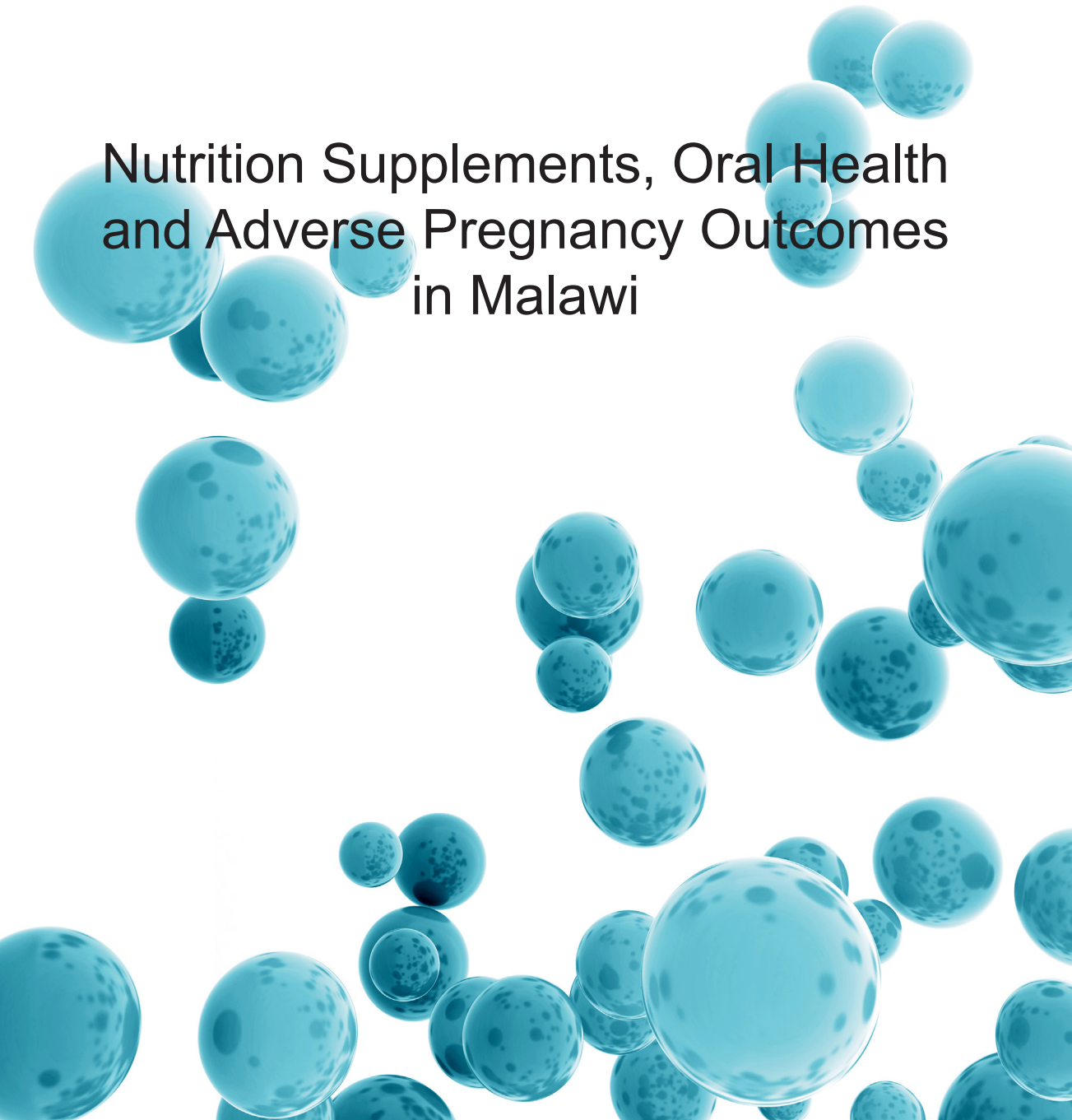
Acta Universitatis Tamperensis 2413

ULLA HARJUNMAA

Nutrition Supplements, Oral Health and Adverse Pregnancy Outcomes in Malawi



ULLA HARJUNMAA

Nutrition Supplements, Oral Health and Adverse Pregnancy Outcomes in Malawi

■

ACADEMIC DISSERTATION
To be presented, with the permission of
the Faculty Council of the Faculty of Medicine and Life Sciences
of the University of Tampere,
for public discussion in the Yellow Hall F025
of the Arvo building, Arvo Ylpön katu 34, Tampere,
on 28 September 2018, at 12 o'clock.

