatment group failed to complete their allocated intervention. Collectively, these constituted a breach in protocol and therefore 253 were included in separate per-protocol analyses. All data were analysed using SAS version 9.3 (Cary, NC, USA).

Ethical approval

The PerioCardio study was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research, the Central Australian Human Research Ethics Committee, Northern Territory Correctional Services Research Committee, University of Adelaide Human Research Ethics Committee, and the Aboriginal Health Council of South Australia. Study participants gave informed consent before participating. Research was conducted in accordance with the World Medical Association Declaration of Helsinki (version VII, 2008).

Trial registration

The PerioCardio study has been registered with the Australian and New Zealand Clinical Trials Register (ANZCTR number: 12610000817044).

Results

Four hundred and twenty-two people were screened for eligibility during the study period with 312 meeting the general and medical inclusion criteria. These 312 were subsequently invited to undergo an oral assessment. Of those, 273 (87.5%) were clinically confirmed periodontitis cases. Reasons for exclusion of the 149 people are shown in Fig. 1. A total of 51 people in the treatment group and a further 53 in the control group were not assessed at the 3-month time-point representing a combined follow-up rate of 61.9%. Four participants in the treatment group and six in the control group received additional dental treatment beyond that of the study intervention. There were no

adverse effects reported by any study participants throughout the trial.

Both groups were comparable in terms of age, educational attainment and mean number of teeth (Table 1). There were no differences in sex distribution between treatment and control group. Approximately, 58% of our sample was male. Overall, 52.7% of study participants self-reported a history of smoking with slightly more participants in the treatment group compared to control. There were no noticeable differences between study arms in the per-protocol analysis at baseline.

Comparisons between those whom completed and those that were lost to follow-up revealed significantly more males completed both appointments ($p = 0.020$) (Table 2). There were no other significant differences when comparing the average age, educational attainment, smoking status, number of teeth and extent PD ≥4 mm. Of those whom completed the 3-month assessments, 48 in the treatment and 39 in the control group were current smokers, 6 and 8, respectively, were former (data not shown). More persons with self-reported diabetes completed the follow-up assessment; however, the difference was not significant.

The extent of sites with PD ≥4 mm and extent of CAL ≥3 mm and PD ≥4 mm were comparable at baseline but significantly lower in the treatment group after 3 months (Table 3). Analysis of the higher disease threshold PD ≥5 mm revealed no significant differences between study groups (mean reduction −0.48, [95% CI −1.78 to 0.82], $p = 0.468$). There was also no significant change in PD ≥4 mm after adjustment for age, lifetime exposure to cigarette smoking, gender and self-reported diabetes status in an exploratory analysis (mean reduction −1.59, [95% CI −4.69 to 1.51], $p = 0.311$) (data not shown). Reduced dental calculus was observed in the treatment group despite no significant difference in changes to total dental plaque scores (mean reduction −0.15, [95% CI −0.34 to 0.03], $p = 0.109$), after 3 months. Negligible changes to effects were observed following per-protocol analysis (Table 4).

Discussion

This study shows that non-surgical periodontal therapy using a single, untimed visit approach leads to improvements in periodontal status in an Indigenous Australian population. The change in extent of periodontal pocketing and CAL is equivalent to a 25% reduction between trial arms which has clinical relevance. It is important to note that the results of this trial are short term and need to be considered in this context. A significant improvement in shallow pockets occurred without concomitant reductions in PD ≥5 mm. The modest improvements in shallow pockets are likely attributed to a reduction in periodontal inflammation in response to the intervention. Plaque presence is not sufficient to cause periodontal disease; however, regular oral hygiene is fundamental to the potential success of any periodontal treatment. Clinically, periodontal health is defined as the absence of periodontal pocketing and gingival bleeding (Offenbacher et al. 2007). Despite a significant improvement in gingival bleeding for the treatment group, moderate inflammation persisted. Although participants were provided oral hygiene instruction, a toothbrush and toothpaste at the conclusion of the baseline appointment, no significant improvements in plaque levels in either group upon recall was detected. Observational studies have reported infrequent tooth brushing practices among sampled Indigenous Australians (Schamschula et al. 1980, Smith et al. 2007, Kruger et al. 2008).

Similar intervention approaches to this study have shown that marked reductions in periodontal pocketing, gingival bleeding and plaque levels can be achieved during a single session. Non-surgical periodontal treatment administered over two appointments within 24 h resulted in a 75% reduction in the mean number of periodontal pockets >4 mm (baseline mean number of

Table 1. Baseline characteristics of study participants

	Complete-case		Per-protocol†	
	Treatment ($n = 138$)	Control ($n = 135$)	Treatment ($n = 124$)	Control ($n = 129$)
Mean age (years)	40.0 ± 10.9	40.3 ± 9.6	40.2 ± 11.0	40.4 ± 9.6
Gender – male (%)	77 (55.8)	81 (60.0)	66 (53.2)	76 (58.9)
Qualification – school level*	69 (53.5)	67 (54.0)	65 (54.6)	63 (53.4)
Trade/apprentice/diploma	49 (38.0)	44 (35.5)	44 (37.0)	42 (35.6)
University degree/higher	11 (8.5)	13 (10.5)	10 (8.4)	13 (11.0)
Mean household income ($)	358.99 ± 324.7	370.64 ± 255.15	345.25 ± 319.46	376.31 ± 256.59
Smoker (pack years)				
None	60 (43.5)	69 (51.1)	50 (40.3)	67 (51.9)
<5 pack years	40 (29.0)	25 (18.1)	39 (31.5)	24 (18.6)
>5 pack years	38 (27.5)	41 (30.4)	35 (28.2)	38 (29.5)
Self-reported diabetes (%)	23 (16.7)	18 (13.3)	22 (17.7)	18 (14.0)
Mean number of teeth	26.2 ± 6.0	26.5 ± 5.9	26.2 ± 6.0	26.4 ± 6.0

Continuous variables are mean ± SD; categorical values are frequency (column %); Household income in Australian dollars derived from total household income divided by the number of occupants in the house.

*Total $n < 273$. ITT: treatment = 129, control = 124; Per protocol: treatment = 119, control = 118

†Per-protocol analysis: Treatment group: 10 participants excluded (did not receive intervention), a further 4 excluded due to additional treatment beyond intervention; Control group: 6 participants excluded due to non-adherence of study protocol (dental extractions and periodontal treatment).

Table 2. Characteristics of completed versus lost-to follow-up participants at 3 months

	Completed (n = 169)	Lost to follow-up (n = 104)	p-value
Mean age (years)	40.2 ± 10.4	40.0 ± 10.1	0.862
Gender			
Male (%)	107 (67.7)	51(32.3)	0.020
Female (%)	62 (53.9)	53 (46.1)	
Qualification – school level	85 (62.5)	51 (37.5)	0.926
Trade/apprentice/diploma	59 (63.4)	37 (36.6)	
University degree/higher	16 (66.7)	8 (33.3)	
Mean household income ($)	350.3 ± 269.1	387.1 ± 324.9	0.467
Smoker (pack years)			
None	50 (57.5)	37 (42.5)	0.487
<5 pack years	34 (64.2)	19 (35.9)	
>5 pack years	67 (65.7)	35 (34.3)	
Self-reported diabetes (%)	29 (70.7)	12 (29..3)	0.207
Mean number of teeth	26.4 ± 6.1	26.4 ± 5.7	0.996
Extent PD ≥4 mm	10.9 ± 11.6	12.8 ± 13.3	0.222

Continuous variables are mean ± SD; categorical values are frequency (row %).
Household income in Australian dollars derived from total household income divided by the number of occupants in the house.

pockets: 64.43 ± 8.37 *versus* 16.43 ± 5.31 at 3 months), in concert with equivalent reductions in full-mouth plaque scores (baseline 68% to 10% at 3 months) (Graziani et al. 2010). Another study using full-mouth ultrasonic debridement reported a 58% decline in the proportion of pockets ≥4 mm and a 45% decrease in full-mouth bleeding on probing scores after 3 months (Wennström et al. 2005). Participants in both the aforementioned studies had, on average, a greater extent and severity of periodontal pocketing and were approximately 10 years older than the present sample making comparisons difficult. It could be postulated that improved oral hygiene in these studies may have contributed to the greater inter-

Table 3. Periodontal parameters at baseline and 3 months (complete-case analysis)

	Treatment		Control		ANCOVA adjusted mean Δ (95% CI)	p-value
	Baseline	3 months	Baseline	3 months		
Extent PD ≥4 mm (%)	13.40 ± 12.84	9.07 ± 10.49	14.50 ± 14.87	12.90 ± 12.37	−2.86 (−5.01 to −0.71)	0.009
Extent CAL ≥3 mm and PD ≥4 mm (%)	13.21 ± 12.68	8.89 ± 10.39	14.32 ± 14.70	12.51 ± 11.73	−2.70 (−4.79 to −0.60)	0.012
Extent PD ≥5 mm (%)	4.41 ± 6.97	3.13 ± 6.88	5.40 ± 8.93	4.21 ± 5.60	−0.48 (−1.78 to 0.82)	0.468
Mean index teeth with calculus	4.20 ± 1.62	2.20 ± 1.79	4.17 ± 1.66	4.01 ± 1.67	−1.70 (−2.14 to −1.26)	<0.001
Mean gingival bleeding score	1.44 ± 0.71	1.04 ± 0.61	1.57 ± 0.65	1.33 ± 0.61	−0.25 (−0.43 to −0.08)	0.005
Mean index teeth with plaque	5.26 ± 1.21	5.27 ± 1.33	5.37 ± 1.17	5.42 ± 1.04	−0.15 (−0.34 to 0.03)	0.109
Extent visible plaque (%)	29.40 ± 36.51	23.43 ± 31.97	28.37 ± 34.41	31.77 ± 38.34	−7.42 (−16.04 to 1.19)	0.091

Values are mean ± SD.
Mean gingival bleeding: modified from Löe (1967) scoring system (number of teeth with BOP/number of teeth periodontally assessed).
Maximum score for index teeth with calculus and plaque = 6.
Extent visible plaque limited to scores ≥2 indicative of moderate/abundant plaque visible with the naked eye (Löe 1967).
PD, pocket depth; CAL, clinical attachment loss.

Table 4. Periodontal parameters at baseline and 3 months (per-protocol analysis)*

	Treatment (n = 124)		Control (n = 129)		ANCOVA adjusted mean Δ (95% CI)	p-value
	Baseline	3 months	Baseline	3 months		
Extent PD ≥4 mm (%)	13.63 ± 12.96	10.43 ± 11.78	14.26 ± 14.86	12.90 ± 14.08	−2.70 (−4.94 to −0.47)	0.018
Extent CAL ≥3 mm and PD ≥4 mm (%)	13.42 ± 12.79	10.20 ± 11.50	14.13 ± 14.79	12.72 ± 13.86	−2.51 (−4.70 to −0.33)	0.024
Extent PD ≥5 mm (%)	4.39 ± 6.90	3.10 ± 6.84	5.40 ± 8.89	4.21 ± 5.60	−0.44 (−1.79 to 0.92)	0.525
Mean index teeth with calculus	4.22 ± 1.61	2.17 ± 1.80	4.20 ± 1.66	4.01 ± 1.66	−1.73 (−2.19 to −1.27)	<0.001
Mean gingival bleeding score	1.44 ± 0.71	1.04 ± 0.61	1.57 ± 0.65	1.33 ± 0.61	−0.26 (−0.44 to −0.07)	0.006
Mean index teeth with plaque	5.26 ± 1.20	5.24 ± 1.35	5.37 ± 1.17	5.42 ± 1.04	−0.17 (−0.37 to 0.03)	0.089
Extent visible plaque (%)	30.79 ± 37.33	23.16 ± 31.88	28.28 ± 34.32	31.77 ± 38.34	−6.00 (−14.90 to 2.91)	0.186

Values are mean ± SD.
Mean gingival bleeding: modified from Löe (1967) scoring system (number of teeth with BOP/number of teeth periodontally assessed).
Maximum score for index teeth with calculus & plaque = 6.
Extent visible plaque limited to scores ≥2 indicative of moderate/abundant plaque visible with the naked eye (Löe 1967).
*Per-protocol analysis: Treatment group: 10 participants excluded (did not receive intervention), a further 4 excluded due to additional treatment beyond intervention; Control group: 6 participants excluded due to non-adherence of study protocol (dental extractions and periodontal treatment).
PD, pocket depth; CAL, clinical attachment loss.

vention effectiveness compared to this study. It is unclear whether additional treatment appointments within the 3-month follow-up period to rescale bleeding sites and to reinforce oral hygiene among the PerioCardio participants would have resulted in a larger treatment effect. Although other studies suggest a multiple-visit approach is warranted (Ide et al. 2003, Vidal et al. 2009, Kamil et al. 2011), this would have been difficult to achieve in this study given challenges in recalling participants, difficulties in accessing dental clinical facilities and staff availability. As this is the first study to investigate a periodontal intervention among Indigenous Australians, it is unclear whether response to therapy differs among Indigenous Australians compared to other populations.

This study employed local Indigenous Australian Health Workers to assist in the initial recruitment and follow-up of participants which included transportation to and from the research centres for assessments. Despite numerous attempts to schedule and reschedule participants who failed to attend pre-arranged appointments, only 62% of the initial sample was completed. Study participants were assigned as lost to follow-up if they could not be reassessed during the 3-month treatment window which was 10–16 weeks from baseline. The periodontal intervention was conducted around existing services provided by the NT government by non-NT government clinical staff. All participants randomized in the treatment group with few exceptions (Fig. 1) received their allocated intervention.

Although Indigenous Australians comprise a relatively small proportion of the Australian population, they have a disproportionately higher odds of developing periodontitis and cardiovascular disease, given the high reported prevalence of smoking and diabetes (AIHW 2010). The effects of cigarette smoking as a risk factor for periodontal disease and its dose-dependent association with both the progression and severity of periodontitis is well understood (Bergström 2006, Do et al. 2008). Similarly, smokers have less favourable outcomes following periodontal therapy compared to non-smokers (Ah et al. 1994, Grossi et al. 1996). While a large proportion of study participants reported a history of smoking in this study, we did not find any significant interactions to effects following post hoc testing (data not presented). Similarly, an explanatory analysis accounting for age, lifetime tobacco exposure and gender attenuated the modest improvement in PD ≥4 mm.

Although some degree of participant attrition in longitudinal studies is expected, we did not anticipate a 38% loss to follow-up after 3 months. A Pub Med literature search for randomized trials among Indigenous Australian adults yielded just two studies. One of pregnant women recruited for a quit-smoking intervention (Eades et al. 2012). In that trial, 67% of women were successfully followed up 36 weeks after recruitment. The other involved adult women in a 12-week exercise and nutrition monitoring programme (Canuto et al. 2012). Loss to follow-up in that study was 33% at 3 months and 47% 6 months from recruitment. In this context, the completion of this study could be considered somewhat comparable.

Missing data in this study arose from lost-to follow-up which was a random event. This determination is made on the basis that there were few differences in the characteristics of those completed and those lost to follow-up (Table 2). For pragmatic trials such as this, the complete-case analysis allows for unbiased estimates when missing values occur randomly at the expense of statistical power (Groenwold et al. 2012). Deviation from assignment in the control group in addition to failure to receive the intervention in the treatment group also has the potential to dilute the treatment effect. Despite 10 study participants breaching protocol by receiving additional dental treatment and 10 participants assigned to the treatment group not receiving the allocated intervention, we did not expect that these would have influenced the effect sizes achieved in our primary results. Findings from the per-protocol analysis (Table 4) are congruent to this. Post hoc multiple imputation for missing periodontal parameters at follow-up using a Markov-chain Monte Carlo method did not notably alter the effect sizes of any outcomes (data not presented).

This study has a number of strengths, including being the largest study to assess the periodontal status of Indigenous Australian adults in the NT, the involvement of Indigenous Australian staff, collaborations with correctional facilities and support from the Northern Territory Government Oral Health services. We were also able to diagnose and provide treatment for periodontitis among a sample of the population that were otherwise unlikely to seek oral health care services. In addition, we provide evidence on the effectiveness of a simplified periodontal intervention in a community setting. A methodological limitation was that allocation concealment was not possible as both clinicians were involved in the oral assessments at baseline and recall and both conducted the treatment of study participants. Although detection bias could not be eliminated, efforts were made to reduce its effects.

In conclusion, this study shows that intensive non-surgical periodontal therapy can improve periodontal status in a high-risk population without changing oral hygiene. These findings provide supporting evidence for the provision of periodontal services as part of regular dental care to Indigenous Australians. It also highlights some difficulties in undertaking longitudinal research in this population that may be of benefit to future investigators.

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Clinical Relevance

Scientific rationale for the study: The prevalence of periodontitis varies among populations, minority groups and people with particular risk factors. Indigenous Australians are a minority group with multiple risk factors. Response to periodontal treatment has not been investigated in this population.
Principal findings: In a sample whom many smoke tobacco, non-surgical periodontal therapy administered in a single appointment reduced the extent of shallow periodontal pocketing and gingival bleeding.
Practical Implications: Full-mouth non-surgical periodontal therapy administered in a single appointment can improve periodontal status in the short term.

6 THE EFFECT OF PERIODONTAL THERAPY ON ARTERIAL STRUCTURE AND FUNCTION AMONG ABORIGINAL AUSTRALIANS: A RANDOMISED CONTROLLED TRIAL

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Author Contributions

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Clinical Trial

Effect of Periodontal Therapy on Arterial Structure and Function Among Aboriginal Australians

A Randomized, Controlled Trial

Kostas Kapellas, Louise J. Maple-Brown, Lisa M. Jamieson, Loc G. Do, Kerin O'Dea, Alex Brown, Tommy Y. Cai, Nicholas M. Anstey, David R. Sullivan, Hao Wang, David S. Celermajer, Gary D. Slade, Michael R. Skilton

Abstract—Observational studies and nonrandomized trials support an association between periodontal disease and atherosclerotic vascular disease. Both diseases occur frequently in Aboriginal Australians. We hypothesized that nonsurgical periodontal therapy would improve measures of arterial function and structure that are subclinical indicators of atherosclerotic vascular disease. This parallel-group, randomized, open label clinical trial enrolled 273 Aboriginal Australians aged ≥18 years with periodontitis. Intervention participants received full-mouth periodontal scaling during a single visit, whereas controls received no treatment. Prespecified primary end points measured 12-month change in carotid intima-media thickness, an indicator of arterial structure, and 3- and 12-month change in pulse wave velocity, an indicator of arterial function. ANCOVA used complete case data to evaluate treatment group differences. End points could be calculated for 169 participants with follow-up data at 3 months and 168 participants at 12 months. Intima-media thickness decreased significantly after 12 months in the intervention group (mean reduction=−0.023 [95% confidence interval {CI}, −0.038 to −0.008] mm) but not in the control group (mean increase=0.002 [95% CI, −0.017 to 0.022] mm). The difference in intima-media thickness change between treatment groups was statistically significant (−0.026 [95% CI, −0.048 to −0.003] mm; P=0.03). In contrast, there were no significant differences between treatment groups in pulse wave velocity at 3 months (mean difference, 0.06 [95% CI, −0.17 to 0.29] m/s; P=0.594) or 12 months (mean difference, 0.21 [95% CI, −0.01 to 0.43] m/s; P=0.062). Periodontal therapy reduced subclinical arterial thickness but not function in Aboriginal Australians with periodontal disease, suggesting periodontal disease and atherosclerosis are significantly associated. (***Hypertension***. **2014;64:702-708.)** • **Online Data Supplement**

Key Words: Aborigines, Australian ■ diabetes mellitus ■ periodontal debridement ■ randomized controlled trial ■ smoking

Periodontal disease is characterized by bacterial infection and chronic inflammation of the tissues around teeth.[1] There is now a large body of evidence, primarily from observational cohort studies and nonrandomized trials, suggesting a possible association among periodontal disease, atherosclerotic vascular disease,[2–4] and arterial stiffness of peripheral arteries.[5–7] Despite published data supporting such associations, some recent reviews have concluded that the current evidence does not support a causative relationship between periodontal disease and atherosclerotic vascular disease.[2–4] However, reviewers cited a lack of evidence from randomized trials investigating periodontal interventions on atherosclerotic disease or cardiovascular events. Currently, no studies have investigated whether treatment of periodontal disease improves arterial function.