UNIVERSITY OF TAMPERE

ULLA HARJUNMAA

Nutrition Supplements, Oral Health and Adverse Pregnancy Outcomes in Malawi

Acta Universitatis Tamperensis 2413
Tampere University Press
Tampere 2018

UNIVERSITY
OF TAMPERE

ACADEMIC DISSERTATION
University of Tampere, Faculty of Medicine and Life Sciences
Center for Child Health Research
Finland

Supervised by
Professor Per Ashorn
University of Tampere
Finland

Reviewed by
Professor Jukka Meurman
University of Helsinki
Finland
Docent Tuula Pelkonen
University of Helsinki
Finland

The originality of this thesis has been checked using the Turnitin OriginalityCheck service in accordance with the quality management system of the University of Tampere.

Cover design by
Mikko Reinikka

Acta Universitatis Tamperensis 2413
ISBN 978-952-03-0834-6 (print)
ISSN-L 1455-1616
ISSN 1455-1616

Acta Electronica Universitatis Tamperensis 1923
ISBN 978-952-03-0835-3 (pdf)
ISSN 1456-954X
http://tampub.uta.fi

Suomen Yliopistopaino Oy – Juvenes Print
Tampere 2018

Abstract

Every year, 32 million infants are born preterm (PTB) or with intrauterine growth restriction (IUGR), predisposing them to a high risk of mortality and morbidity. Most of these infants are born in low-income countries. Maternal malnutrition and infections are among the most important interlinked risk factors for adverse pregnancy outcomes. Therefore, nutritional supplementation with multiple micronutrients during pregnancy is increasingly recommended in low-resource settings. Periodontitis, i.e. infection in the tooth-supporting tissues, has been extensively investigated as a risk for poor pregnancy outcomes, but the role of dental caries and its sequel, periapical infection (PAI), is almost unknown. As with periodontitis, the pathway between PAI and adverse pregnancy outcomes is biologically plausible.

The aim of this thesis was to investigate the interlinkage between nutrition supplementation, oral diseases and pregnancy outcomes in a rural Malawian population. The thesis was based on three studies: Study I assessed whether supplementation with multiple micronutrients (MMN) or lipid-based nutrient supplement (LNS) influence dental caries development or periodontal health when compared with commonly used iron and folic-acid supplementation (IFA). Study II investigated the associations between maternal caries, PAI or periodontitis and pregnancy duration and birth size of the infant. Study III explored two possible causal mechanisms underlying the association between PAI and adverse pregnancy outcomes detected in Study II: spread of periapical bacteria to the fetoplacental unit (direct pathway) and systemic inflammation (indirect pathway).

For the study, 1391 pregnant women were enrolled before their twentieth gestational week. They were provided with a daily capsule of IFA or MMN or a sachet of LNS containing proteins, carbohydrates, essential fatty acids and 21 micronutrients. Maternal blood and salivary samples were collected at enrollment and at 36 gestational weeks, and placental tissue after delivery. Infant weight, length and head circumference were measured after birth. Of the enrolled participants, 1024 underwent clinical and radiological oral health examination within six weeks after delivery and were included in the analysis.

When comparing the nutrition supplement groups, the prevalence of caries was significantly higher in the MMN and LNS groups than in the IFA group (69.1%, 63.3% and 56.7% respectively; P = 0.004). When assessing the birth outcomes by infection group, the mean (95% CI) pregnancy duration was 0.4 weeks (0.1–0.8) shorter and infants' mean birth weight 79 g (13–145) lower and length 0.5 cm (0.2–0.9) shorter in women who had PAI compared to those without. PTB incidence was 3.5% (95%; CI 1.1–8.1%) and prevalence of stunting 9.0% higher (95% CI 2.7–15.2%) in women who had PAI. No significant differences in the periodontal disease parameters were seen between supplementation groups or in birth outcomes between groups of women with or without periodontitis. PAI was associated with c-reactive protein (CRP), alpha-1-acid glycoprotein (AGP) and cortisol concentrations in a dose-dependent manner at 36 gestation weeks. When CRP, AGP or cortisol concentration was added into regression models, the association between PAI and duration of pregnancy or infant birth weight and length was attenuated, suggesting that AGP, CRP and cortisol were on the causal pathway between PAI and birth outcomes.

In conclusion, maternal diet supplementation with multiple micronutrients during pregnancy in low-resource environments in which malnutrition and oral diseases are common may enhance caries development. PAI, the common sequel of caries, elevates systemic inflammation, which can lead to PTB and IUGR. This study thus suggests that PAI is a common, previously unidentified and relatively easily modifiable risk factor for adverse pregnancy outcomes. Caries prevention should be considered, especially when planning nutrient intervention programs for pregnant women in low-resource environments.

Tiivistelmä

Vuosittain 32 miljoonaa lasta syntyy ennenaikaisesti tai liian pieninä raskausviikkoihin nähden, pääasiassa matalan tulotason maissa. Näillä lapsilla on suuresti kohonnut riski sekä kuolemalle että pitkäaikaiselle sairastavuudelle. Äidin aliravitsemus ja infektiosairaudet ovat eräitä tärkeimpiä, tunnistettuja riskitekijöitä, jotka myös altistavat toinen toiselleen. Monipuolista lisäravinteiden antamista raskauden aikana suositellaankin enenevässä määrin etenkin matalan tulotason maissa. Hampaiden kiinnityskudossairautta parodontiittia on tutkittu runsaasti ennenaikaisen synnytyksen riskitekijänä mutta hammaskarieksen ja sen seurannaissairauden, periapikaali-infektion rooli on lähes tuntematon. Kuten parodontiitin, periapikaali-infektion ja raskauskomplikaatioiden yhteys on biologisesti mahdollinen.

Tämän väitöskirjatyön tavoitteena oli tutkia raskaudenaikaisen rasvitsemuslisän, suusairauksien ja raskauskomplikaatioiden välisiä yhteyksiä Malawin maaseudulla, eteläisessä Afrikassa. Väitöskirjatyö perustuu kolmeen osatutkimukseen. Ensimmäisessä osatutkimuksessa arvioitiin sekä monipuolisen hivenaine- ja monivitamiinilisän (MMN) että maapähkinäpohjaisen lisäravinnon (LNS) vaikutusta karieksen ja parodontaalisairauksien esiintyvyyteen verrattuna yleisesti käytettyyn rauta- ja foolihappo-lisään (IFA). Toisessa osatutkimuksessa selvitettiin äidin suusairauksien yhteyttä raskauden kestoon ja lapsen syntymäkokoon. Kolmas osatutkimus selvitti toisessa osatutkimuksessa havaitun periapikaali-infektion ja lyhentyneen raskauden keston sekä lapsen pienen syntymäkoon välisen yhteyden taustalla vaikuttavia syy-seuraussuhteita ja kahta mahdollista mekanismia, hammasperäisten bakteerien aiheuttamaa kohdunsisäistä inflammaatiota (suora mekanismi) sekä systeemistä inflammaatiota (epäsuora mekanismi).

Tutkimukseen rekrytoitiin 1391 raskaana olevaa naista ennen 20 raskausviikkoa. Heidät satunnaistettiin kolmeen ryhmään, joissa tutkittavat saivat päivittäin IFA kapselin, MMN kapselin tai LNS annoksen, joka sisälsi proteiinia, hiilihydraatteja, välttämättömiä rasvahappoja sekä 21 hivenainetta. Äidiltä kerättiin veri- ja sylkinäytteet ennen 20 raskausviikkoa sekä 36 raskausviikolla. Synnytyksen jälkeen kerättiin istukka ja sikiökalvonäytteet sekä mitattiin lapsen paino, pituus ja pään

ympärys. Analyyseihin sisällytettiin 1024 naista, jotka osallistuivat kliiniseen sekä radiologiseen suuterveystutkimukseen kuuden viikon sisällä synnytyksestä.

Karieksen esiintyvyys oli tilastollisesti merkitsevästi korkeampi MMN (69,1 %) ja LNS (63,3 %) ryhmissä verrattuna IFA ryhmään (56,7 %, P = 0.004). Keskimääräinen raskauden kesto (95 % luottamusväli) oli 0,4 viikkoa (0,1–0,8) lyhyempi, lapsen syntymäpaino 79 g (13–145) alhaisempi ja pituus 0,5 cm (0,2–0,9) lyhyempi naisilla, joilla oli periapikaali-infektio kuin naisilla, joilla ei ollut infektiota. Ennenaikaisuuden ilmaantuvuus oli 3,5 % (95 % luottamusväli 1,1–8,1 %) ja lyhytkasvuisuuden 9,0 % (95 % luottamusväli 2,7–15,2 %) korkeampi periapikaali-infektioryhmässä. Lisäravinnon saannin ja parodontaalisairauksien tai parodontiitin ja syntymätulosten välillä ei havaittu yhteyttä. Periapikaali-infektioilla oli annos-vasteyhteys c-reaktiivisen proteiinin (CRP), hapan alpha-1-glykoproteiinin (AGP)- ja kortisolipitoisuuksien kanssa 36 raskausviikolla. CRP:n, AGP:n tai kortisolin lisääminen regressiomalleihin heikensi periapikaali-infektion ja raskauden keston sekä lapsen painon ja pituuden välistä yhteyttä. Nämä tulokset viittaavat siihen että periapikaali-infektioiden ja raskaustulosten yhteys välittyy osin CRP:n, AGP:n ja kortisolin kautta.