Coronary heart disease typically occurs much earlier among Aboriginal and Torres Strait Islanders compared with their non-Aboriginal counterparts.[8] Periodontal disease is more common among Aboriginal Australians according to Australian national survey data.[9]

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A randomized, controlled trial was conducted to determine the effect of a periodontal intervention on the progression of carotid intima-media thickness (IMT), an established noninvasive measure of subclinical atherosclerosis,[10] and central arterial stiffness measured via carotid-dorsalis pedis pulse wave velocity (PWV) in Aboriginal adults.

Methods

A detailed description of the methods is given in the online-only Data Supplement.

Recruitment commenced in June 2010 and completed in January 2012. Participants were recalled at 3 months and 12 months for repeat assessments of periodontal status and cardiovascular end points. Eligibility criteria were as follows: Aboriginal Australian participants aged ≥18 years without a previous history of cardiovascular disease, a minimum of 5 natural teeth, and moderate periodontitis defined as ≥2 interproximal sites with clinical attachment loss ≥4 mm or ≥2 interproximal sites with pocket depth ≥5 mm.[11] Exclusion criteria were as follows: individuals receiving periodontal treatment in the preceding 6 months, those with a history of any cardiovascular condition, rheumatic fever or any other cardiac or medical conditions requiring preventive antibiotic prophylaxis, pregnant women, or people with clinically visible endodontic or orofacial infections.

Interventions

The periodontal intervention consisted of an untimed single episode of nonsurgical periodontal therapy as described in detail elsewhere.[12,13] Briefly, 2 clinicians conducted the intervention (see online-only Data Supplement) with the use of Gracey hand scalers (Hu-Friedy, Chicago, IL) and piezoelectric ultrasonic device (Kyungwon Ferrite, Gyeonggi-do, Korea) using universal tips. All participants received oral hygiene instruction along with toothbrush and toothpaste.

Arterial Function

The functional outcome was the short-term 3-month change in carotid-dorsalis pedis PWV, whereas 12-month PWV provided long-term information on functional changes.[14] Applanation tonometry was used to measure PWV via a Millar transducer, the SphygmoCor-PVMx device, and software (version 8.2, AtCor Medical, Sydney, Australia).[15]

Carotid Intima-Media Thickness

The long-term structural outcome measure was the 12-month post-baseline change in maximum carotid IMT, as previously described.[13,16] Briefly, the common carotid arteries are scanned in longitudinal section by high-resolution ultrasound (10-5 MHz linear array transducer; Sonosite MicroMaxx, Bothell, WA) using a standardized scanning protocol that was consistent for all study visits. The scanning protocol consisted of sequential imaging of anterior, lateral, and posterior views of the carotid artery. Carotid IMT was assessed from the view with the greatest wall thickness.

Randomization

Participants were randomized on a 1:1 basis to either intervention or control group using permuted block randomization with variable block sizes, stratified by recruitment site (Darwin, Katherine, Darwin, and Alice Springs correctional facilities). Randomization was allocated by the study clinicians unaware of block sizes following baseline measures. Because of the mode of intervention, clinicians and study participants could not be blinded from allocation grouping.

Sample Size

Sample size was based on the 12-month structural outcome measure, carotid IMT. Estimates of carotid IMT were derived from published data for Aboriginal Australian adults from the same region (mean [SD], 0.67±0.12 mm)[17] and an estimated 10% difference between groups in maximum carotid IMT after intervention, based on prior evidence from a nonrandomized study.[18] Based on a power of 80% and α of 0.05, calculations indicated that a sample size of 144 participants randomized equally would be required. An a priori change of 10% reduction in PWV was proposed to indicate a clinically significant improvement in this surrogate measure of arterial stiffness. Based on carotid-femoral PWV of a similar sample,[19] a mean (SD) of 8.0 (1.7) m/s was used to estimate the sample size.

Statistical Analysis

Primary analyses for both PWV and IMT were based on the complete case approach. ANCOVA measured between-group change for PWV at 3 and 12 months and both mean and maximum IMT at 12 months, with randomization as the factor after adjustment for baseline values as covariates.

Change in carotid IMT from baseline to 12 months was normally distributed; however, cross-sectional measures of carotid IMT at baseline and 12 months were positively skewed. As such, cross-sectional measures were log-transformed for analysis. Differences in proportions and means between completed and lost to follow-up participants were analyzed using χ^2 and independent t tests. Specific to IMT, subgroup analyses were undertaken within groups stratified according to baseline measures of sex, age, overweight/obesity, diabetes mellitus, smoking status, and severity of periodontal disease. As a secondary analysis to enable inclusion of the baseline data of participants lost to follow-up and 3 participants who had carotid IMT data available from 12 months but not baseline, we constructed a linear mixed model including carotid IMT data from both baseline and 12 months, with the effect of the intervention determined by the interaction between visit number and allocated group.

Ethical Approval

The PerioCardio study was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research, the Central Australian Human Research Ethics Committee, Northern Territory Correctional Services Research Committee, University of Adelaide Human Research Ethics Committee, and the Aboriginal Health Council of South Australia. Study participants gave informed consent before participating, which included acknowledgment that should they be randomized into the control group they would not receive immediate periodontal therapy. Research was conducted in accordance with the World Medical Association Declaration of Helsinki (version VII, 2008).

Results

Four hundred twenty-two people were initially screened for eligibility, and of those, 312 met the inclusion criteria and underwent oral assessment. Of those, 273 were clinically confirmed periodontitis cases. Reasons for exclusion of 149 people are included in Figure S1 in the online-only Data Supplement. Fifty-one people in the treatment and 53 in the control group were not assessed at the 3-month time point, and 49 and 56 in the treatment and control groups, respectively, were lost to follow-up at the 12-month time point. Follow-up rates did not differ by randomized group (3 months: intervention 63%, control 61%, P=0.70; 12 months: intervention 65%, control 59%, P=0.26). One participant randomized to the treatment group and self-reported experiencing a myocardial infarction during the study period. There were no other adverse events reported.

Participant characteristics stratified by randomized groups are shown in Table 1, and for those participants who attended the 3- and 12-month recalls compared with those who were lost to follow-up, see Table S1. Baseline PWV, IMT, and periodontal parameters were similar in those lost to follow-up compared with those who completed follow-up at 3 months and 12 months.

Table 1. Baseline Demographic, Periodontal, Anthropometric, and Cardiovascular Risk Factors and Metabolic Markers by Randomization Status

Participant Characteristics	Treatment (n=138)	Control (n=135)
Mean age, y	40.2 (10.9)	40.3 (9.6)
No. of men, %	77 [55.8]	81 [60.0]
Current smoker, %*	87 [69.6]	74 [62.2]
Former/never-smoker, %*	38 [30.4]	45 [37.8]
Diabetes mellitus, %†	35 [25.4]	27 [20.0]
HbA1c, mmol/mol	46.5 (17.9)	43.4 (13.0)
Systolic BP, mm Hg	124.4 (16.8)	125.5 (16.6)
Diastolic BP, mm Hg	80.2 (10.4)	80.2 (10.2)
Total cholesterol, mmol/L	5.01 (1.05)	4.96 (1.02)
Non-HDL cholesterol, mmol/L	3.99 (1.04)	3.89 (1.03)
HDL cholesterol, mmol/L	1.01 (0.31)	1.07 (0.32)
hsCRP, mg/L	4.90 (5.22)	4.88 (5.51)
IL-6, pg/mL	3.02 (2.26)	2.5 (2.26)
ADMA, μmol/L	0.42 (0.12)	0.43 (0.11)
Body mass index	28.93 (6.33)	29.32 (7.99)
Mean PWV, m/s	8.23 (1.22)	8.45 (1.27)
Mean IMT, mm‡	0.62 (0.10)	0.63 (0.11)
Maximum IMT, mm‡	0.76 (0.14)	0.76 (0.16)
Mean number of teeth	26.20 (5.96)	26.56 (5.93)
Extent CAL ≥3 mm, %	53.10 (24.84)	53.50 (26.40)
Extent PD ≥4 mm, %	13.49 (12.95)	14.51 (14.93)
Mean sites with calculus	4.20 (1.62)	4.13 (1.69)
Mean gingival index	1.49 (0.65)	1.41 (0.68)
No. of index teeth with plaque	5.26 (1.21)	5.37 (1.21)

Continuous variables are mean (SD); categorical values are presented as n [%].Gingival index: modified from Löe and Silness scoring system (number of teeth with bleeding on probing/number of teeth periodontally assessed); maximum score for plaque and calculus=6. ADMA indicates asymmetrical dimethylarginine; BP, blood pressure; CAL, clinical attachment loss; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; IMT, intima-media thickness; PD, pocket depth; and PWV, pulse wave velocity.

*Complete data are only available for n=244 for smoking status.

†Diabetes mellitus via self-report (n=41) or when HbA1c ≥47.5 mmol/mol (additional 21).

‡Geometric mean (interquartile range) for non-normally distributed variables.

Pulse Wave Velocity

After the periodontal intervention, PWV values improved to a greater degree at both 3 and 12 months in the control group (3-month between-group ANCOVA Δ, +0.06 [95% confidence interval {CI}, −0.17 to 0.29] m/s; *P*=0.60 and 12-month between-group ANCOVA Δ, +0.21 [95% CI, −0.01 to 0.43] m/s; *P*=0.06) but were not significantly different than the treatment group (Tables 2 and 3). Stratified analysis by sex revealed no significant treatment effects at 3 months; however, there was a tendency for PWV being higher among men in the treatment group after 1 year (Table S5).

Carotid IMT

In the periodontal intervention group, the maximum carotid IMT was less at 12 months than at baseline (mean reduction=−0.02 [SD 0.07] mm; *P*=0.003). In the control group, maximum carotid IMT was marginally greater at 12 months than at baseline (mean increase=0.002 [SD 0.09] mm; *P*=0.820). In our primary analysis, the periodontal intervention produced a statistically significant reduction in maximum carotid IMT compared with the control group (−0.02 [95% CI, −0.05 to −0.002] mm; *P*=0.031). The effect of the intervention on mean carotid IMT was consistent, albeit less marked (−0.01 [−0.03 to 0.004] mm; *P*=0.134). Analysis incorporating data for 3 participants excluded from the above analysis because of missing carotid IMT data at baseline, in addition to baseline data for all participants lost to follow-up, found similar results (less IMT progression at 12 months; *P*=0.020).

Subgroup analyses are shown in Table S2, stratifying by sex, age, overweight/obesity, diabetes mellitus, smoking status, and severity of periodontal disease. Briefly, there was some evidence that the intervention was of no benefit, compared with the control, in participants with diabetes mellitus and may have been of greater benefit in current smokers. Closer examination of current smokers suggests that the carotid IMT regression in the intervention group was similar to that seen with the intervention in the entire study population (−0.027 [95% CI, −0.046 to −0.009] mm); however, the current smokers who were randomized to the control group had more marked progression of carotid IMT (0.020 [95% CI, −0.001 to 0.040] mm).

The effect of the periodontal intervention on carotid IMT in a per-protocol analysis, excluding 5 participants randomized to the intervention group who did not complete the periodontal intervention and 23 who had additional periodontal treatment or dental extractions during follow-up, was similar if not slightly strengthened (−0.030 [95% CI, −0.056 to −0.004] mm; *P*=0.02).

As an additional post hoc sensitivity analysis, we examined whether the change in carotid IMT differed among those in the treatment group with improved periodontal status versus those who experienced periodontal disease recurrence. Although underpowered, maximum IMT was lower among those with periodontal improvement compared with those where periodontal disease regressed (mean IMT change, −0.033 [−0.220 to 0.154] mm; *P*=0.723, which was consistent with our main findings).

Periodontal Parameters

Results of changes to the periodontal parameters are shown in Table S3. Periodontal therapy resulted in modest improvements in periodontal parameters in the short-term, which were no longer significant by 12 months (consistent with disease recurrence). Short-term effects of the periodontal intervention on periodontal health at 3 months have been described in detail in a previous publication.[12]

Discussion

In this randomized, controlled trial of Aboriginal Australians with periodontitis, nonsurgical periodontal therapy significantly reduced progression of carotid IMT during a 1-year period in the absence of changes in PWV.

Carotid IMT measures the extent and severity of localized atherosclerosis in the carotid artery,[20] is independently

Table 2. Change in Pulse Wave Velocity, Carotid Intima-Media Thickness, and Inflammatory Markers (Complete Case Analysis) at 3 Months

	Treatment		Control		ANCOVA Least Squares	
Cardiovascular Risk Markers	Baseline	3 Mo	Baseline	3 Mo	Mean Δ (95% CI)	*P* Value
hsCRP, mg/L	4.84 (5.37)	4.80 (4.80)	4.71 (5.71)	4.15 (4.70)	0.78 (−0.40 to 1.97)	0.19
IL-6, pg/mL	3.21 (2.35)	2.58 (2.21)	2.39 (2.45)	2.10 (2.49)	0.25 (−0.67 to 1.16)	0.59
Total cholesterol, mmol/L	4.97 (1.12)	4.78 (0.97)	4.82 (0.87)	4.73 (0.94)	−0.05 (−0.21 to 0.11)	0.53
Non-HDL cholesterol, mmol/L	3.97 (1.11)	3.79 (0.95)	3.81 (0.90)	3.75 (0.94)	−0.13 (−0.31 to 0.06)	0.18
HDL cholesterol, mmol/L	1.00 (0.30)	0.99 (0.30)	1.01 (0.32)	0.98 (0.30)	0.01 (−0.04 to 0.07)	0.65
ADMA, μmol/L	0.42 (0.13)	0.43 (0.12)	0.43 (0.12)	0.44 (0.10)	−0.01 (−0.05 to 0.02)	0.50
HbA1c, mmol/mol	48.02 (19.79)	46.57 (17.28)	44.75 (14.74)	43.42 (12.94)	0.62 (−1.59 to 2.83)	0.58
PWV, m/s	8.22 (1.30)	8.15 (1.09)	8.43 (1.34)	8.18 (1.17)	0.06 (−0.17 to 0.29)	0.59

Data for baseline/follow-up means presented as mean (SD). Reported values limited to those that have completed data at 3 months post-intervention. ADMA indicates asymmetrical dimethylarginine; CI, confidence interval; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; and PWV, pulse wave velocity.

associated with incidence of cardiovascular events,[21] and is considered to be the best noninvasive marker of global burden of atherosclerosis.[22] Consistent with the pathophysiology of atherosclerosis, carotid IMT generally increases as people age. The increase in carotid IMT in the control group is consistent with the annual progression described in observational cohort studies.[23] Increased IMT is associated with the presence of high counts of periodontitis etiologic bacteria.[24] Prospective analysis of the Oral Infections and Vascular Disease Epidemiology Study cohort for a median of 3 years (range, 2–7 years) has recently reported that natural progression of IMT is significantly lower when either an improvement in periodontal status is observed or when a decrease in the amount of etiologic bacteria occurs compared with baseline.[25] Progression of carotid IMT derived from sequential measures may be a valid surrogate measure of cardiovascular disease events for use in clinical trials.[26] Maximum carotid IMT is thought to better reflect focal atherosclerosis[10] and may respond to therapy more rapidly and more markedly than mean carotid IMT.[27,28]

The magnitude of reduction in maximum carotid IMT in response to the periodontal intervention, relative to the control group, is of a similar degree to that observed in other clinical trials in high-risk populations (eg, with a ≈30% reduction in low-density lipoprotein levels because of statin therapy).[29,30] Furthermore, the magnitude of the reduction in carotid IMT with periodontal intervention in this trial is equivalent to the effects of reversal of 4 years of aging, 8 kg/m^2 lower body mass index, or 25 mm Hg lower systolic blood pressure.[31]

Carotid-femoral PWV is associated with increased risk of cardiovascular disease in otherwise healthy adults[32] and is the recommended direct measure of arterial stiffness in trials.[15,33] Arterial stiffness has both structural and functional determinants.[34] Structural determinants include the breakdown of elastin and increased deposition of collagen,[15] which are more likely to influence long-term changes in arterial stiffness. Short- to mid-term changes in arterial stiffness, however, are more likely because of changes in arterial function. Accordingly, the present findings on the effects of periodontal therapy on PWV at 3 months likely reflect changes in arterial function. Findings from our validation study reveal a small absolute difference between PWV derived from carotid-femoral and carotid-dorsalis pedis measurements (Table S7 and

Table 3. Change in Pulse Wave Velocity, Carotid Intima-Media Thickness, and Inflammatory Markers (Complete Case Analysis) at 12 Months

	Treatment		Control		ANCOVA Least Squares	
Cardiovascular Risk Markers	Baseline	12 Mo	Baseline	12 Mo	Mean Δ (95% CI)	*P* Value
hsCRP, mg/L	4.68 (5.41)	5.28 (6.46)	4.84 (6.18)	4.25 (4.38)	0.84 (−0.63 to 2.32)	0.26
IL-6, pg/mL	3.24 (2.27)	1.87 (2.64)	1.99 (2.05)	2.22 (2.33)	−0.28 (−1.38 to 0.82)	0.62
Total cholesterol, mmol/L	5.01 (1.09)	4.97 (1.14)	5.04 (1.13)	4.82 (1.06)	0.07 (−0.11 to 0.24)	0.44
Non-HDL cholesterol, mmol/L	3.98 (1.11)	3.96 (1.10)	3.97 (1.14)	3.67 (1.09)	0.20 (−0.07 to 0.48)	0.15
HDL Cholesterol, mmol/L	1.03 (0.33)	1.04 (0.31)	1.08 (0.36)	1.11 (0.48)	0.00 (−0.07 to 0.06)	0.88
ADMA, mmol/L	0.42 (0.12)	0.48 (0.11)	0.43 (0.11)	0.44 (0.10)	0.05 (0.004 to 0.10)	0.03
HbA1c, mmol/mol	48.12 (19.61)	46.81 (19.30)	44.98 (15.40)	43.22 (12.08)	1.29 (−0.86 to 3.44)	0.24
PWV, m/s	8.27 (1.30)	8.44 (0.92)	8.37 (1.36)	8.33 (1.04)	0.21 (−0.01 to 0.43)	0.06
Maximum IMT, mm	0.79 (0.19)	0.76 (0.16)	0.79 (0.15)	0.78 (0.15)	−0.025 (−0.047 to −0.002)	0.03
Mean IMT, mm	0.64 (0.14)	0.63 (0.14)	0.64 (0.12)	0.65 (0.11)	−0.013 (−0.030 to 0.004)	0.13

Data for baseline/follow-up means presented as mean (SD). Reported values limited to those that have completed data at 12 months post-intervention. ADMA indicates asymmetrical dimethylarginine; CI, confidence interval; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; IMT, intima-media thickness; and PWV, pulse wave velocity.

Figure S4). Furthermore, the 2 measures of PWV are linearly correlated such that the carotid-femoral PWV may be estimated using measurements of the carotid-dorsalis pedis PWV.

Periodontal disease may be associated with PWV through persistent inflammation. Elevated C-reactive protein levels inhibit release of nitric oxide by the endothelium in patients with arterial disease,[35] and periodontitis stimulates C-reactive protein.[36] Despite modest short-term improvements in periodontal status after therapy in this study,[12] persistent inflammation is thought to remain as evidenced by residual periodontal pocketing and the continuation of moderate gingivitis in the intervention group.[12] In the present study, periodontal disease is likely to be one of several sources of inflammation. Previous research shows that adiposity and cigarette smoking contribute to systemic inflammation and PWV in Aboriginal Australians.[19,37,38] Vessel function was not amenable to a single session of periodontal therapy in this instance.

The specific reasons that describe significant improvements in carotid IMT but not PWV remain unclear. It is possible that the significant increase found in plasma asymmetrical dimethylarginine, an endogenous inhibitor of endothelial NO synthesis, in the treatment but not control group at 12 months may have contributed to the lack of improvement in vessel function. We also cannot exclude the possibility of a threshold effect. Inclusion criteria for some periodontal intervention trials that have reported significant improvements to inflammatory markers[39] and endothelial function[40] have only included participants with severe periodontal disease. By comparison, only 3 of the 273 participants in the present study would be eligible using the same classification.[39,40] Although the predominance of moderate periodontitis in this trial might have attenuated the observed effects of periodontal treatment on arterial end points, inclusion of people with moderate periodontitis means that these results are more readily generalizable to the Aboriginal adult population.

Previous trials provide important contextual information for our findings. Intensive periodontal treatment involving periodontal therapy and removal of teeth combined with localized antibiotic administration improved endothelial function at 60 and 180 days postintervention.[40] Another study including extraction of hopeless teeth for the secondary prevention of cardiovascular disease reported no benefit for subsequent cardiovascular events.[41] Such improvements in endothelial function are consistent with slower progression of carotid IMT,[42] as observed in the current trial, and reduced risk of cardiovascular disease events.[43] Evidence suggests that IMT may be a stronger predictor of future cardiovascular events than endothelial dysfunction, particularly in relation to patients otherwise considered at low risk.[22] Another uncontrolled trial of 35 participants with mild to moderate periodontal disease described the effects of a periodontal intervention on carotid IMT, finding marked reductions in carotid IMT at 6 months and 12 months after periodontal intervention.[18] These reductions, although limited by the lack of a control group and the small sample size, are consistent with those of the current randomized, controlled trial.

No prior trials have investigated change in PWV after periodontal therapy. Associations between periodontal disease and arterial stiffness have been limited to observational studies.[5,6,44–46] Aside from considerable variations in the criteria defining periodontitis between studies, the anatomic sites for arterial stiffness measurement differ, meaning each study measured separate segments of the arterial tree and are thus not directly comparable. Aging, for example, results in a greater collagen content of central arteries relative to elastin, whereas the elastin content of peripheral arteries supported by muscle has a tendency to increase with age.[14]

Loss to follow-up was ≈35% at both 3 and 12 months, and as such the potential for attrition bias cannot be completely eliminated. There were no significant differences, however, in baseline PWV, IMT, or periodontal parameters when compared between those lost to follow-up and those completing the follow-up visits. Aside from the present study, 2 other randomized trials limited to Aboriginal Australian adult women have been completed and reported comparable loss to follow-up rates.[47,48] We made concerted efforts to minimize detection bias in this study by not informing examining clinicians of the original study participant group allocation. For outcomes of interest such as PWV, the 2 assessors achieved sufficient reliability, whereas reproducibility of blinded IMT measurements was excellent. In addition, because of staff changes during the study, examiner 1 collected the majority of the annual measurements, increasing consistency (Figure S1).

We have previously demonstrated that modest improvements to periodontal status can be achieved and maintained for ≤3 months after 1-stage periodontal therapy, irrespective of oral hygiene.[12] However, those findings suggest that, without periodontal maintenance to remove newly formed deposits of calculus and disturb the dental biofilm, the short-term response to the periodontal tissues has a tendency to regress.

Perspectives

The present study reveals that conventional periodontal therapy is sufficient to reduce carotid IMT. This finding is robust even when considering subgroup analyses. Future investigations may determine whether a more intensive approach to periodontal therapy, including regular periodontal maintenance schedules, may result in more marked improvements in vascular structure. Extrapolation of these results, if repeated in other studies, may suggest that treatment of periodontal disease is important for cardiovascular disease risk reduction.

Conclusions

In conclusion, carotid IMT regressed in Aboriginal Australian adults with moderate to severe periodontal disease after periodontal therapy, suggesting periodontal disease and atherosclerosis are significantly associated. However, a single session of nonsurgical periodontal therapy may be insufficient to alter the functional aspects of vascular health that contribute to and modify PWV in the short-term. These findings provide some evidence to suggest that periodontal therapy may have a systemic impact beyond the oral environment. Future studies may also seek to reproduce these findings in other populations and assess cardiovascular events.

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Disclosures

None.

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Novelty and Significance

What Is New?

- This study shows, using a randomized, controlled trial design, that conventional periodontal therapy attenuates progression of carotid intima-media thickening in a sample at high risk for cardiovascular disease.

What Is Relevant?

- If periodontal disease contributes to atherosclerosis, then treatment of periodontitis should be included in patient management.
- Periodontal disease is highly prevalent worldwide; therefore, the public health importance of these findings may be high.

Summary

Periodontal disease and atherosclerosis seem to be significantly associated. These findings should be examined in other populations. Prospective cardiovascular event data are required.

6.2 Online supplement

The effect of periodontal therapy on arterial structure and function among Aboriginal Australians: a randomised controlled trial

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6.2.1 Expanded methods

The PerioCardio study is a parallel-group, randomised, open label controlled trial investigating the effect of non-surgical periodontal therapy on surrogate markers of cardiovascular disease among Aboriginal Australian adults residing in the Northern Territory, Australia.

Participants were recruited from two regional centres in Australia's Northern Territory; one the largest urban centre (Darwin) and the other a regional town approximately 300 km south (Katherine). Participants were also recruited from two correctional facilities; one in Darwin and the other in Alice Springs (the second largest regional centre in the Northern Territory, approximately 1,500 km south of Darwin).

6.2.2 Changes to original study protocol

The original study protocol can be found online http://www.anzctr.org.au/ [ANZCTR number: 12610000817044].

Three changes to the protocol were made during the study period. Firstly, the age for inclusion was reduced from 25 to 18 years. Eight study participants aged 22 through 24 years were subsequently included. Secondly, the periodontal intervention was altered from two sessions of half-mouth mechanical debridement, to a single, full-mouth untimed session due to operational issues relating to the availability of dental clinical facilities and study participant preference. Thirdly, change in carotid IMT from baseline to 3-month recall was not assessed following cardiologist advice as the timeframe was considered too short to represent true changes to common carotid structural wall.

6.2.3 Participant enrolment and allocation

Enrolment of study participants was conducted by non-dental research staff. We attempted to minimise the effect of detection bias at each recall appointment by not informing examining clinicians of the original study participant group allocation. One of the dental clinicians (KK) and one investigator not involved in data collection (MRS) were responsible for assessing outcomes using de-identified data.

6.2.4 Oral assessment methods to measure periodontal status

Periodontal probing depth (PPD) and gingival recession was measured at four sites at every tooth excluding third molars. These included the mesio-buccal, mid-buccal, disto-buccal and disto-lingual. Oral plaque scores were recorded for six index teeth (if present) based on published criteria (Silness and Löe, 1964) which included the most anterior molar in each quadrant, tooth 11 and tooth 31. The same six index teeth were assessed for calculus presence. A single gingival bleeding on probing score was collected for each tooth

periodontally assessed and was scored based on the Gingival Index criteria (Löe and Silness, 1963).

6.2.5 Statistical methods to analyse periodontal parameters

Extent of PPD and CAL were calculated as the percentage of sites examined based on the methods of Carlos and colleagues (Carlos et al., 1986). For ordinal measures of calculus and plaque assessed on six index teeth, the mean number of each was calculated. Extent of visible plaque (equivalent to Plaque Index (PI) values ≥2 (Löe, 1967) determined degree of accumulation relative to total plaque. A modified Gingival Index score (Löe, 1967) was calculated by dividing the number of teeth with gingival bleeding by the number of teeth periodontally assessed.

6.2.6 Periodontal Intervention

The periodontal intervention consisted of an untimed single-visit full-mouth non-surgical removal of sub and supragingival calculus and plaque biofilm following administration of local anaesthesia if requested by participants. The time required to provide the intervention varied from 45 minutes through to three hours depending on treatment complexity. Two clinicians, one oral health therapist (provider 1) and one dentist (provider 2) (Figure 6.1) conducted the intervention with the use of Gracey hand scalers (Hu-Friedy, Chicago, USA), and piezoelectric ultrasonic device (Kyungwon Ferrite, Gyeonggi-Do, Korea) using universal tips. The same intervention was provided to those randomised to the control group following completion of annual assessments. Oral hygiene instruction was provided to participants along with toothbrush and toothpaste upon completion of the treatment.

Periodontitis status was determined during a clinical oral examination in which periodontal probing depth (PPD) and gingival recession was measured at four sites at every tooth excluding third molars.

6.2.7 Vascular measures

PWV

Validation of the carotid-dorsalis pedis PWV against carotid-femoral PWV was conducted on an age and sex-matched control group of participants not involved in the primary study (details included in Supplement Table 8 and Figure 6.4). A pressure tonometer was placed transcutaneously over the carotid followed by the DP arteries. The subtraction method was used to determine the path length between measurement sites (O'Rourke et al., 2002), and the resultant PWV score was calculated using the 'foot-to-foot' method.

IMT

A minimum of three loops were acquired from each of the left and right common carotid arteries, for later batch analysis using semi-automated and validated software in a central reading laboratory (Carotid Analyzer, Medical Imaging Applications, USA), by an observer blinded to participant details, including randomization status. The single thickest section of intima-media on the far wall of the common carotid artery over a 1 cm long portion (0-1 cm proximal to the bulb) was measured from each frame, and averaged over the entire loop. Two loops were obtained from each side (left/right carotid), and averaged to obtain the maximum carotid IMT.

The spatial resolution of high-resolution ultrasound is generally considered to be 100-150 microns. The use of automated analysis systems, however, can effectively provide measures of carotid IMT with greater accuracy, in the order of 0.01 for the mean IMT from a given frame and 0.025 mm for the IMT of a single point, as a result of analysis of grey-

levels between pixel pairs and by averaging the IMT along a 1cm long segment of the arterial wall.

6.2.8 Cardiovascular risk assessment

Non-fasting venous blood samples were collected via the antecubital vein. Samples were transported to a local commercial pathology clinic for analysis of lipid profile: total cholesterol (TC), high-density lipoprotein (HDL) and glycated haemoglobin (HbA1c). Direct methods were used to determine lipid profile using an ADVIA 2400 Chemistry System (Siemens, Tarrytown, USA). NonHDL cholesterol was the difference between TC and HDL. Serum and plasma was stored at -80°C until batch analysis. Serum high-sensitivity C-reactive protein (hsCRP) was measured by particle-enhanced immunonephelometry using the BN II system. HbA1c was determined by turbidimetric inhibition immunoassay with a COBAS INTEGRA (Roche Diagnostics, Indianapolis, USA). Plasma asymetric dimethylarginine was assessed using high-performance liquid chromatography with simultaneous UV and fluorescence detection as previously described (Jones et al., 2010). Plasma IL-6 was measured via commercial ELISA assay (Human IL-6 Quantikine kit, R&D Systems Inc., Minneapolis, USA).

Brachial blood pressure was measured while seated using an automated device (Welch Allyn Medical Products, Skaneateles Falls, USA). Three measurements of systolic and diastolic pressure were collected with three minutes between readings. The mean values of the final two recordings were used as sitting blood pressure. Height was measured to the nearest 0.1cm using a stadiometer. Weight was measured to the nearest 0.1 kilogram using a portable weight scale (Tanita HD-351, Arlington Heights, USA) with participants lightly clothed. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (metres).

6.2.9 Self-reported questionnaire

Information on socio-demographic characteristics, tobacco smoking and self-reported health status was obtained via questionnaire. Individuals with self-reported diabetes or HbA1c ≥47.5 mmol/mol were defined as having diabetes for this analysis.

6.2.10 Reproducibility of measurements

Both clinicians were trained in the collection of PWV and calibrated for periodontal assessment procedures prior to study commencement. For the periodontal assessments, five healthy volunteers were subsequently examined by both clinicians each blinded to the assessment of the other. Periodontal measurements were standardised to within 1mm. Where differences in the assessments occurred, agreement was attained via discussion. Inter-examiner reliability between the two clinicians during the study was assessed using volunteers not involved in the present trial. Precise agreement on the number of sites with PPD ≥2 mm was used as a cut-point with weighted kappa statistic of 0.75 (95% CI 0.70 to 0.80) representing very good agreement (Landis and Koch, 1977).

For PWV, inter-observer repeatability was tested throughout the study on 20 volunteers and was rated as ‘moderate’ (intra-class correlation = 0.72). Intra-observer repeatability was equally comparable between the two examiners and rated ‘good’; examiner 1: ICC = 0.86, examiner 2: ICC = 0.83. Inter-reader reproducibility for IMT measurement, derived from 20 measures assessed by two experience readers, was excellent (ICC = 0.99 (95% CI 0.98, 1.00).

6.2.11 Sample size

To account for an *a priori* participant attrition of 25%, the original baseline sample size was set at 200 participants. Following one year of recruitment, almost 40% of the initial sample was lost to follow-up necessitating an increase in the sample. A final sample size of

273 people was subsequently recruited to ensure that the required sample size would be attained.

6.2.12 Statistical analysis

Specific to IMT, sub-group analyses were undertaken within groups stratified according to baseline measures of sex, age, overweight/obesity, diabetes, smoking status and severity of periodontal disease. As a secondary analysis to enable inclusion of the baseline data of participants lost to follow-up and three participants who had carotid IMT data available from 12-months but not baseline, we constructed a linear mixed model including carotid IMT data from both baseline and 12-months, with the effect of the intervention determined by the interaction between visit number and allocated group.

A sex-specific comparison of baseline characteristics was undertaken due to significantly more males being completed at the 3-month follow-up (presented in Supplementary Table 5) and sex-stratified change to the two primary outcomes, PWV and IMT are presented in Supplementary Table 6. A baseline-carried-forward approach was also conducted as an additional sensitivity analysis (results presented in Supplementary Table 7).

Data were analysed using a combination of SAS version 9.3 (Cary, North Carolina, USA) and IBM SPSS Statistics (version 21.0; IBM Corp., Somers, NY). Statistical significance was inferred at two-sided P-value <0.05.

6.3 Validation of carotid-dorsalis pedis PWV with carotid-femoral PWV

6.3.1 Methods

Participants

We recruited 30 non-Indigenous Australians, aged ≥18 years, with no history of rheumatic heart disease, prior myocardial infarction, stroke or coronary revascularisation,

as a comparator group for the PerioCardio study (Skilton et al., 2011a). All participants provided written informed consent, and the study was approved by the joint Menzies School of Health Research - Northern Territory Department of Health Human Research Ethics Committee.

Pulse wave velocity

PWV was measured using applanation tonometry (SphygmoCor-PVMx device, AtCor Medical, Sydney, Australia), as previously described (Laurent et al., 2006). Both carotid-femoral and carotid-dorsalis pedis PWV were systematically measured in that order, and calculated via computer algorithm using the mean time difference between the R-wave and pressure wave at the measurement sites and the arterial path length between the recording sites and the suprasternal notch.

Cardiovascular risk factors

Cardiovascular risk factors were assessed as per the PerioCardio study protocol, as described in detail above and elsewhere (Skilton et al., 2011a). In brief, blood pressure was taken three times from seated subjects using an automated device (Welch Allyn Medical Products, Skaneateles Falls, NY, USA). The average of the last two measures was used. A non-fasting blood sample was collected by routine venous blood sampling techniques. Total cholesterol was assayed by enzymatic methods, and high-density lipoprotein cholesterol was measured directly (ADVIA chemistry system, Siemens, Tarrytown, USA). Smoking status was assessed by questionnaire, and pack years calculated. Weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm.

Statistical analysis

Of the 30 participants, three participants were excluded due to technical issues or poor measurement quality of carotid-femoral PWV, leaving 27 participants for this analysis. The two PWV measures were compared by paired samples t-test, Pearson correlation and Bland-Altman plot (Bland and Altman, 1996). Total cholesterol: HDL-cholesterol ratio

(TC:HDL) was calculated. Associations of PWV with cardiovascular risk factors were by Pearson correlation. Statistical analyses were performed using SPSS version 21 (SPSS Inc, Chicago, IL, USA). Statistical significance was inferred at P <0.05.

6.4 Expanded results

6.4.1 Periodontal parameters

Periodontal therapy improved periodontal parameters 3-months post-intervention, described in detail elsewhere (Kapellas et al., 2013). The periodontal intervention resulted in a significant improvement in periodontal health at 3-months post intervention (mean pocket depth: -0.16 mm [95% CI -0.25, -0.07] in the intervention group relative to control, P = 0.0008). There was some evidence for a sustained improvement in periodontal health at 12-months post intervention (mean pocket depth: -0.09 mm [95% CI -0.19, 0.01] in intervention group relative to control, P = 0.08). In summary however, the single-visit non-surgical periodontal intervention did not result in sustained improvements in periodontal status long-term (Supplement Table 4).

There was no significant correlation between the 12-month change in maximum IMT & the 3-month change in the extent of periodontal pocket depths ≥4mm (Pearson's r = 0.07, *P*=0.39). The corresponding scatter plot is presented in Figure 6.5.

6.4.2 Blood lipids, Asymetric dimethylarginine (ADMA) & high sensitivity C-reactive protein (hsCRP)

There were no significant differences to total cholesterol, nonHDL cholesterol, HDL cholesterol or hsCRP following the intervention (Table 6.2). In contrast, ADMA was significantly higher in the treatment group 12-months post-intervention (12-month change 0.05μM/L [95% CI 0.004, 0.10], compared to control, P= 0.03) but not at 3-months (Table 6.2). Periodontal treatment did not alter hsCRP in the treatment group, however there was

a non-significant reduction in the control group following the 3-month assessments (3-month change +0.78 mg/L [95% CI -0.40 to 1.97], p=0.19).

6.5 Validation of carotid-dorsalis pedis PWV with carotid-femoral PWV

6.5.1 Results

Participants were non-diabetic, aged between 20-66 years (mean 40 years [SD 15]), 16 of 27 were male, and had generally good cardiovascular risk profiles (mean systolic blood pressure 114 mm Hg [SD 9], BMI 24.6 kg/m^2 [SD 4.0]), TC:HDL 3.8 [SD 1.2]). Two of the participants were current smokers, while a further 10 were ex-smokers.

The two measures of PWV were significantly correlated (r = 0.496, *P* = 0.008). Carotid-dorsalis pedis PWV was higher than carotid-femoral PWV (7.4 m/s [SD 1.0] vs 6.8 m/s [SD 1.1], *P* = 0.005). Agreement between the two PWV measures was similar across the spectrum of measures (Figure 6.4). Carotid-femoral PWV could be estimated from carotid-dorsalis pedis PWV using the equation:

$$\textit{Carotid-femoral PWV} = 0.565 * \textit{carotid-dorsalis pedis PWV} + 2.601,$$

where PWV is measured in m/s.

In general, the two PWV measures shared similar associations with risk factors (Supplementary Table 8). Pulse Wave Velocity was most strongly associated with age and systolic blood pressure; with carotid-femoral PWV being more strongly associated with age, and carotid-dorsalis pedis more strongly associated with systolic blood pressure. Associations of PWV with TC:HDL and body mass index were similar. Neither measure of PWV differed by sex (carotid-femoral PWV: -0.3 m/s [95% CI -1.2, 0.6] in males *vs* females, *P* = 0.47; carotid-dorsalis pedis PWV: 0.3 m/s [95% CI -0.5, 1.1] in males *vs* females, *P* = 0.46).

6.6 Expanded discussion

Inflammation contributes to endothelial dysfunction (Ross, 1999) and impaired function of the endothelium has been reported in people with periodontitis (Amar et al., 2003). Accumulating evidence suggests both conventional non-surgical (Blum et al., 2007) and intensive periodontal therapies involving dental extractions, antibiotics and/or surgery can improve endothelial-dependent vasodilation (Elter et al., 2006, Higashi et al., 2008, Tonetti et al., 2007). The intensive interventions in these aforementioned studies resulted in marked reduction in systemic inflammation. In contrast, the one-stage non-surgical periodontal intervention provided in the present investigation did not significantly alter inflammation, potentially consistent with the lack of a short-term effect of the intervention on PWV in the present study.

The original study protocol planned for the intervention to be spread over two appointments, whereby two quadrants were to be treated at each session (Skilton et al., 2011a). The intention was to reduce discomfort for study participants and the physical burden on clinicians. However, participant preference towards a single session led to modification of the intervention protocol early in the study. A single session had the added advantage of ensuring that study participants randomised to the treatment arm completed their assigned therapy in a timely manner and this alteration ensured that 128 of 138 in the intervention group were completed. In relation to short-term periodontal tissue responses, Cochrane systematic review evidence indicates that single sessions of non-surgical periodontal treatment yield comparable outcomes to multiple visits (Eberhard et al., 2008). Therefore, it was not expected that the alteration from an intervention administered over two appointments would differ in terms of efficacy to an intervention administered in a single session, with regards to short-term changes in periodontal health.