Loppupäätelmänä totean että hivenaine- ja monivitamiinilisän antaminen raskaana oleville naisille ympäristössä, jossa sekä aliravitsemus että suusairaudet ovat yleisiä saattaa lisätä karieksen esiintymistä tai etenemistä. Hoitamattoman karieksen aiheuttama periapikaali-infektio puolestaan aiheuttaa systeemisen inflammaation, joka voi johtaa ennenaikaiseen syntymään ja kohdunsisäiseen kasvuviiveeseen. Tämän tutkimuksen tulokset viittaavat siis siihen, että periapikaali-infektio on yleinen, aiemmin tunnistamaton ja suhteellisen helposti hoidettava raskauskomplikaatioiden riskitekijä. Karieksen ehkäisy tulisi ottaa huomioon erityisesti suunniteltaessa ravitsemusinterventio-ohjelmia raskaana oleville naisille matalan tulotason maissa.

Contents

Abbreviations

AGP	Alpha-1-acid glycoprotein
CI	Confidence interval
CRP	C-reactive protein
DALY	Disability-adjusted life years
gw	Gestational weeks
HAZ	Height-for-age z-score
Hb	Hemoglobin
HIV	Human immune-deficiency virus
IFA	Iron and folic-acid supplement
IGF	Insulin-like growth factor
IL	Interleukin
iLiNS-DYAD	A study of the International Lipid-Based Nutrient Supplement Project: Supplementing maternal and infant diet with high-energy, micronutrient fortified lipid-based nutrient supplements
IUGR	Intrauterine growth restriction
LAZ	Length-for-age z-score
LBW	Low birth weight
LNS	Lipid-based nutrient supplement
LPS	Lipopolysacharide
MMN(s)	Multiple micronutrient(s)
MMP	Matrix metalloproteinase
MUAC	Mid-upper arm circumference
PAI	Periapical infection
PGE_2	Prostaglandin E_2
PMNs	Polymorphonuclear leukocytes
PTB	Preterm birth
SD	Standard deviation
TNF-α	Tumor necrosis factor alpha
WAZ	Weight-for-age z-score
WHO	World Health Organization

WHZ	Weight-for-height z-score
WLZ	Weight-for-length z-score

List of Original Publications

The thesis is based on the following original articles, referred to in the text by the Roman numerals I-III.

I Harjunmaa U, Järnstedt J, Dewey KG, Ashorn U, Maleta K, Vosti SA, Ashorn P. Nutrient supplementation may adversely affect maternal oral health – a randomised controlled trial in rural Malawi. Maternal and Child Nutrition 2015;12:99-110. doi: 10.1111/mcn.12204

II Harjunmaa U, Järnstedt J, Alho L, Dewey KG, Cheung YB, Deitchler M, Ashorn U, Maleta K, Klein NJ, Ashorn P. Association between maternal dental periapical infections and pregnancy outcomes: results from a cross-sectional study in Malawi. Tropical Medicine and International Health 2015; 20(11): 1549-1558. doi 10.1111/tmi.12579

III Harjunmaa U, Doyle R, Järnstedt J, Kamiza S, Stewart CP, Jörgensen J, Shawn L, Alho L, Ashorn U, Klein N, Dewey KG, Maleta K, Ashorn P. Periapical infection may affect birth outcomes via systemic inflammation. Oral Diseases 2018;00:1–9. doi: 10.1111/odi.12817

1 Introduction

Preterm birth (PTB) and intrauterine growth restriction (IUGR) compromise the survival of 32 million newborns every year (1). In addition to high mortality, infants born too early or too small have a high risk of serious short- and long-term morbidity, including vulnerability for infections, neurodevelopmental disorders, reduced cognitive ability and growth failure, and even socio-economic problems in adulthood (2,3). Most of these infants are born in low-income countries where resources for healthcare are generally scarce, and the possibility of meeting the special needs of these children is almost nonexistent (1). In addition to the resultant hardship for the children and their families, the economic consequences of PTB and IUGR are huge due to long-term needs for medical care, special education and other support, imposing a significant public health burden upon all countries in the world (4).