Access to and the provision of dental services for Indigenous Australian adults in the Northern Territory is challenging. For example, those living in rural or remote locations

may have dental practitioners visit as little as twice yearly and due to seasonal conditions, some locations can become temporarily inaccessible by road for weeks or months. Dental service attendance for Indigenous Australians living in Darwin is heavily weighted towards problem-associated visiting patterns. Reasons for this have not been extensively investigated. Among the PerioCardio study sample, almost 75% reported their last visit to a dentist was for a problem (Amarasena et al., 2014) which is similar to that reported in Western Australia (Smith et al., 2007). Further, one-third of the PerioCardio study sample was recruited from correctional facilities in Darwin and Alice Springs. By way of explaining the unmet treatment need, at the time of the PerioCardio study, participants in the Darwin correctional facility had, on average, four teeth with untreated caries (Kapellas et al., 2014b). For these individuals, sourcing an appointment was dependent on having a problem in the first instance and preventive services were extremely limited if non-existent. Measures from the Northern Territory government have since been implemented to improve access to dental services in this population With this considered, a periodontal treatment regimen requiring multiple appointments for initial treatment and additional appointments for periodontal maintenance was, in real terms, not suitable in this setting.

It was not expected that the periodontal therapy provided in this study would be sufficient to completely eliminate periodontal disease, especially after one year. Nevertheless, it was expected that the response of the intervention to the periodontal tissues would have been greater than that observed. Teeth were scaled using ultrasonic and manual scalers until a smooth surface was confirmed with a dental explorer. Oral hygiene was discussed and reinforced immediately following the periodontal therapy and again upon follow-up visits. Despite these efforts, there were negligible and clinically insignificant changes to oral hygiene at both recall appointments for both study arms. The smaller-than-expected periodontal treatment effect in the treatment group at 3-months can be partially attributed to the lack of improvement in oral hygiene (Kapellas et al., 2013),

which contributed to the perpetuation of periodontal inflammation as evidenced by a Gingival Index >1 for all periods of the study. There was no correlation between the 3-month change in pocket depth and the 12-month change in carotid IMT, although it is likely that the pocket depth variable does not fully capture the improvements in periodontal disease due to the treatment, both with regards to pathology and time course.

Several operational issues impacted on the delivery of the study which would have precluded the provision of periodontal maintenance even if it was initially included within the study protocol. Firstly, the PerioCardio study was conducted around existing services provided by government departments and Aboriginal medical services. As such, access to dental facilities was not available at all times and a subsequent increase in the number of staff employed by the Northern Territory dental service during the study meant that access to dental facilities had to be arranged on a daily basis and was dependent on clinic availability on a given day. Secondly, scheduling participants for appointments was often complicated by changes to residential addresses and contact details, the management of which was often an overwhelming task for investigators and staff involved. Finally, failure to attend scheduled appointments was a regular occurrence for many study participants which negatively impacted on completion rates. In all likelihood, adoption of a periodontal maintenance regimen as opposed to a one-stage approach for this study protocol would have led to few participants completing the required number of appointments due to the aforementioned factors.

At the time of recruitment many study participants were unaware of their periodontal disease status. There was no limitation on external periodontal treatment, and as such all participants were free to receive periodontal treatment through their usual service providers during the course of the study. Only a few however, accessed periodontal therapy external to the study. For example, only six participants randomised to the treatment group and seven participants in the control group sought additional periodontal treatment during the

12-month follow-up period. At the completion of the 12-month study visit, control group participants were invited to receive the periodontal treatment. Of the 79 control participants who attended the 12-month follow-up, only 24 opted to receive periodontal therapy.

There are few alternatives available for a control-arm in a periodontal intervention other than the community care approach, such as that utilized in this study. An alternative study design would be to treat all study participants at baseline, with periodontal maintenance in the intervention group throughout the duration of the study, however this would likely dilute the overall effect size of our arterial outcome measures, which may be especially problematic given the relatively short time duration of the follow-up. Accordingly, adoption of the community care approach was the pragmatic option in this study.

6.7 References

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Supplement Table 2: Baseline Characteristics of Completed versus Lost to Follow-up (Baseline Data Reported).

		3 month			12 month		
Participant characteristics	**Group (n=273)**	**Completed (n=169)**	**LTF (n=104)**	**P value**	**Completed (n=168)**	**LTF (n=105)**	**P value**
Mean age (years)	40.3 (10.3)	40.2 (10.4)	40.3 (10.1)	0.93	42.2 (10.5)	38.0 (9.4)	<0.01
Sex – Male [%]	158 [57.3]	107 [63.3]	51 [49.0]	0.02	96 [57.1]	62 [59.0]	0.84
Female [%]	115 [42.7]	62 [36.7]	53 [51.0]		72 [42.9]	43 [41.0]	
Current Smoker [%]*	161 [66.0]	102 [66.7]	59 [64.8]	0.77	97 [64.7]	64 [68.1]	0.58
Former/Never smoker [%]*	83 [34.0]	51 [33.3]	32 [35.2]		53 [35.3]	30 [31.9]	
Diabetes - Yes (%)†	62 [22.7]	44 [26.0]	18 [17.3]	0.09	45 [26.6]	17 [16.4]	0.049
Mean HbA1c (mmol/mol)	45.0 (15.8)	46.5 (17.6)	42.1 (11.1)	0.02	46.6 (17.7)	41.9 (10.9)	0.01
Total cholesterol (mmol/L)	4.98 (1.03)	4.90 (1.01)	5.14 (1.07)	0.08	5.03 (1.11)	4.91 (0.88)	0.37
nonHDL cholesterol (mmol/L)	3.95 (1.03)	3.90 (1.02)	4.03 (1.06)	0.33	3.97 (1.12)	3.90 (0.87)	0.56
HDL cholesterol (mmol/L)	1.04 (0.32)	1.00 (0.30)	1.11 (0.33)	0.01	1.05 (0.35)	1.01 (0.26)	0.31
Mean hsCRP (mg/L)	4.89 (5.35)	4.78 (5.52)	5.11 (5.05)	0.65	4.72 (5.74)	5.19 (4.62)	0.49
Mean IL-6 (pg/mL)	2.79 (2.27)	2.82 (2.42)	2.72 (1.95)	0.73	2.66 (2.24)	3.00 (2.32)	0.27
ADMA (μM/L)	0.43 (0.12)	0.42 (0.12)	0.43 (0.10)	0.76	0.43 (0.12)	0.42 (0.12)	0.82
Mean BMI	29.12 (7.18)	29.67 (6.39)	28.15 (8.35)	0.13	29.06 (6.15)	29.20 (8.77)	0.89
Mean PWV (m/s)	8.34 (1.25)	8.32 (1.32)	8.38 (1.11)	0.72	8.32 (1.32)	8.39 (1.10)	0.66
Mean carotid IMT (mm)‡	0.63 (0.12)	-	-	-	0.64 (0.13)	0.62 (0.10)	0.18
Maximum carotid IMT (mm)‡	0.78 (0.16)	-	-	-	0.79 (0.17)	0.76 (0.14)	0.18
Mean systolic BP (mmHg)	124.9 (16.7)	124.7 (14.4)	125.4 (20.1)	0.76	125.1 (16.9)	124.6 (16.4)	0.81
Mean diastolic BP (mmHg)	80.2 (10.3)	80.1 (10.2)	80.4 (10.6)	0.82	80.3 (10.5)	80.0 (10.1)	0.82

Mean number of teeth	26.34 (5.96)	26.38 (6.08)	26.27 (5.80)	0.89	25.94 (5.83)	27.11 (6.01)	0.12
Extent CAL ≥3mm	46.79 (25.46)	52.24 (25.18)	55.02 (26.24)	0.38	53.07 (26.59)	53.68 (23.89)	0.85
Extent PPD ≥4mm	14.00 (13.96)	14.29 (14.48)	13.51 (13.07)	0.66	13.79 (13.71)	14.20 (14.20)	0.81

Data for means presented as mean (SD); difference in means via independent samples t-test.
Proportions presented as column N [%]; Difference in proportions via $\chi 2$ test
*Reported values limited to those that have completed data (n=244).
†Diabetes via self-report (n=41) or when HbA1c ≥ 6.5% (n=21).
‡Geometric mean (IQR) for non-normally distributed variables.

Supplement Table 3: Periodontal Therapy and Change in Carotid Intima-Media Thickness – Subgroup Analyses.

Subgroups	**Effect of periodontal therapy on carotid IMT, mm (95% CI)**
Sex	
Males (n=93)	-0.031 (-0.061, -0.000)
Females (n=72)	-0.019 (-0.053, 0.016)
Age	
Younger (≤40.5 y)(n=81)	-0.026 (-0.055, 0.002)
Older (>40.5 y)(n=84)	-0.022 (-0.057, 0.013)
Adiposity	
Healthy weight (n=39)	-0.025 (-0.063, 0.014)
Overweight (n=62)	-0.027 (-0.064, 0.010)
Obese (n=64)	-0.028 (-0.067, 0.010)
Diabetes status	
Diabetic (n=44)	-0.001 (-0.048, 0.047)
Non-diabetic (n=121)	-0.032 (-0.058, -0.006)
Smoking status	
Current smoker (n=95)	-0.047 (-0.075, -0.019)
Never or ex-smoker (n=52)	-0.013 (-0.054, 0.027)
Severity of periodontal disease	
Moderate (n=112)	-0.030 (-0.057, -0.004)
Severe (n=53)	-0.015 (-0.058, 0.029)

Data are mean (95% CI) for change in maximum carotid IMT with periodontal therapy, relative to control group, adjusting for baseline maximum carotid IMT (log transformed) by ANCOVA

Supplement Table 4: Periodontal Parameters at Baseline and 12 Months (Complete-Case Analysis).

	Treatment		Control			
Periodontal health measures	**Baseline**	**12 month**	**Baseline**	**12 month**	**ANCOVA least squares mean Δ (95% CI)**	**P value**
Extent CAL ≥3mm	53.07 (25.84)	46.37 (25.71)	53.07 (27.59)	50.37 (24.20)	-4.00 (-8.01, 0.02)	0.05
Mean PPD mm	2.38 (0.53)	2.26 (0.52)	2.41 (0.60)	2.37 (0.55)	-0.09 (-0.19, 0.01)	0.08
Extent PPD ≥4mm (%)	13.38 (12.67)	11.69 (12.82)	14.26 (14.86)	13.61 (13.65)	-0.16 (-0.33, 0.01)	0.06
Extent PPD ≥5mm (%)	4.45 (6.36)	4.24 (6.82)	5.45 (8.67)	5.25 (8.25)	-0.63 (-2.09, 0.84)	0.40
Extent CAL ≥3mm & PPD ≥4mm	13.18 (12.50)	11.27 (12.28)	14.18 (14.62)	13.10 (13.19)	-1.27 (-2.87, 0.32)	0.12
Mean index teeth with calculus	4.11 (1.67)	2.97 (1.94)	4.06 (1.64)	3.94 (1.76)	-1.02 (-1.48, -0.56)	<0.01
Mean gingival bleeding score	1.44 (0.67)	1.35 (0.74)	1.43 (0.68)	1.50 (0.67)	-0.13 (-0.32, 0.05)	0.16
Mean index teeth with plaque	5.22 (1.25)	5.18 (1.37)	5.39 (1.06)	5.29 (1.23)	-0.06 (-0.05, 0.17)	0.30
Extent visible plaque (%)	28.66 (36.22)	28.54 (36.31)	25.25 (33.29)	32.14 (38.49)	-6.12 (-16.30, 4.05)	0.24

Data for means presented as mean (SD)
Reported mean (SD) values limited to those that have completed data 12-months post-intervention.
CAL= Clinical attachment loss; PPD = probing pocket depth.
Mean gingival bleeding: modified from Löe & Silness scoring system (1967) (number of teeth with BOP / number of teeth periodontally assessed);
Maximum score for index teeth with calculus & plaque=6;
Extent visible plaque limited to scores ≥2 indicative of moderate/abundant plaque visible with the naked eye (Löe, 1967).

Supplement Table 5: Baseline Comparisons of Means Stratified by Sex.

Participant characteristics	Male (n=158)	Female (n=115)	P value
Mean age (years)	37.90 (9.28)	43.19 (10.71)	<0.01
Current smoker [%]*	96 [69.1]	65 [61.9]	0.24
Former/Never smoker [%]*	43 [30.9]	40 [38.1]	
Diabetes [%]†	24 [15.2]	17 [14.8]	0.93
HbA1c (mmol/mol)	44.8 (15.5)	45.2 (16.3)	0.86
Systolic BP (mmHg)	126.3 (12.7)	123.0 (20.9)	0.13
Diastolic BP (mmHg)	81.2 (9.7)	78.8 (10.9)	0.07
Total cholesterol (mmol/L)	4.88 (0.96)	5.12 (1.12)	0.08
nonHDL cholesterol (mmol/L)	3.96 (0.98)	3.92 (1.11)	0.78
HDL cholesterol (mmol/L)	0.92 (0.27)	1.20 (0.31)	<0.01
hsCRP (mg/L)	3.29 (3.97)	7.05 (6.18)	<0.01
IL-6 (pg/mL)	2.74 (2.44)	2.84 (2.01)	0.74
ADMA (μM/L)	0.41 (0.10)	0.45 (0.13)	0.04
Body mass index	27.69 (5.34)	31.08 (8.78)	<0.01
Mean PWV (m/s)	8.42 (1.19)	8.23 (1.32)	0.24
Mean IMT (mm)‡	0.64 (0.17)	0.61 (0.15)	0.02
Maximum IMT (mm)‡	0.78 (0.18)	0.74 (0.17)	0.03
Mean number of teeth	27.42 (5.10)	24.95 (6.68)	0.001
Extent PPD ≥ 4mm (%)	16.63 (14.87)	10.24 (11.42)	<0.01

Continuous variables are mean (SD); Categorical values presented as column N [%].
*Reported values limited to those that have completed data (n=244).
†Diabetes via self-report (n=41) or when HbA1c ≥47.5 mmol/mol (additional 21).
ǂGeometric mean (IQR) for non-normally distributed variables.
PWV: pulse wave velocity; BP: blood pressure; HDL: high-density lipoprotein; hsCRP: high-sensitivity C-reactive protein; HbA1c: Glycated haemoglobin; ADMA: Asymmetric dimethylarginine.

Supplement Table 6: Change in Pulse Wave Velocity Stratified by Sex.

	Treatment		Control			
Sex & outcome	**Baseline**	**3 month**	**Baseline**	**3 month**	**ANCOVA least squares mean Δ (95% CI)**	**P value**
Male PWV (m/s)	8.15 (1.30)	8.06 (1.07)	8.44 (1.19)	8.13 (1.21)	0.04 (-0.29, 0.38)	0.79
Female PWV (m/s)	8.32 (1.31)	8.15 (1.09)	8.42 (1.68)	8.18 (1.17)	0.05 (-0.25, 0.35)	0.73
	Baseline	**12 month**	**Baseline**	**12 month**		
Male PWV (m/s)	8.22 (1.26)	8.56 (0.87)	8.44 (1.93)	8.39 (1.07)	0.31 (0.01, 0.62)	0.05
Female PWV (m/s)	8.34 (1.37)	8.29 (0.99)	8.27 (1.52)	8.21 (0.96)	0.09 (-0.22, 0.40)	0.57
Male max. IMT (mm)	0.77 (0.18)	0.76 (0.16)	0.81 (0.16)	0.81 (0.15)	-0.029 (-0.060, 0.0004)	0.05
Female max. IMT (mm)	0.79 (0.20)	0.77 (0.17)	0.74 (0.11)	0.75 (0.13)	-0.017 (-0.051, 0.018)	0.33

Data for baseline/follow-up means presented as mean (SD). Reported values limited to those that have completed data post-intervention.

Supplement Table 7: Baseline-Carried-Forward Analysis.

	Treatment		Control			
Risk markers	**Baseline**	**3 month**	**Baseline**	**3 month**	**ANCOVA least squares mean Δ (95% CI)**	**P value**
Extent CAL ≥3mm	53.10 (24.84)	45.44 (25.61)	53.50 (26.40)	51.30 (24.54)	-5.55 (-9.04, -2.06)	<0.01
Mean PPD mm	2.39 (0.52)	2.23 (0.47)	2.41 (0.59)	2.36 (0.53)	-0.12 (-0.19, -0.05)	<0.01
Extent PPD ≥4mm (%)	13.40 (12.84)	10.07 (10.82)	14.50 (14.87)	13.25 (13.34)	-2.39 (-4.04, -0.73)	0.01
Extent PPD ≥5mm (%)	4.40 (6.90)	3.87 (6.75)	5.40 (8.89)	5.04 (8.13)	-0.01 (-0.03, 0.01)	0.16
Extent CAL ≥3mm & PPD ≥4mm	13.21 (12.68)	9.90 (10.76)	14.32 (14.70)	12.97 (12.95)	-2.28 (-3.91, -0.66)	0.01
PWV (m/s)	8.23 (1.22)	8.23 (1.08)	8.45 (1.27)	8.28 (1.14)	0.06 (-0.16, 0.28)	0.61
	Baseline	**12 month**	**Baseline**	**12 month**		
Extent CAL ≥3mm (%)	53.10 (24.84)	47.26 (25.21)	53.50 (26.40)	51.41 (24.66)	-3.81 (-6.74, -0.89)	0.01
Mean PPD mm	2.39 (0.52)	2.29 (0.51)	2.41 (0.59)	2.38 (0.58)	-0.08 (-0.14, -0.01)	0.03
Extent PPD ≥4mm (%)	13.40 (12.84)	11.68 (12.69)	14.50 (14.87)	13.97 (14.72)	-1.34 (-2.94, 0.27)	0.10
Extent PPD ≥5mm (%)	4.40 (6.90)	4.34 (7.39)	5.40 (8.89)	5.06 (8.28)	0.01 (-0.03, 0.05)	0.47
Extent CAL ≥3mm & PPD ≥4mm	13.21 (12.68)	11.45 (12.51)	14.32 (14.70)	13.70 (14.50)	-1.30 (-2.87, 0.28)	0.11
PWV (m/s)	8.23 (1.22)	8.37 (0.96)	8.45 (1.27)	8.28 (1.09)	0.19 (-0.01, 0.40)	0.07
Max. IMT (mm)	0.78 (0.18)	0.77 (0.17)	0.77 (0.13)	0.77 (0.13)	-0.015 (-0.029, -0.0006)	0.04
Mean IMT (mm)	0.64 (0.14)	0.63 (0.13)	0.63 (0.10)	0.63 (0.10)	-0.007 (-0.018, 0.003)	0.17

Data for means presented as mean (SD)
CAL= Clinical attachment loss; PPD = probing pocket depth; PWV=Pulse Wave Velocity; IMT=Intima-Media Thickness

Supplement Table 8: Correlation of Cardiovascular Risk Factors with Carotid-Femoral and Carotid-Dorsalis Pedis Pulse Wave Velocity.

	Carotid-femoral PWV		Carotid-dorsalis pedis PWV	
Risk Factors	**Correlation coefficient**	**P-value**	**Correlation coefficient**	**P-value**
Age	0.523	0.005	0.441	0.017
SBP	0.562	0.003	0.615	0.001
BMI	0.179	0.371	0.235	0.220
Pack years	0.381	0.060	0.063	0.757
TC:HDL	0.327	0.103	0.183	0.351

Results from Pearson correlation

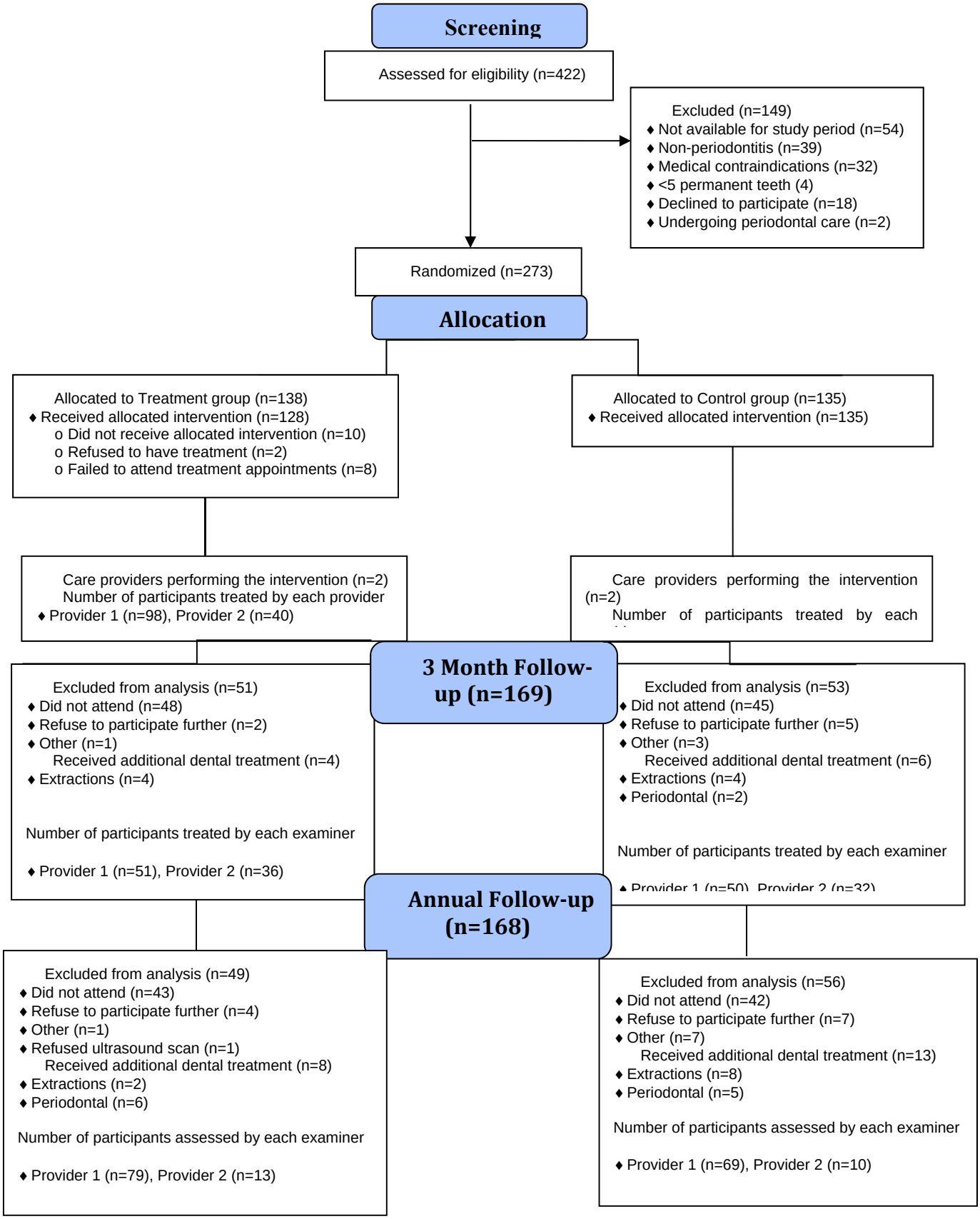

Figure 6.1: CONSORT flow diagram (3-month PWV n=169) & (Annual cIMT n=168).

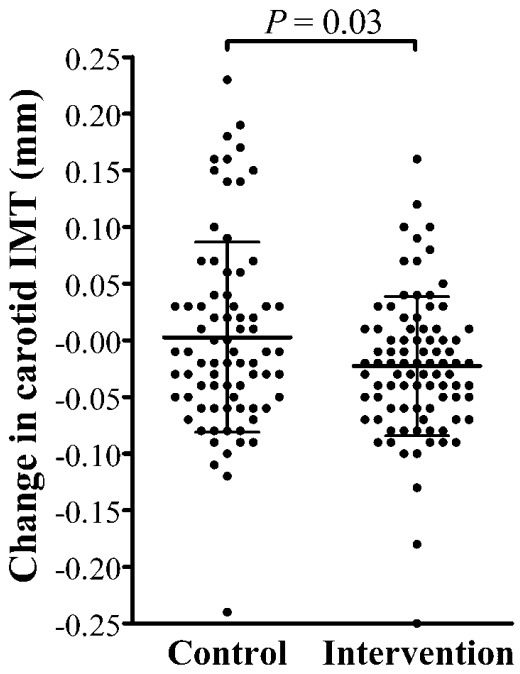

Figure 6.2: Periodontal therapy and 12-month change in carotid intima-media thickness.

Scatter plot of change in maximum carotid intima-media thickness from baseline to 12-months post randomization adjusting for baseline carotid intima-media thickness, stratified by randomized group. *P*-value for effect of intervention by analysis of covariance adjusting for baseline carotid intima-media thickness. Carotid intima-media thickness regressed in the intervention group ($P = 0.003$), but did not change significantly in the control group ($P = 0.82$). Line and error bars represent mean and standard deviation.

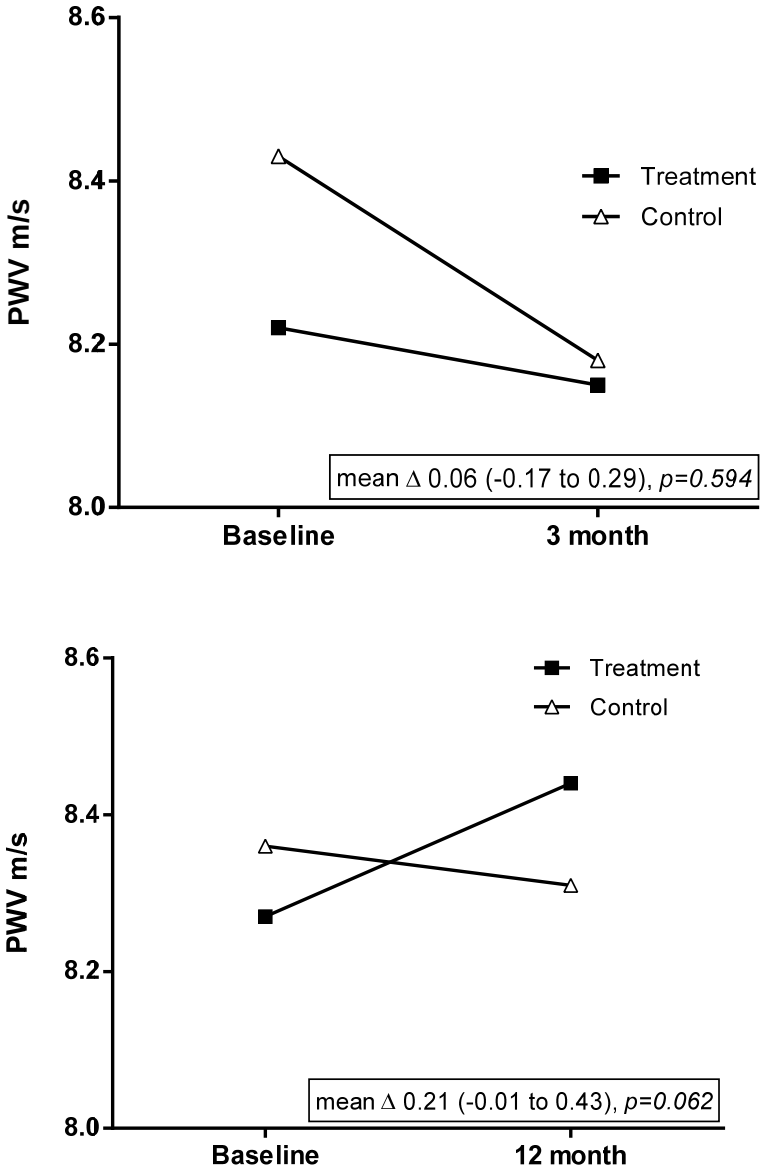

Figure 6.3: Change in PWV [baseline - 3-month] (top panel) & [baseline - 12-month] (bottom panel).

Reported values in box refer to differences in within-group change and corresponding (95% CI). Positive values denote greater difference in control group.

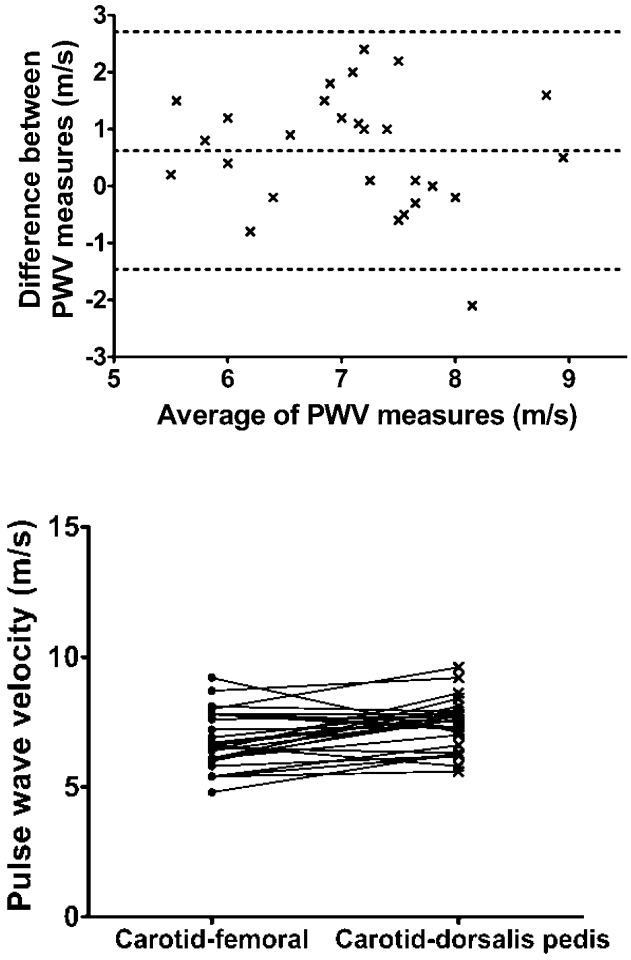

Figure 6.4: Agreement between carotid-femoral pulse wave velocity and carotid-dorsalis pedis pulse wave velocity.

A) Bland-Altman plot, and B) individual participant data for carotid-femoral pulse wave velocity and carotid-dorsalis pedis pulse wave velocity. Dotted lines on Bland-Altman plot represent mean difference ±2SD.

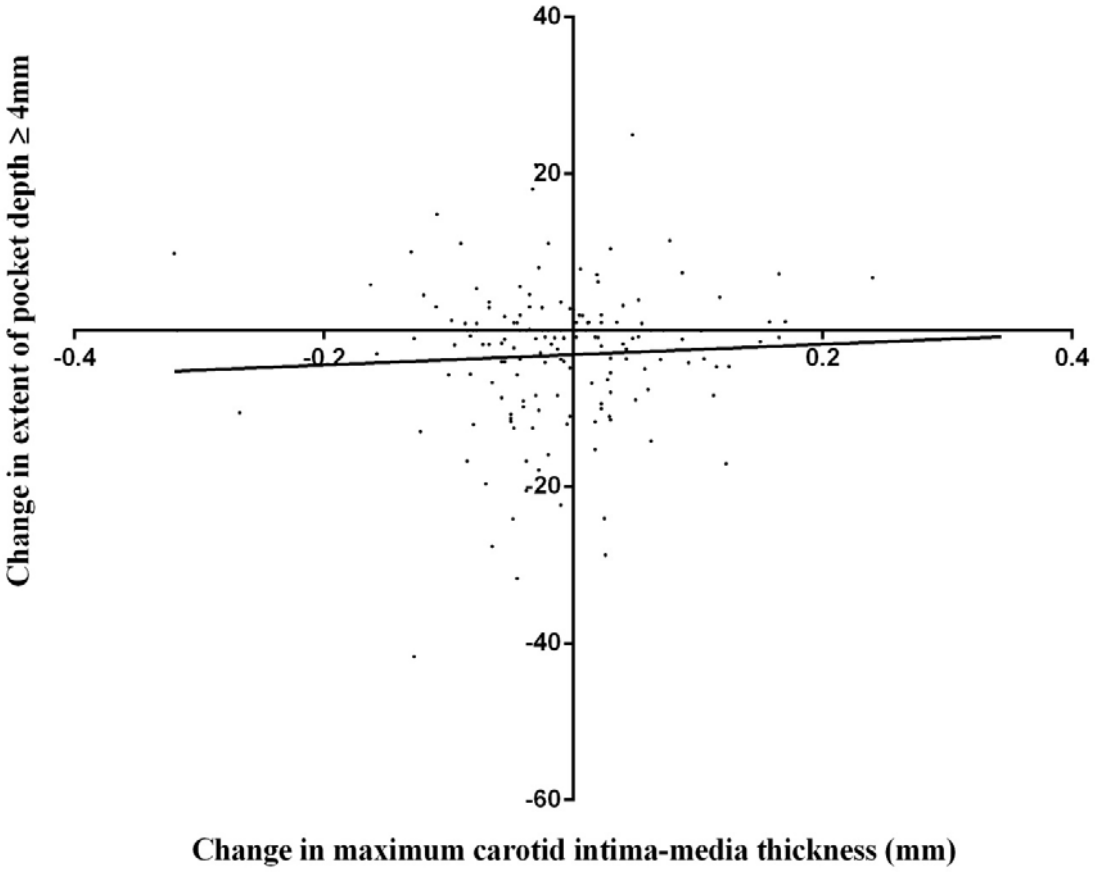

Figure 6.5: Scatter plot of change in extent PPD ≥ 4mm and change in maximum carotid intima-media thickness (mm).

7 GENERAL DISCUSSION AND CONCLUSIONS

7.1 Summary of findings

This study used a parallel-group randomised controlled design to investigate whether non-surgical periodontal therapy influenced central arterial stiffness in a sample of Indigenous Australian adults with moderate to severe periodontal disease. Cross-sectional analysis of baseline data from this study revealed that periodontal disease was positively associated with male sex, a history of smoking cigarettes and Type 2 diabetes. Additionally, PWV was linearly associated with increasing severity of periodontal disease. Analysis of the periodontal intervention showed that a marginal improvement in periodontal status was obtained but that the small improvement did not influence PWV.

Chapter 3 presents the first manuscript, a descriptive article that confirms periodontal disease is associated with male sex, cigarette smoking and diabetes in line with current literature. Cross-sectional analysis of our convenience sample suggest that the prevalence of periodontal disease among Indigenous Australians in the Northern Territory may be higher than previous national estimates.

Prior to study commencement, study investigators decided to not collect anthropometric measurements for study participants ineligible for the primary study on the basis that it would be considered unethical to subject people to unnecessary investigations. Consequently, PWV measurements were not collected for non-periodontitis cases. The manuscript presented in Chapter 4 was prepared to address Aim 1 and Hypothesis 1 of this study. Without non-periodontitis cases as a comparison group, we proposed that establishing a dose-dependent association would be tantamount (if not more informative) to a crude comparison of ‘non-periodontitis case’ versus ‘periodontitis case’. Using cross-sectional analysis of baseline data, we were able to successfully show that a dose-dependent association exists between increasing extent of periodontal pocketing and PWV after adjusting for common confounders. The association attenuated with the inclusion of

systolic blood pressure within the regression modelling suggesting that the aetiology of arterial stiffening is strongly influenced by hypertension.

Chapters 5 and 6 were prepared to address Aim 2 and Hypothesis 2 of this study. Rejection of the null hypothesis would imply that periodontal disease is causally related to arterial stiffness and indicate that control of periodontal infection/inflammation is important for maintenance of vascular health. Chapter 5 presented the short-term response of periodontal parameters to the periodontal intervention. In the absence of notable improvements to oral hygiene and without facilities to extract teeth with extensive bone loss, a single untimed session of non-surgical periodontal therapy resulted in a statistically significant but clinically marginal improvement of shallow but not deep periodontal pockets and reduced gingival bleeding for the treatment group compared to control.

The manuscript presented in Chapter 6 along with the supplementary tables presented within Chapter 6 and those comprising the supplementary material of the thesis report the results of the periodontal intervention on PWV. Without periodontal maintenance for the duration of the study and with few participants accessing dental care on their own volition, periodontal pocketing reverted towards baseline in the treatment group and was not significantly different to control after one year. The small short-term reduction in periodontal pocketing at 3-months was insufficient to influence PWV. Despite our cross-sectional finding that periodontal pocketing is linearly associated with increasing arterial stiffness, the small magnitude of periodontal pocket reduction following a single session of periodontal therapy, and the high number of residual sites with pocketing leads to the conclusion that the single session of periodontal therapy is insufficient to effectively treat periodontitis in this sample. Thus, there is insufficient evidence to determine whether periodontal disease is causally related to arterial stiffness.

7.2 General discussion

Many studies have investigated the impact of periodontal treatment on numerous surrogate cardiovascular markers to warrant comprehensive review and meta-analyses (D'Aiuto et al., 2013). Supplementary analysis to Chapter 6 showed that glycaemic control and other systemic measures of inflammation were not significantly altered at any period following the periodontal intervention. However, a statistically significant reduction in diastolic blood pressure was noted after 3-months in the treatment group which was predominantly driven by change within males despite the sex-stratified analysis being insufficiently powered. In contrast, the sex-stratified analysis revealed that hsCRP significantly increased among males only in the treatment group at 3-month follow-up and that PWV was also higher in the intervention arm among males after one year.

HsCRP behaved differently in the treatment and control groups in the primary analysis and by sex in the stratified analysis. In the primary analysis, hsCRP concentration did not change at 3-months in the treatment group, but was higher at 12-months compared to baseline whereas hsCRP levels in the control group was lower than baseline for both the 3-month and 12-month re-assessments. This pattern was replicated in the PWV measures suggesting that the level of inflammation may be related to PWV via oxidant stress within vessels and consequent reduction in nitric oxide availability. In contrast, the stratified analysis showed that women had significantly higher hsCRP concentrations compared to men (Appendix Tables 2-5), but did not have higher PWV values. The effects of periodontal therapy on inflammatory markers such as hsCRP or IL-6 are short-term. Without periodontal maintenance therapy, any changes to inflammatory markers owing to a periodontal intervention will regress toward baseline beyond 12-weeks (D'Aiuto et al., 2006, Tonetti et al., 2007, Buhlin et al., 2009, Piconi et al., 2009). It is therefore unlikely that the periodontal intervention, provided as a single session at baseline, was the stimuli for higher hsCRP concentrations in the treatment group at the annual follow-up. A recent

systematic review with meta-analysis including 20 studies and 2,561 randomised patients confirmed that effective periodontal treatment commonly leads to significant reductions of hsCRP concentrations irrespective of treatment modality (Demmer et al., 2013) which is contrary to findings of the present investigation. While periodontal therapy administered in this study was insufficient to effectively treat periodontitis, other factors for example, central abdominal adiposity are known to stimulate hsCRP production (Shemesh et al., 2007). Indigenous Australians commonly have higher CRP levels than other populations (Wang and Hoy, 2006) Indigenous Australian women often exhibit higher concentrations of hsCRP than their male counterparts (Hodge et al., 2010), consistent with present findings. A low periodontal disease threshold for inclusion in the study may also explain the minimal change in hsCRP levels post-intervention in the treatment group given other extraneous sources contribute to systemic inflammation.

Acute and chronic inflammation contributes to increased arterial stiffness. Among patients with rheumatoid arthritis for example, chronic inflammation has been attributed to increased PWV (Mäki-Petäjä et al., 2007, Mäki-Petäjä et al., 2006). Medical interventions in the form of statin (Mäki-Petäjä et al., 2007, Matsuo et al., 2005) or anti-inflammatory (Angel et al., 2010) therapy in these patients and mild exercise regimens in a general population considered overweight (Kearney et al.) have been shown to reduce PWV. Whether PWV could be modified following periodontal therapy had never been investigated. Non-surgical periodontal therapy, in particular when accompanied with long-term maintenance is an effective mode of periodontal treatment (Axelsson et al., 2004). It was expected that periodontal therapy provided in this study would have been successful in treating periodontal disease and an improvement in PWV would eventuate.

Arterial function of peripheral vasculature such as the brachial artery assessed in previous periodontal intervention trials (Blum et al., 2007, Elter et al., 2006, Higashi et al., 2008, Tonetti et al., 2007) may respond differently to central arteries measured in the

present study. Peripheral arteries have higher elastin content relative to central arteries (Greenwald, 2007) and thus may be more sensitive to inflammatory stimuli. While inflammation has little influence on arterial function in people with established vascular disease (Blann et al., 2013), a cause and effect relationship has been modelled among young and healthy individuals following vaccination with bacterial lipopolisacharide which led to temporary increases in hsCRP and IL-6 and a concomitant increase in PWV that was attenuated by pre-treatment with aspirin (Vlachopoulos et al., 2005). In a more recent study, high median hsCRP was associated with higher PWV but this relationship was complicated by an interaction with adiponectin (Tsioufis et al., 2007) suggesting other, non-inflammatory mechanisms also impart on arterial stiffness.

Whereas the stimuli for inflammation differs from periodontitis to intra-muscular injection, the work by Vlahopoulos and colleagues shows that central arteries free from atherosclerosis can become stiffer in the presence of acute inflammation. Findings from the present study presented in (Chapter 5) contribute additional information relating periodontitis (a potential chronic source of inflammation) and arterial stiffness in people free from cardiovascular disease (Kapellas et al., 2014a). We demonstrated that PWV increased linearly with the severity of periodontal pocketing after controlling for age and adjustment for smoking exposure. This association was found in the absence of differences in both IL-6 and hsCRP suggesting that chronic inflammation also contributes to arterial stiffness. As this is the first to show a dose-dependent association, other studies are needed to confirm whether periodontitis indeed contributes to arterial stiffness. An important caveat to note is that the PerioCardio study did not measure PWV in those who were not found to have periodontal disease following screening. It is currently unclear whether those without periodontitis have lower PWV values compared to those with periodontal disease.

The effects of periodontal therapy on blood pressure have recently been reviewed (D'Aiuto et al., 2013) and there is limited evidence beyond the findings of the present study

and one other to suggest periodontal treatment has any notable influence on blood pressure (D'Aiuto et al., 2006). Although both systolic and diastolic blood pressure reduced in the short-term in the present study, only diastolic blood-pressure was statistically significant. Stratified analysis by sex revealed that blood pressure was significantly reduced among males only. It is not expected that sex differences exist in terms of the effects of periodontal treatment. Rather, males had a greater extent of PPD ≥4mm compared to females. A higher degree of periodontal disease could manifest with more systemic inflammation influencing blood pressure and periodontal treatment may counteract this. Alternatively, periodontal therapy may impact blood pressure via other means unrelated to inflammation. To elucidate this, future investigations could examine the effects of periodontal treatment on blood pressure in both sexes which have comparable levels of periodontal disease severity.

Although not specifically an outcome of this study, the modified intervention provided an opportunity to examine the effectiveness of single-visit non-surgical periodontal therapy within this context. As an extension, it also allowed us to examine for the first time, the treatment response which could be achieved from periodontal treatment provided by the local public dental service. Results from Chapters 5 and 6 indicate that short-term improvements can be obtained however without subsequent appointments to manage the periodontal state, treatment of periodontal disease is likely to fail long-term.

7.3 Study Limitations

Although it would have been ideal to adopt a periodontal maintenance regimen as opposed to a one-stage approach for this study protocol it was not feasible for the reasons elaborated in the discussion of Chapter 6. From a data collection perspective, implementation of a periodontal maintenance regimen would have resulted in a fragmented dataset compiled of study participants randomised to the intervention group in receipt of a

varying number of periodontal therapy sessions. Analysis and interpretation of the data would then have likely been compromised for the following reasons: 1) the results of the primary analysis comparing treatment versus control would be influenced by the varying number of periodontal therapy sessions, and; 2) the likelihood of being insufficiently powered to assess comparisons of complete treatment versus incomplete treatment versus none (complete non-adherence) within the intervention arm of the study would be high. The latter point is of interest where the effectiveness of periodontal therapy is important. Statistically, a properly-conducted intention-to-treat analysis would enable appropriate handling of data where loss to follow-up is small. In the case of loss to follow-up in the PerioCardio study, an intention-to-treat analysis is likely to yield biased estimates.

The potential bias arising from lack of assessor blinding may be considered by some to be an issue in this study. Examiner #1 was heavily involved in the recruitment of participants, conducted the majority of the oral and cardiovascular assessments throughout the study, provided the majority of the periodontal interventions, cleaned and analysed the data and subsequently wrote the manuscripts presented within this thesis. In terms of blinding to randomisation status throughout the study, this was unattainable. The following measures were implemented during the study to minimise this influence:

1) Randomisation was allocated following collection of all measures by research staff not involved with the oral or cardiovascular assessments using a randomisation database provided by a third party.

2) For follow-up measures of periodontal and vascular status, both examiners were requested to not view the randomisation status of study participants prior to conducting said assessments.

3) Although PWV was measured directly on study participants, the values obtained arise from a computer algorithm combining data from a 3-lead ECG tracing, measurements

of arterial tree lengths and the specific pulses. Calculations for this and the subsequent values are beyond the influence of any clinicians.

4) All laboratorial assessments were blinded; a) blood lipids and HbA1c were conducted by a commercial laboratory in Darwin; b) assessment of IL-6 and ADMA was conducted by research staff at the Menzies School of Health Research who, for the most part were not involved in the PerioCardio study; c) assessment of hsCRP and apolipoproteins were conducted in laboratories affiliated with the Royal Prince Alfred Hospital, Sydney.

7.4 Concluding statement

After conducting the present investigation to determine the effect of providing periodontal therapy on arterial stiffness, one is still left with a conundrum on the basis that the periodontal intervention provided was insufficient to effectively treat and stabilise the periodontal condition long-term. Although we were able to successfully show within Chapter 4 that there is a linear association between the extent of shallow periodontal pocketing and PWV and that this association persisted following adjustment for common confounders, our prospective findings presented in Chapter 6 cannot ascribe or refute any causal inferences. To this end, it is unclear if elimination of active periodontal disease will result in improvements of PWV. The statistical and clinically significant improvement in carotid IMT (also presented within Chapter 6) indicates that multiple mechanisms are likely to interact between periodontal, cardiovascular and possibly other underlying chronic conditions. The following sub-section proposes future directions into periodontal and cardiovascular disease research.

7.5 Future directions

This study was solely conducted on Indigenous Australian adults who as a population have unique risk factors that predispose to developing chronic conditions such as cardiovascular disease, diabetes and periodontitis earlier than non-Indigenous Australians. The dose-dependent association between increasing periodontal pocketing severity and PWV needs to be examined on other populations to determine whether this association exists elsewhere.

As the periodontal therapy provided was insufficient to effectively treat the periodontal disease in this study, it is not possible to determine if a causal relationship exists between periodontitis and PWV. Future studies intending to investigate this relationship will need to consider the two primary mechanisms by which periodontal disease potentially influences arterial stiffness and function.

Firstly, chronic periodontitis may be considered a systemic source of bacteria which can translocate and subsequently enter the blood stream, adhere to arterial walls to then initiate or contribute to atheroma formation. This process will consequently lead to changes of wall structure, destruction of elastin, and repair with collagen and eventually result in arteriosclerosis. Structural changes to arterial walls via this mechanism would require a trial that treats periodontal disease and maintains a healthy periodontium for many years. In this instance, a causal relationship between periodontal disease and PWV would arise as a result of a difference in PWV occurring due to natural progression of arterial stiffness with age in the untreated control group whereas progression would be minimal in the treatment group. Secondly, periodontitis may influence PWV via chronic inflammation whereby inflammatory mediators produced locally in the periodontium enter the systemic circulation, or stimulate production of inflammatory mediators at distant sites. In this instance, inflammation alters arterial function via the inhibition of nitric oxide for example.

If a true effect of periodontal treatment on cardiovascular disease is to be determined, it may be mandatory that the periodontal disease be successfully treated with verified elimination of inflammation and significant reduction in the infectious burden. This effect of disease stability or return to periodontal health needs to be sustained over a significant period of time; years or possibly even decades. To achieve this, trials investigating these mechanisms would require a periodontal intervention that eliminates periodontal pocketing and gingival inflammation as part of the initial treatment and subsequently maintenance of a healthy periodontal state.

An example of a future trial may be as follows: Participants with periodontal disease but without a history of cardiovascular disease will need to be recruited, randomised, and followed-up over many years. To overcome any potential ethical concerns, all recruited participants will have their periodontal condition treated and stabilised in the first instance. After which, study participants will be randomised into the maintenance group whereby periodontal disease will be regularly monitored and retreated as required or to the non-maintained group whereby participants will be monitored in line with the community-control approach. Like conventional drug trials, participants will then be followed up until a sufficient number of primary cardiovascular events occur as determined by power calculations.

Future investigations aiming to determine any causative relationship between periodontal disease and cardiovascular disease will need to account for several elements as listed herein:

1. From a periodontal disease perspective, if the association between periodontitis and cardiovascular disease is inflammatory driven then it would be best to recruit people with severe periodontitis as more inflammation intuitively may pose a higher future risk for future cardiovascular events. These people then potentially may receive a greater benefit from the periodontal intervention than a person with milder forms of

periodontal disease. This has not been extensively investigated presently so is purely and educated speculation.

2. It would be good to recruit people without periodontal disease and cardiovascular disease and see if periodontal disease precedes the incidence of cardiovascular events. A prospective cohort of this type would be incredibly long, expensive and difficult to manage. It may be possible however, to incorporate dental components to existing cohort studies.
3. From a cardiovascular disease perspective, recruiting people who have recently had a cardiovascular event may be ideal as these people are at an increased risk for future events than the general population. This will mean that a smaller sample size could be recruited in the first instance. The randomised trial will need to be similar to that proposed above. A study of this type however, will need to consider how to handle anti-platelet medications and statins which are likely to be prescribed to all patients.

Periodontal disease aside, this study also highlighted that untreated dental caries in this sample was five times higher than previous age-matched national Australian estimates while overall DMFT was 30% lower. (Roberts-Thomson and Do, 2007, Kapellas et al., 2014b) Collectively, this suggests that factors such as availability and receipt of timely dental care may be an issue for participants in this study and for Indigenous Australians. It would be beneficial to understand what factors influence the dental care visiting patterns of Indigenous Australians. Published findings from this study (Amarasena et al., 2014) suggest that Indigenous Australians may tend to be problem-associated visitors, the reasons for why this is the case are yet to be discovered.

7.6 General overview of periodontal disease/cardiovascular associations

Research into periodontal/cardiovascular disease associations has been hampered due to the slow progression of both cardiovascular disease and periodontitis. Both conditions

often require decades of development before symptoms are noted. Certainly, the fatty streak which is the earliest detectable change to the vascular wall occurs within the first years of life (Napoli et al., 1997). There are common confounders such as age and common risk factors including cigarette smoking and poor management of diabetes that make it inherently difficult to analyse specific associations for both conditions. Prospective studies are required to quantify the extent to which periodontal disease contributes to cardiovascular disease.

7.7 References

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8 APPENDICES

8.1 Manuscripts relevant to thesis

Skilton M, Maple-Brown L, Kapellas K, Celermajer D, Bartold M, Brown A, O'Dea K, Slade G, Jamieson L. The effect of a periodontal intervention on cardiovascular risk markers in Indigenous Australians with periodontal disease: The PerioCardio study. BMC Public Health. 2011;11:729

[This manuscript presented the PerioCardio study methods.]

Skilton et al. BMC Public Health 2011, 11:729
http://www.biomedcentral.com/1471-2458/11/729

BMC Public Health

STUDY PROTOCOL Open Access

The effect of a periodontal intervention on cardiovascular risk markers in Indigenous Australians with periodontal disease: the PerioCardio study

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Abstract

Background: Indigenous Australians experience an overwhelming burden of chronic disease, including cardiovascular diseases. Periodontal disease (inflammation of the tissues surrounding teeth) is also widespread, and may contribute to the risk of cardiovascular diseases via pathogenic inflammatory pathways. This study will assess measures of vascular health and inflammation in Indigenous Australian adults with periodontal disease, and determine if intensive periodontal therapy improves these measures over a 12 month follow-up. The aims of the study are: (i) to determine whether there is a dose response relationship between extent and severity of periodontal disease and measures of vascular health and inflammation among Indigenous Australian adults with moderate to severe periodontal disease; and (ii) to determine the effects of periodontal treatment on changes in measures of vascular health and inflammation in a cohort of Indigenous Australians.

Methods/Design: This study will be a randomised, controlled trial, with predominantly blinded assessment of outcome measures and blinded statistical analysis. All participants will receive the periodontal intervention benefits (with the intervention delayed 12 months in participants who are randomised to the control arm). Participants will be Indigenous adults aged ≥25 years from urban centres within the Top End of the Northern Territory, Australia. Participants assessed to have moderate or severe periodontal disease will be randomised to the study's intervention or control arm. The intervention involves intensive removal of subgingival and supragingival calculus and plaque biofilm by scaling and root-planing. Study visits at baseline, 3 and 12 months, will incorporate questionnaires, non-fasting blood and urine samples, body measurements, blood pressure, periodontal assessment and non-invasive measures of vascular health (pulse wave velocity and carotid intima-media thickness). Primary outcome measures are pulse wave velocity and carotid intima-media thickness.

Discussion: The study will assess the periodontal-cardiovascular disease relationship among Indigenous Australian adults with periodontal disease, and the effectiveness of an intervention aimed at improving periodontal and cardiovascular health. Efforts to understand and improve Indigenous oral health and cardiovascular risk may serve as an important means of reducing the gap between Indigenous and non-Indigenous health in Australia.

Trial Registration: Australia and New Zealand Clinical Trials Register (ANZCTR): ACTRN12610000817044

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Background

The health of Indigenous Australians

Indigenous Australians are disadvantaged on almost every health and social indicator relative to their non-Indigenous counterparts. They have 15-20 years shorter life expectancy, much higher levels of cardiovascular disease, diabetes and other chronic conditions including poor oral health status, and are more likely to experience disability and reduced quality of life due to ill health [1-4]. They also have a high burden of infectious diseases alongside significant rates of metabolic risk [5].

Cardiovascular disease

Cardiovascular diseases are a leading cause of morbidity and mortality in developed countries. The disease process that underlies the majority of cardiovascular events is atherosclerosis, an inflammatory disease of the blood vessel wall. The earliest physical evidence of atherosclerosis are fatty streaks, which are typically present in childhood. In the presence of arterial endothelial dysfunction, which is involved in the initiation and progression of atherosclerosis, these early lesions progress through to complex atheromatous lesions in adulthood, finally resulting in occlusion, plaque rupture and ischaemic events [6].

Periodontal disease

Periodontal disease is inflammation of the tissues surrounding teeth and results from a complex interplay between bacteria and host risk factors such as long-term smoking, poor oral hygiene, poorly controlled diabetes, stress and genetic predisposition [7]. Not only have periodontal organisms adapted to survive within an environment that is constantly besieged by host defences, but they flourish in the presence of inflammation, enabling their capacity to invade host tissues and gain direct access to the circulation [8]. Repeated bacteremias and endotoxemias are characteristic of periodontal infection, and periodontal organisms have been found to co-localise within atheromatous plaques [9]. The constant exposure of the vasculature to these pathogens provides an opportunity for endothelial inflammatory activation and functional impairment. Clinically, periodontal disease manifests as deepening of the epithelial attachment around teeth, loss of periodontal attachment and, ultimately, tooth loosening.

Periodontal disease is widespread and poses a substantial problem among Australian Aboriginal populations. In Australia's second National Survey of Adult Oral Health (NSAOH), mild forms of periodontal disease were reported to affect 20 percent of the adult population, while more severe forms affected about 1 in 40 adults [10], increasing to 3 in 10 adults when the Indigenous population were considered in isolation. Similarly, Endean and colleagues reported that among Aboriginal adults seeking dental care in several Central Australian remote communities, 30.2 percent had a history of this condition [11]. The prevalence of tooth loss due to periodontal disease among Aboriginal populations is also high [12].

Periodontal disease has been associated with atherosclerosis [13], cardiovascular disease [14], diabetes [15], pre-term low birth weight [16], stroke [17], and premature death [18]. Accordingly, periodontal disease may account for a portion of the risk for cardiovascular disease via a shared pathogenic underlying inflammatory response (figure 1) [8].

Periodontal therapy decreases systemic inflammation and improves endothelial function

Treating periodontal disease results in a functional improvement in cardiovascular status [19-22]. These studies are consistent with the concept that periodontal disease may be an important source of infectious and inflammatory vascular stress, and that periodontal therapy may be of particular clinical relevance in populations with high prevalence of both periodontal disease and cardiovascular disease.

Aims & hypotheses

The PerioCardio study will assess measures of vascular health and inflammation in Indigenous Australian adults with periodontal disease, and determine if intensive periodontal therapy improves these measures over a 12 month follow-up. The specific aims and hypotheses are:

Aim 1: To describe the extent and severity of measures of vascular health and inflammation in Indigenous Australian adults with moderate or severe periodontal disease.

Hypothesis: The extent and severity of cardiovascular surrogate endpoints in an Indigenous population with periodontal disease will be high.

Aim 2: To determine whether there is a dose-response relationship between extent and severity of periodontal disease and measures of vascular health and inflammation among Indigenous Australian adults.

Hypothesis: The extent and severity of periodontal disease has a dose-response relationship with vascular health and inflammatory measures.

Aim 3: To determine whether periodontal treatment influences vascular health and inflammation in Indigenous Australians with moderate to severe periodontal disease.

Hypothesis: Periodontal therapy will improve vascular health and inflammatory markers in Indigenous Australians.

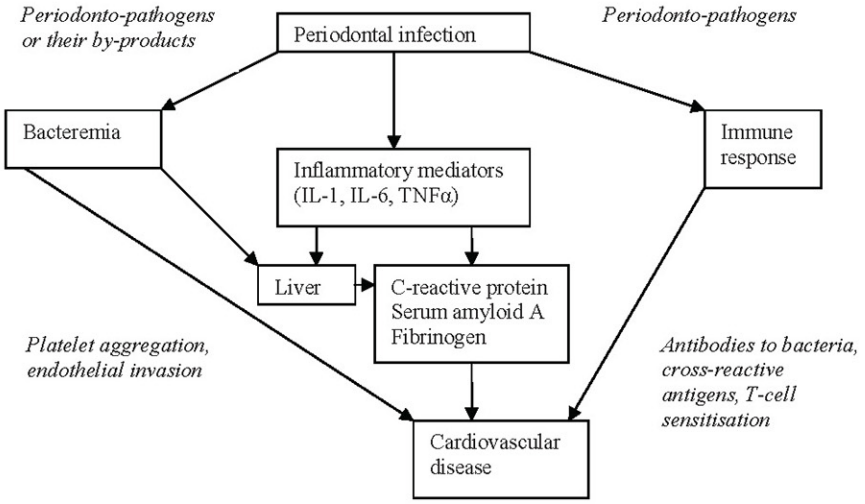

Figure 1 Conceptual model of periodontal disease and cardiovascular surrogate endpoint response.

Methods/design

Study design and overview

This will be a randomised, controlled trial, with predominantly blinded measurement of cardiovascular endpoints, and blinded statistical analysis. All participants will receive, or be offered, the periodontal intervention benefits. A schema outlining the study design is presented in Figure 2. The PerioCardio study has been registered with an international clinical trials registry (ANZCTR; ACTRN12610000817044), and will be reported as per the CONSORT statement. Recruitment for the study commenced in June 2010.

Setting & location

Participants will be recruited from urban centres within the Top End of the Northern Territory, Australia, facilitated through the following Aboriginal Medical Services or health facilities: Danila Dilba Health Services (Darwin and Palmerston), Bagot Community Health Centre (Darwin), Royal Darwin Hospital, Darwin Dental Centre (Northern Territory Oral Health Services, Department of Health), Northern Territory Correctional Services (Darwin), and Wurli Wurlinjang (Katherine).

Participants and recruitment

Participants will be Indigenous Australians aged ≥25 years that have lived in their current location for ≥2 years and who plan to live at their current location for the next 2 years. Those participants who have moderate or severe periodontal disease will be randomised to the intervention or control arm of the study. Those participants without periodontal disease, or only mild periodontal disease, will not be included in the trial. Baseline questionnaires will, however, be assessed in these participants in order to study the social, demographic, lifestyle and general health correlates of periodontal disease in Indigenous Australians.

Exclusion criteria include: a history of rheumatic heart disease, prior myocardial infarction, stroke or coronary revascularisation, other cardiac conditions requiring antibiotic prophylaxis for prevention of subacute bacterial endocarditis, obvious endodontic lesions, other sources of oral infection, treatment for periodontal disease within the previous six months.

Feedback to participants will be provided at the end of each examination. Participants with unexpected abnormal results will be referred to an appropriate health care provider. In addition, written results will be provided in a plain language and culturally appropriate manner to individual participants and to their nominated primary health care provider. Whenever possible, participants with abnormal results will be contacted by telephone or in person. The results packet will then be mailed or hand-delivered, as appropriate. A $50 gift voucher to a local store will be provided to participants at the end of the examination or with their results as a gift of appreciation for their participation in the study.

Funding

Funding was provided by the National Health and Medical Research Council of Australia (NHMRC, project grant #627100).

Ethics

The study was approved by the joint Menzies School of Health Research - Northern Territory Department of Health Human Research Ethics Committee. The project

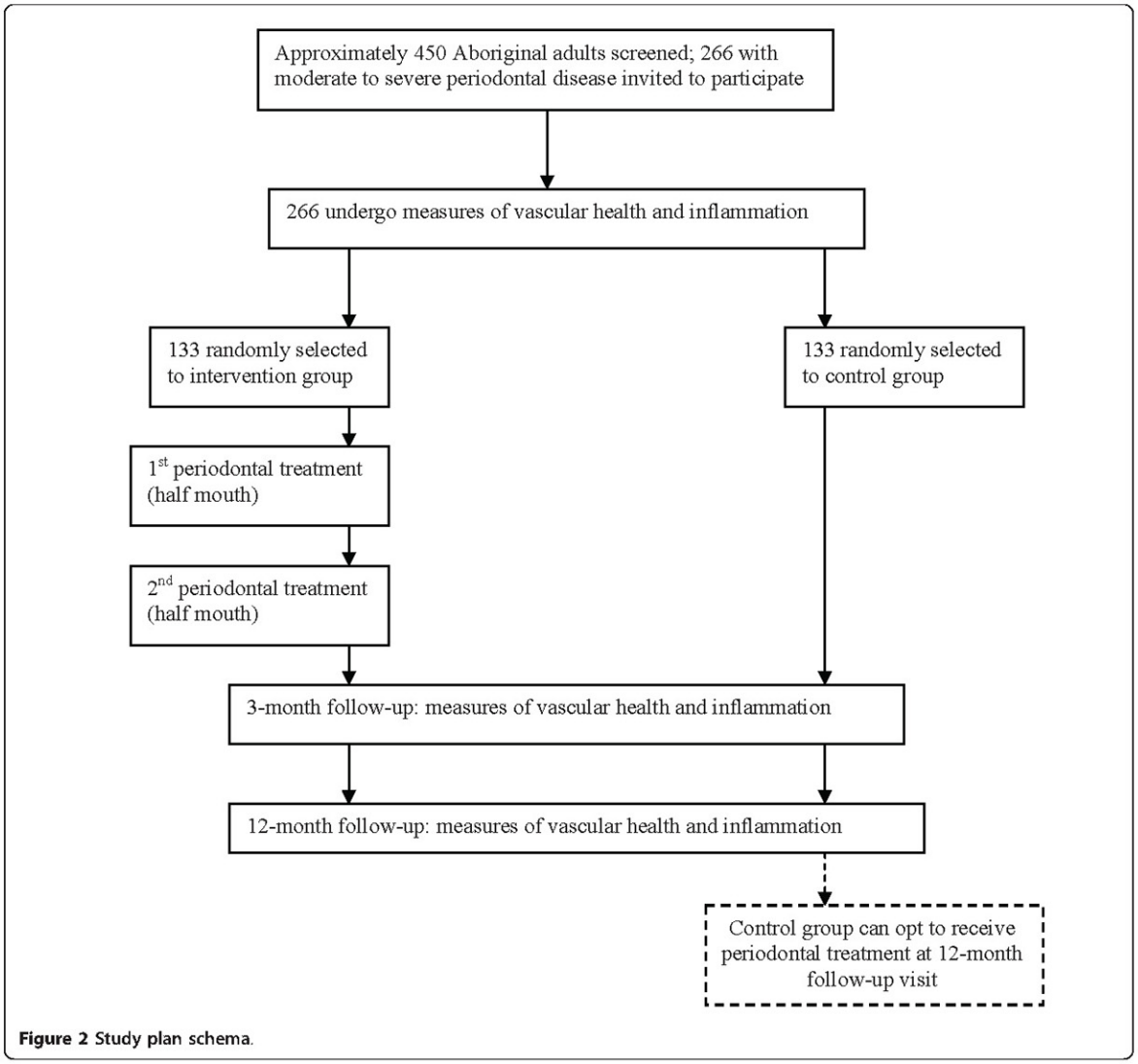

Figure 2 Study plan schema.

was considered and approved by both the Aboriginal sub-committee, which has absolute right of veto, and by the main committee. The study was also approved by the Central Australian Human Research Ethics Committee, Northern Territory Correctional Services Research Committee, University of Adelaide Human Research Ethics Committee, and the Aboriginal Health Council of South Australia.

Staff

Three people are employed on contracts for the course of the study. Of these, two are Indigenous and one is non-Indigenous. Additional people are employed on a casual basis during visits to discrete locations or communities; these community members act as local facilitators. One post-graduate research student also participates in the study and is substantially involved in data collection, taking on the role of project manager. The majority of staff members have prior health qualifications and experience: one is an Aboriginal Health Worker, one has overseas dental qualifications and the post-graduate research student is an oral health therapist.

Consent

Potential participants are provided with written information about the study, face-to-face discussion with a staff member, and given an opportunity to ask questions. If required, an interpreter explains all information relating

to the study. Those who indicate that they wish to participate are then asked to complete and sign a consent form. Consent is obtained using the NHMRC Guidelines for Ethical Conduct in Aboriginal and Torres Strait Islander Health Research. All participants are informed that their participation is voluntary and that they can refuse or withdraw from participating and need give no reason or justification for their decision and that it will not affect their medical or dental care. Participants are asked to provide separate consent for various elements of the study, including: vascular assessments, blood and urine samples, body measurements, blood pressure and questionnaires. They are also asked whether they want a report sent to their primary health care provider and whether their blood and urine samples can be stored at Menzies School of Health Research for 3 years.

Screening for periodontal disease

Following confirmation of contact details and eligibility, and after obtaining informed consent, a participant will be screened for periodontal disease, using the US Centers for Disease Control and Prevention and the American Academy of Periodontology definitions [23]. Thus, a case of moderate periodontitis is considered as the presence of either two sites between adjacent teeth with ≥4 mm attachment loss or at least two such sites with ≥5 mm pockets, and severe periodontitis as having at least two sites between adjacent teeth with ≥6 mm attachment loss and at least one pocket ≥5 mm.

Randomisation

After screening for periodontal disease, those with moderate or severe periodontal disease are randomised on a 1:1 basis to either the treatment or control group. A computer generated permuted block randomisation sequence is used, stratified by recruitment site (Darwin/Palmerston, Katherine).

Intervention

Individuals randomised into the 'treatment group' undergo a periodontal intervention based on the technique described previously [20]. This involves intensive removal of subgingival and supragingival calculus and plaque biofilm by scaling and root-planing. Participants are offered local anaesthesia prior to the procedure. The procedures are carried out with the use of Hu Friedy hand scalers and a piezoelectric ultrasonic scaler with universal tips. Dental extraction of teeth that cannot be saved is not performed as part of the treatment protocol. The intervention is performed by two oral health practitioners who have received additional training with these techniques. The periodontal intervention occurs during a single untimed visit to the study clinic.

Oral hygiene instruction and an oral hygiene pack containing a toothbrush and tooth paste are provided to all participants at the baseline visit. Information concerning dental extractions during the course of the study is recorded.

Participant involvement

Each participant randomised will attend the study clinic a total of 3 times (figure 2).

Periodontal treatment will be offered to those in the control group at 12 months post-randomisation, to ensure that all participants are able to receive the oral health benefits of the periodontal treatment. The periodontal treatment offered to participants in this study is beyond that which forms routine treatment within the government funded system. Participants are not restricted from seeking periodontal treatment during the study through the private health system.

Data collection techniques

Dental assessment

including tooth presence, caries experience, periodontal destruction, gingivitis and calculus, plaque, oral mucosal lesions and trauma.

Clinical and anthropometric measures

Blood pressure is measured while seated using an automated device (Welch Allyn Medical Products, Skaneateles Falls, USA). Three measurements of systolic and diastolic pressure are made, with three minutes between readings. The mean values of the final two recordings will be used in statistical analysis.

Height is measured to the nearest 0.1 cm using a wall-mounted stadiometer.

Weight is measured to the nearest 0.1 kilogram, using a portable weight scale (Tanita HD-351, Arlington Heights, USA) with participants lightly clothed. Two separate measurements of weight are recorded and if they differ, then a third measurement of weight is taken.

Waist and hip circumference are measured to the nearest 0.1 centimetre using a 2-metre non-stretch flexible steel tape (Model W606PM Lufkin, USA), as previously described [24].

Common carotid intima-media thickness (IMT) and M-mode imaging

External ultrasound of the common carotid artery is performed with the patient lying in a supine position, neck slightly extended and the head tilted away from the side being scanned whilst connected to 3-lead ECG, to monitor cardiac cycle. Bilateral longitudinal images of the carotid bulb and common carotid artery, up to 15 mm proximal to the bulb, are obtained using a portable ultrasound (Sonosite MicroMaxx, Bothell, USA), with a 10-5 MHz linear array transducer. Loops are obtained of each artery,

and stored in a digital format for batch analysis by an observer blinded to participant group, characteristics and study visit. Measurement of carotid IMT will be performed on the far wall of the common carotid artery at end diastole, within 10 mm proximal to the carotid bulb, on two consecutive cardiac cycles.

M-mode imaging of the arterial diameter during the cardiac cycle is used to assess regional stiffness of the common carotid artery. The area of interest for the stiffness measure is between 10 mm and 15 mm proximal to the bulb. Loops of each side are obtained, ensuring that both the near and far walls are clearly visible and the artery is perpendicular to the angle of insonation. Internal diameter of the artery during systole and end-diastole will be measured over three cardiac cycles to generate the following indices: pressure-strain elastic modulus, Young's modulus, cross-sectional compliance, distensibility coefficient.

Pulse wave velocity (PWV)

PWV is measured between the common carotid and dorsalis pedis arteries using applanation tonometry (SphygmoCor-PVMx device, AtCor Medical, Sydney, Australia), with participants in a supine position for at least 10 minutes prior to commencement of measures. The common carotid artery is located via lateral palpation along the thyroid cartilage while the dorsalis pedis artery is located between the first and second metatarsals on the dorsal surface of the foot. A pressure tonometer is placed transcutaneously above the corresponding artery to record the pulse pressure waveform while simultaneously recording the ECG signal, which provides an R-wave timing reference. The arteries are assessed consecutively. The resultant PWV score is calculated via computer algorithm as the mean time difference between the R-wave and the pressure wave per heart/pulse beat and the arterial path length between the two recording sites. To correct against measurement error, the distance from the common carotid location and the sternal notch will be subtracted from the distance between the sternal notch and the dorsalis pedis site. Data quality control for this measurement requires that standard deviation not exceed 10%. In the instances that this occurs, PWV will be remeasured. Measurement of PWV is undertaken automatically by the acquisition machine, and as such the observer will not be blinded to study participant characteristics.

Blood and urine derived measures of cardiovascular risk

non-fasting blood and urine samples will be collected to assess established measures of cardiovascular risk, inflammation and vascular health, including glycosylated haemoglobin (HbA_{1c}), lipids and lipoproteins (total cholesterol, high-density lipoprotein cholesterol, apolipoprotein A-1 and apolipoprotein B), high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), asymmetric dimethylarginine (ADMA), and urine albumin to creatinine ratio.

Collected blood samples are centrifuged for 10 min at 1300 g within 30 minutes of collection. If unable to be centrifuged immediately, blood is stored in a fridge or on ice packs in a cool-box until centrifugation a maximum of 2-hours after collection. Following centrifugation, samples are transported on ice to be processed by Westerns Pathology, Darwin for lipids and HbA1c. Total cholesterol is assayed by enzymatic methods, and high-density lipoprotein cholesterol measured directly, with an ADVIA chemistry system (Siemens, Tarrytown, USA). HbA1c is determined by turbidimetric inhibition immunoassay with a COBAS INTEGRA (Roche Diagnostics, Indianapolis, USA). Other samples are stored at -80 degree Celsius. For samples collected in remote locations, storage for transportation is either on dry ice or in liquid nitrogen ("Biological Shipper", CryoPak Series, Taylor-Wharton, USA). Samples are stored for analysis at a later date for hs-CRP, IL-6, ADMA, apolipoproteins and glucose.

Self-administered questionnaires

Self-reported information pertaining to socio-demographic, oral health behaviours and general health factors is gathered at baseline, with the assistance of study personnel as required.

Socio-demographic details include age, sex, Aboriginal/Torres Strait Islander status, grandparental Indigenous status, education level, employment, English-as-first-language, house ownership, number of children, number of people who stayed in house the previous night and car ownership.

Oral health covariates include periodontal status and a self-report item pertaining to whether or not participants think they have gum disease.

General health covariates include behaviours such as smoking, current medication, current diagnoses (for diabetes etc), status of current diagnoses (controlled, uncontrolled etc) and stress (measured by the Kessler 10+ scale; a validated measure of distress that has been validated in a national health survey for Indigenous adults) [5].

Primary and secondary outcomes

The primary outcome measures for purposes of this investigation will be carotid IMT and PWV. Secondary outcome measures will include CRP, IL-6, ADMA, and HbA1c.

Sample size

Based on recent studies of Indigenous adults residing in the Northern Territory, we estimate that a sample size of 144 will provide 0.80 power to detect a 10 percent difference between groups in carotid IMT progression over 12-months, and a 10 percent reduction in PWV in the

intervention group compared to the control group, at $P < 0.05$. The literature indicates that it is reasonable to expect effects of this magnitude following the periodontal intervention [25,26]. Allowing for an attrition rate of 45 percent over 12 months, 266 participants are required at baseline. In NSAOH, the proportion of Aboriginal adults with periodontal disease was estimated at 30 percent [10], however, it is likely that Aboriginal participants in NSAOH are not representative of the Aboriginal population in which we are interested. There are two main reasons: i) NSAOH participants were required to complete a telephone interview (ie to have an operating telephone and be registered in the electronic white pages); and ii) NSAOH participants were required to organise their own transport and to present for a scheduled dental examination at a local dental clinic. We posit that the NSAOH estimate is an under-estimation of periodontal disease prevalence in our target population, with anecdotal evidence suggesting that the prevalence is closer to 60 percent. We have estimated the prevalence of periodontal disease in our target population as 40 percent, meaning approximately 450 Indigenous adults will need to be screened in order to obtain 266 with moderate or severe periodontal disease.

Data handling and statistical methods

At the time of baseline assessment, data is collected on paper forms and then entered into a Microsoft Access database. Data is stored securely at Menzies School of Health Research. In brief, the plan for the analysis is:

Aim 1: descriptive statistics including mean and standard deviation for normally distributed variables, geometric mean and 95% confidence interval for non-normally distributed data, and percentages for categorical variables.

Aim 2: associations of the extent and severity of periodontal disease with measures of vascular health and inflammation from cross-sectional analysis at baseline, using correlation analysis, and multivariable regression modelling adjusted for cardiovascular risk factors.

Aim 3: intention to treat analysis comparing cardiovascular surrogate endpoints between the two randomised groups. Primary outcomes are carotid IMT at 12-months and PWV at both 3- and 12-months. Correlations between the changes from baseline to 3- and 12-months in carotid IMT, PWV and secondary outcomes will be determined.

Appropriate transformation of the data will be performed as required. Crude correlations will be detailed, followed by multivariable modelling. Statistical significance will be inferred at $2P \leq 0.05$.

Discussion

The PerioCardio study will provide answers to three key questions: (1) the extent of severity of cardiovascular surrogate endpoints in an Indigenous adult population with periodontal disease; (2) whether the extent and severity of periodontal disease correlates with measures of vascular health and inflammation in Indigenous Australian adults and; (3) whether intense periodontal therapy improves these markers of vascular health and inflammation.

Furthermore the PerioCardio study will be the first investigation examining the effectiveness of periodontal therapy in changing cardiovascular surrogate endpoint levels in an Indigenous Australian population, and the first investigation to monitor changes in cardiovascular surrogate endpoints following periodontal intervention at both 3- and 12-months.

As such, the PerioCardio study will have significance for policy and planning by providing evidence of the relationship between periodontal disease, periodontal therapy, and cardiovascular disease among Indigenous Australian adults with periodontal disease, and the effectiveness of an intervention aimed at improving periodontal and cardiovascular health in an Indigenous population. Efforts to understand and improve Indigenous oral health and cardiovascular risk may serve as an important means of reducing the gap between Indigenous and non-Indigenous health in Australia.

The findings may help raise the profile of the role of periodontal disease in cardiovascular health; thus increasing the knowledge base of those working intimately with patients with cardiovascular disease. Ultimately it is hoped that the findings might encourage greater dialogue between oral health and medical professionals so that periodontal treatment might become a routine part of care in the treatment of cardiovascular disease among Indigenous as well as non-Indigenous populations.

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Funding bodies had no role in the study design, in the collection, analysis or interpretation of data, in the writing of the manuscript or the decision to submit the manuscript for publication.

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Authors' contributions

MRS participated in study design, ethics applications, data collection, data management and manuscript preparation. LMB participated in study design, ethics applications, data collection, data management and manuscript preparation. KK participated in study design, data collection, project management, data management and manuscript preparation. DSC, MB, AB, KO, GS provided important intellectual input into the study design and revision of the manuscript. LJ drove the design of the study protocol for funding and ethics applications, coordinated data collection and data management, and participated in manuscript preparation.
All authors were involved in revising the manuscript for important intellectual content and read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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8.2 Supplementary analysis to Chapter 6

8.2.1 Results

The difference in between-group mean diastolic blood pressure was significantly lower in the treatment group after 3-months (ANCOVA Δ -2.85 mm/Hg, [95% CI -5.03 to -0.68] $p = 0.010$) (Appendix Table 1). In contrast, there were no significant differences to systolic blood pressure (ANCOVA Δ -0.43 mm/Hg, [95% CI -4.05 to 3.18], $p = 0.814$) or brachial pulse pressure (ANCOVA Δ -1.04 mm/Hg [95% CI -3.86 to 1.77], $p = 0.464$) (Figure 8.1 & 8.2). Periodontal treatment did not alter hsCRP in the treatment group, however there was a non-significant reduction in the control group following the 3-month assessments (ANCOVA Δ +0.78 mg/L [-0.40 to 1.97], p=0.194).

8.2.2 Stratified analysis by sex

Stratified analysis by sex revealed no significant treatment effects for females, however diastolic blood pressure was significantly lower in males while. One year from baseline, PWV was significantly higher among males in the treatment group compared to control (ANCOVA Δ 0.31 m/s (0.01 to 0.62), p= 0.045.

8.2.3 Conclusion

In conclusion the present study shows that a single session of non-surgical periodontal therapy is insufficient to alter PWV in the short to medium term in a high-risk population. Periodontal treatment appears to reduce blood pressure among males only. These findings provide some evidence to suggest that periodontal disease may have a systemic impact beyond the oral environment. Future investigations are needed to determine whether periodontal treatment combined with regular periodontal maintenance will result in reduced arterial stiffness or a slower progression of arterial stiffening. Similarly, the effects of periodontal treatment on blood pressure require further investigation.

Appendix Table 1: Change in Anthropometric, Cardiovascular Risk Factors and Metabolic Markers – 3 Months Post-Intervention*.

	Treatment		Control			
	Baseline	**3 month**	**Baseline**	**3 month**	**ANCOVA least squares mean Δ (95% CI)**	***P* value**
Systolic BP (mmHg)	123.7 (13.9)	120.7 (15.0)	125.7 (14.8)	122.7 (15.3)	-0.43 (-4.05 to 3.18)	0.814
Diastolic BP (mmHg)	79.5 (9.9)	75.0 (8.1)	80.7 (10.4)	78.5 (8.7)	-2.85 (-5.03 to -0.68)	0.010
Brachial PP (mmHg)	44.35 (11.03)	42.23 (7.73)	44.83 (9.60)	43.63 (10.49)	-1.04 (-3.86 to 1.77)	0.464
Apolipoprotein-A1 (g/L)	1.25 (0.31)	1.17 (0.30)	1.21 (0.26)	1.18 (0.31)	-0.02 (-0.09 to 0.05)	0.597
Apolipoprotein-B (g/L)	0.98 (0.27)	0.94 (0.38)	0.92 (0.21)	0.88 (0.28)	-0.02 (-0.13 to 0.08)	0.661
Arginine/ADMA ratio	206.54 (64.95)	196.99 (78.92)	206.33 (67.80)	192.39 (55.27)	1.01 (-20.87 to 22.90)	0.927
HbA1c (mmol/mol)	48.02 (19.79)	46.57 (17.28)	44.75 (14.74)	43.42 (12.94)	0.62 (-1.59 to 2.83)	0.578

Data for means presented as mean (SD)
*Reported mean (SD) values limited to those that have completed data 3-months post-intervention.

Appendix Table 2: Change in Anthropometric, CVRFs & Metabolic Markers – 3 Months Post-Intervention Limited to Males*

	Treatment		Control			
	Baseline	**3 month**	**Baseline**	**3 month**	**ANCOVA least squares mean Δ (95% CI)**	***P* value**
PWV (m/s)	8.15 (1.30)	8.06 (1.07)	8.44 (1.19)	8.13 (1.21)	0.04 (-0.29 to 0.38)	0.793
Systolic BP (mmHg)	125.4 (12.8)	122.2 (13.7)	127.0 (12.6)	122.9 (14.7)	-0.36 (-4.02 to 3.3)	0.848
Diastolic BP (mmHg)	80.6 (11.1)	74.9 (8.9)	80.9 (9.8)	77.9 (8.9)	-2.21 (-4.72 to 0.31)	0.085
Brachial PP (mmHg)	44.56 (7.03)	44.15 (6.92)	45.80 (9.18)	44.49 (11.61)	0.19 (-3.77 to 4.16)	0.923
Total cholesterol (mmol/L)	4.83 (1.09)	4.65 (0.96)	4.82 (0.84)	4.65 (0.85)	-0.14 (-0.34 to 0.06)	0.166
nonHDL cholesterol (mmol/L)	3.96 (1.09)	3.76 (0.92)	3.90 (0.88)	3.76 (0.88)	-0.12 (-0.32 to 0.08)	0.238
HDL cholesterol (mmol/L)	0.87 (0.23)	0.89 (0.29)	0.91 (0.27)	0.88 (0.28)	-0.003 (-0.05 to 0.05)	0.922
TC/HDL ratio (mmol/L)	5.97 (2.03)	5.60 (1.72)	5.80 (2.10)	5.72 (1.91)	-0.19 (-0.58 to 0.19)	0.319
Apolipoprotein-A1 (g/L)	1.06 (0.23)	1.06 (0.29)	1.16 (0.28)	1.15 (0.26)	-0.02 (-0.10 to 0.06)	0.605
Apolipoprotein-B (g/L)	0.97 (0.27)	0.89 (0.26)	0.94 (0.24)	0.90 (0.24)	-0.04 (-0.11 to 0.03)	0.227
Interleukin-6 (pg/mL)	3.56 (2.51)	2.47 (2.27)	2.37 (2.62)	1.66 (1.91)	0.73 (-0.21 to 1.67)	0.127
hsCRP (mg/L)	3.06 (4.79)	3.50 (3.76)	3.26 (3.31)	2.72 (2.78)	1.32 (0.16 to 2.47)	0.026
ADMA (μM/L)	0.39 (0.09)	0.43 (0.13)	0.43 (0.11)	0.43 (0.08)	-0.01 (-0.05 to 0.04)	0.774
Arginine/ADMA ratio	228.07 (56.68)	232.30 (70.71)	222.75 (66.22)	208.10 (48.75)	18.87 (-7.49 to 45.23)	0.158
HbA1c (mmol/mol)	45.10 (16.43)	42.62 (12.47)	46.22 (16.94)	44.25 (14.69)	-0.13 (-2.26 to 2.01)	0.905

Data presented as mean (SD)

* Reported mean (SD) values limited to those that have completed data 3 months post-intervention.

Appendix Table 3: Change in Anthropometric, CVRFs & Metabolic Markers – 12 Months Post-Intervention Limited to Males*

	Treatment		Control			
	Baseline	**12 month**	**Baseline**	**12 month**	**ANCOVA least squares mean Δ (95% CI)**	***P* value**
PWV (m/s)	8.22 (1.26)	8.56 (0.87)	8.44 (1.93)	8.39 (1.07)	0.31 (0.01 to 0.62)	0.045
Systolic BP (mmHg)	125.9 (12.2)	123.6 (11.8)	125.3 (12.5)	124.5 (13.7)	0.41 (-4.43 to 5.24)	0.868
Diastolic BP (mmHg)	81.1 (9.8)	80.0 (9.1)	80.6 (9.3)	80.3 (9.6)	-0.50 (-2.94 to 1.94)	0.684
Brachial PP (mmHg)	43.57 (6.98)	44.02 (8.60)	44.96 (8.39)	42.10 (7.59)	2.36 (-0.97 to 5.69)	0.163
Total cholesterol (mmol/L)	4.85 (1.09)	4.87 (1.12)	4.87 (0.94)	4.66 (1.12)	0.07 (-0.16 to 0.31)	0.542
nonHDL cholesterol (mmol/L)	3.98 (1.10)	3.92 (1.12)	3.95 (0.99)	3.68 (1.17)	0.12 (-0.24 to 0.49)	0.503
TC/HDL ratio (mmol/L)	5.97 (2.04)	5.53 (1.63)	5.85 (2.24)	5.29 (1.97)	0.05 (-0.44 to 0.53)	0.847
Apolipoprotein-A1 (g/L)	1.04 (0.21)	1.15 (0.20)	1.18 (0.27)	1.24 (0.28)	-0.01 (-0.07 to 0.09)	0.865
Apolipoprotein-B (g/L)	0.96 (0.27)	0.94 (0.23)	0.95 (0.25)	0.90 (0.30)	-0.02 (-0.08 to 0.11)	0.739
Interleukin-6 (pg/mL)	3.62 (2.46)	0.88 (1.92)	1.65 (1.93)	1.57 (1.56)	-1.12 (-2.29 to 0.05)	0.059
hsCRP (mg/L)	3.26 (5.02)	3.93 (5.47)	3.42 (4.26)	3.48 (4.22)	0.37 (-1.49 to 2.23)	0.693
ADMA (μM/L)	0.39 (0.09)	0.46 (0.07)	0.43 (0.09)	0.43 (0.09)	0.04 (-0.01 to 0.09)	0.139
Arginine/ADMA ratio	222.80 (54.18)	189.46 (66.69)	219.83 (62.72)	195.90 (56.52)	-1.04 (-40.83 to 38.74)	0.958
HbA1c (mmol/mol)	46.18 (16.82)	44.71 (15.12)	46.63 (18.30)	43.04 (12.29)	1.76 (-0.80 to 4.31)	0.176

Data presented as mean (SD);
*Reported mean (SD) values limited to those that have completed data 12-months post-intervention.

Appendix Table 4: Change in Anthropometric, CVRFs & Metabolic Markers – 3 Months Post-Intervention Limited to Females*

	Treatment		Control			
	Baseline	**3 month**	**Baseline**	**3 month**	**ANCOVA least squares mean Δ (95% CI)**	***P* value**
PWV (m/s)	8.32 (1.31)	8.15 (1.09)	8.42 (1.68)	8.18 (1.17)	0.05 (-0.25 to 0.35)	0.727
Systolic BP (mmHg)	121.3 (15.2)	118.5 (16.6)	122.7 (18.9)	122.3 (17.0)	-0.64 (-5.15 to 3.87)	0.780
Diastolic BP (mmHg)	77.9 (8.0)	75.1 (7.0)	80.2 (11.8)	79.7 (8.5)	-1.10 (-4.03 to 1.83)	0.459
Brachial PP (mmHg)	44.06 (15.03)	40.13 (8.13)	42.64 (10.33)	41.95 (7.88)	-1.36 (-5.13 to 2.41)	0.473
Total cholesterol (mmol/L)	5.16 (1.14)	4.97 (0.96)	4.83 (0.97)	4.92 (1.13)	0.05 (-0.21 to 0.32)	0.689
nonHDL cholesterol (mmol/L)	3.99 (1.15)	3.85 (1.01)	3.58 (0.92)	3.72 (1.11)	-0.19 (-0.58 to 0.19)	0.321
TC/HDL ratio (mmol/L)	4.62 (1.45)	4.57 (1.53)	3.99 (1.01)	4.26 (1.30)	-0.18 (-0.44 to 0.08)	0.179
Apolipoprotein-A1 (g/L)	1.43 (0.25)	1.37 (0.23)	1.42 (0.26)	1.38 (0.25)	-0.01 (-0.13 to 0.10)	0.799
Apolipoprotein-B (g/L)	1.02 (0.27)	1.02 (0.48)	0.90 (0.24)	0.93 (0.29)	-0.01 (-0.22 to 0.17)	0.890
Interleukin-6 (pg/mL)	2.72 (2.04)	2.85 (2.16)	2.45 (2.00)	3.37 (3.43)	-0.56 (-2.22 to 1.10)	0.500
hsCRP (mg/L)	7.18 (5.26)	6.66 (5.53)	8.29 (8.40)	7.70 (6.43)	-0.69 (-3.36 to 1.98)	0.604
ADMA (μM/L)	0.46 (0.16)	0.44 (0.10)	0.44 (0.15)	0.45 (0.13)	-0.03 (-0.09 to 0.04)	0.444
Arginine/ADMA ratio	178.04 (64.95)	153.66 (66.54)	163.66 (52.47)	153.57 (52.30)	-6.64 (-42.55 to 29.27)	0.711
HbA1c (mmol/mol)	51.95 (23.24)	52.14 (21.26)	41.33 (6.68)	41.38 (6.88)	-0.02 (-2.38 to 2.34)	0.984

Data presented as mean (SD);
*Reported mean (SD) values limited to those that have completed data 3-months post-intervention.

Appendix Table 5: Change in Anthropometric, CVRFs & Metabolic Markers – 12 Months Post-Intervention Limited to Females*

	Treatment		Control			
	Baseline	**12 month**	**Baseline**	**12 month**	**ANCOVA least squares mean Δ (95% CI)**	***P* value**
PWV (m/s)	8.34 (1.37)	8.29 (0.99)	8.27 (1.52)	8.21 (0.96)	0.09 (-0.22 to 0.40)	0.570
Systolic BP (mmHg)	124.6 (23.7)	119.0 (17.9)	124.1 (19.2)	120.4 (16.0)	-1.39 (-5.58 to 2.80)	0.513
Diastolic BP (mmHg)	79.5 (12.1)	75.5 (10.7)	79.8 (11.3)	79.3 (11.9)	-1.97 (-5.08 to 1.15)	0.213
Brachial PP (mmHg)	44.92 (15.00)	39.92 (8.88)	42.45 (10.27)	40.53 (9.25)	-1.77 (-5.36 to 1.82)	0.328
Total cholesterol (mmol/L)	5.19 (1.09)	5.18 (1.08)	5.31 (1.35)	4.98 (1.03)	0.01 (-0.36 to 0.38)	0.969
nonHDL cholesterol (mmol/L)	3.98 (1.15)	4.00 (1.10)	3.99 (1.35)	3.67 (0.95)	0.33 (-0.09 to 0.76)	0.125
HDL cholesterol (mmol/L)	1.22 (0.34)	1.17 (0.32)	1.33 (0.30)	1.31 (0.52)	0.003 (-0.11 to 0.11)	0.959
TC/HDL ratio (mmol/L)	4.56 (1.57)	4.71 (1.63)	4.19 (1.43)	4.15 (1.42)	-0.18 (-0.44 to 0.08)	0.179
Apolipoprotein-A1 (g/L)	1.46 (0.26)	1.38 (0.26)	1.45 (0.25)	1.54 (0.41)	-0.13 (-0.29 to 0.03)	0.103
Apolipoprotein-B (g/L)	1.00 (0.26)	1.00 (0.25)	0.98 (0.34)	0.92 (0.24)	0.07 (-0.03 to 0.17)	0.156
Interleukin-6 (pg/mL)	2.76 (1.94)	2.62 (2.88)	2.66 (2.12)	2.94 (2.82)	0.11 (-1.69 to 1.92)	0.899
hsCRP (mg/L)	6.16 (5.44)	6.71 (7.07)	7.19 (7.98)	5.33 (4.43)	1.35 (-1.14 to 3.85)	0.282
ADMA (μM/L)	0.46 (0.14)	0.52 (0.15)	0.45 (0.14)	0.44 (0.12)	0.06 (-0.03 to 0.16)	0.199
Arginine/ADMA ratio	187.46 (65.49)	174.72 (52.93)	151.25 (59.46)	180.62 (61.35)	-28.93 (-70.23 to 12.38)	0.163
HbA1c (mmol/mol)	50.22 (22.30)	49.41 (236.43)	42.36 (8.22)	43.51 (117.96)	-0.59 (-4.04 to 2.86)	0.735

Data presented as mean (SD);
* Reported mean (SD) values limited to those that have completed data 12-months post-intervention.

Appendix Table 6: Multiple Imputation of Blood Pressure (3 & 12-months).

	Treatment		Control		ANCOVA least squares mean Δ (95% CI)	*P* value
	Baseline	**3 month**	**Baseline**	**3 month**		
Systolic BP (mmHg)	124.30 (16.76)	121.78 (16.50)	125.47 (16.48)	123.09 (16.48)	-0.47 (-3.22 to 2.27)	0.741
Diastolic BP (mmHg)	80.16 (10.38)	76.96 (9.54)	80.19 (10.16)	78.67 (9.61)	-1.69 (-3.53 to 0.15)	0.076
	Baseline	**12 month**	**Baseline**	**12 month**		
Systolic BP (mmHg)	124.30 (16.76)	121.64 (14.58)	125.47 (16.48)	123.59 (16.22)	-1.21 (-3.90 to 1.49)	0.389
Diastolic BP (mmHg)	80.16 (10.38)	78.15 (9.39)	80.19 (10.16)	79.47 (10.05)	-0.81 (-3.16 to 0.55)	0.175

Data for baseline/follow-up means presented as mean (SD). Least squares means, 95% CI and p-value is mean of 5 MI iterations.

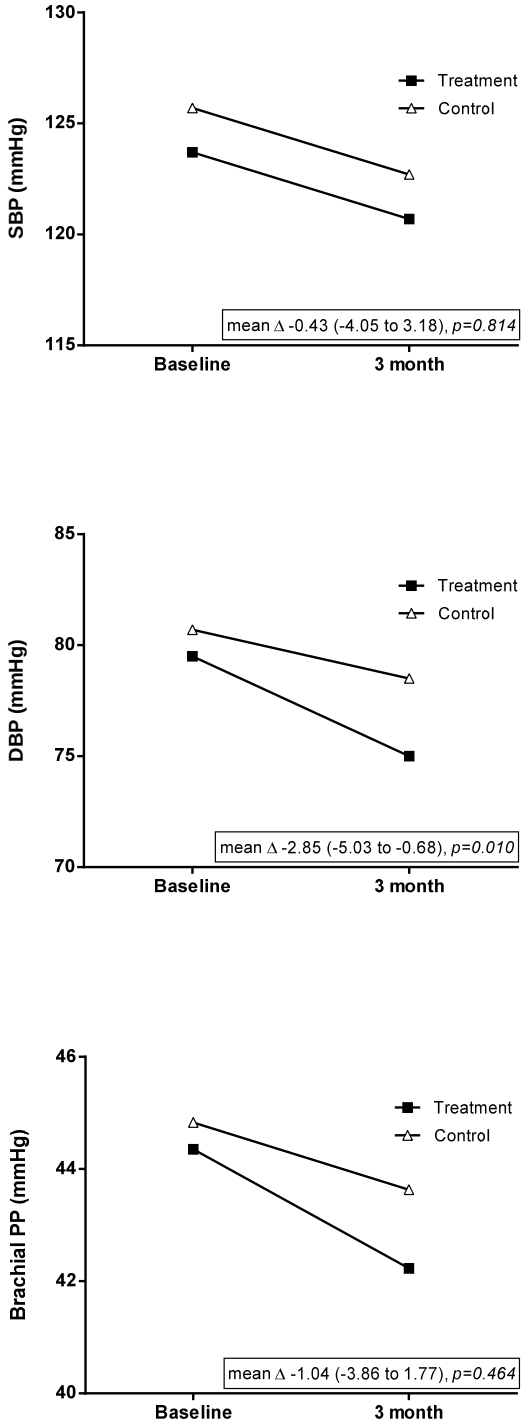

Figure 8.1: Change in PWV and blood pressure (baseline to 3-month).

Reported values in box refer to differences in within-group changes (baseline to 3-month) (95% CI). Negative values denote greater difference in treatment group.

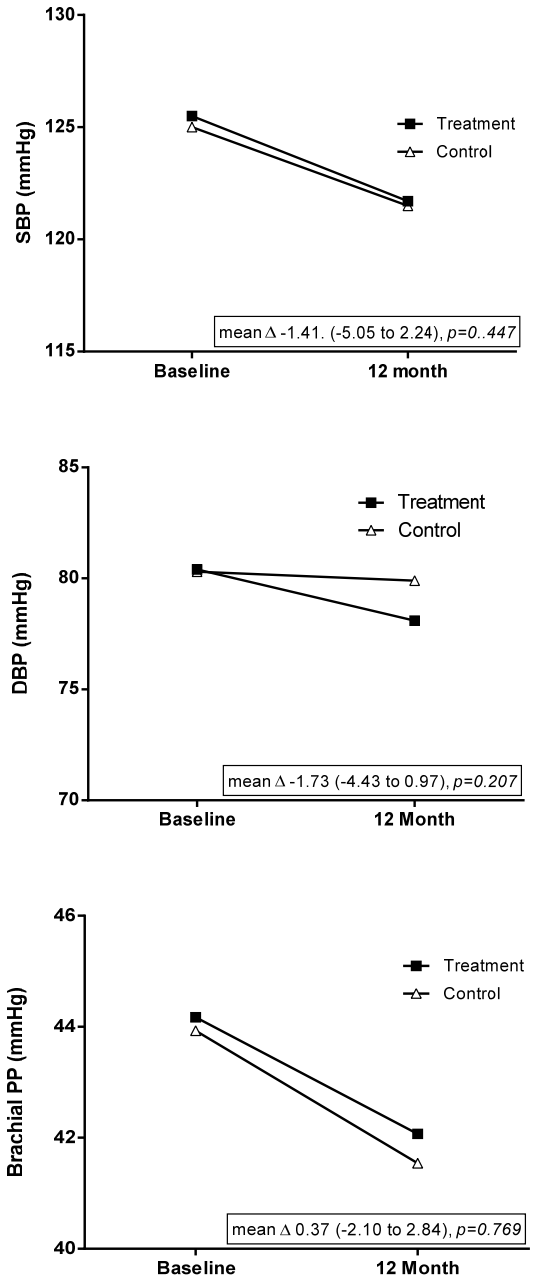

Figure 8.2: Change in PWV and blood pressure (baseline to 12-month).

Reported values in box refer to differences in within-group changes (baseline to 12-month) (95% CI). Negative values denote greater difference in treatment group.