PTB and IUGR are consequences of a complex interplay of physiological, behavioral, environmental, psycho-social and genetic risk factors. Although many of the risk factors have been identified and targeted, the interventions applied have not been successful in reducing the incidence of the poor birth outcomes noticeably. This implies that some of the risk factors and underlying mechanisms leading to PTB or IUGR are yet not known or fully understood. (5)

Two major and well-known risk factors for PTB and IUGR are poor maternal nutritional status and maternal infections, which are also interlinked, one predisposing to the other (5). Because of the known importance of maternal nutrition to the development of the fetus, supplementation of pregnant women's diet with iron and folic acid (IFA) has long been recommended all over the world (6). Recently, even more comprehensive diet supplementation with multiple micronutrients (MMNs) – preferably combined with protein, essential fatty acids and other macronutrients – is increasingly utilized in many low-resource environments for its better impact on maternal mortality reduction and improved birth outcomes (7-9). Nutrient interventions may, however, have also other, unknown health effects, for example in the oral cavity.

Oral health and disease pathology are closely related to nutrition, which affects the oral microflora, saliva flow and composition, and local immunological reactions

(10-12). The effects may be even more pronounced in pregnancy due to the impaired immune responses (13). Therefore, nutrition interventions may have considerable effects, also on oral health and disease development. Oral diseases may in turn have an impact on pregnancy, especially in resource-poor environments due to their high prevalence (14,15), combined with lack of preventive methods and treatment options (16,17). Of the oral diseases, periodontitis – infection in the tooth-supporting tissues – has been associated with PTB and IUGR in several studies, although contradictory results also exist (18). Nevertheless, studies on dental caries and its sequel periapical infection (PAI) are almost non-existent (19), although caries is among the most common chronic infectious diseases globally (15), and the pathway between PAI and adverse pregnancy outcomes is biologically plausible. It is thus possible that caries and PAI form previously unidentified, modifiable risk factors for adverse pregnancy outcomes.

The present study was designed to evaluate the role of dental caries, periapical infection, and periodontal disease in adverse pregnancy outcomes and to explore possible underlying mechanisms linking these conditions in low-resource environments. In addition, the study assessed the effect of multiple micronutrient invention on the oral health of the pregnant women.

2 Review of the Literature

2.1 Approach to the Literature Review

The purpose of the literature review is to provide background information on the research presented in this thesis. The review includes a description of the oral diseases studied and the adverse pregnancy outcomes, in addition to their global epidemiology and risk factors. It also discusses the effects of pregnancy on oral health, along with the possible interrelations and underlying mechanisms linking oral infections and pregnancy outcomes.

The literature was searched electronically using mainly Medline Ovid and PubMed interfaces, and to a lesser extent Google Scholar. The key words used were periodontitis, caries, periapical infection, apical periodontitis, nutrient supplement, nutrient intervention, micronutrient, pregnancy duration, preterm birth, low birth weight, infant length, stunting, and head circumference. The reference lists of relevant literature were then further reviewed to identify additional literature. The last searches were done in March 2018. When discussing other studies in this field, the literature review was limited to those published before 2015, when the first articles included in this thesis were published. More recent findings are included in the discussion chapter.

2.2 Etiology, Epidemiology, and Treatment of Dental Caries, Periapical Infection, and Periodontal Diseases

2.2.1 Structure of Teeth and Supporting Tissues

Adult dentition comprises 32 teeth that are embedded in the tooth-supporting tissues, the periodontium. The crown of the tooth consists of three layers: enamel, dentine, and the pulp that extends to the dental roots and contains nerves and blood vessels. The pulp is connected to the rest of the body through the apical foramen at

the apex (tip) of the roots. The periodontium comprises gingiva (gums), alveolar bone that surrounds the roots, and periodontal ligaments that attach the root cementum to the alveolar bone. The dental crown is surrounded by free gingiva leaving a 1-3 mm deep "pocket", sulcus, between the tooth and the gingiva. At the bottom of the sulcus, the junctional epithelium attaches to the enamel at a distance of 1 mm. This junctional epithelium layer forms the main barrier between the oral cavity and the rest of the body. (20)

2.2.2 Oral Microbiota and Oral Cavity Defense Mechanisms

The oral cavity has a rich and diverse microbiome that comprises more than 600 identified bacterial species (21) and other microorganisms such as viruses, fungi, protozoa and archaea in smaller amounts (22). One oral cavity may harbor 100 to 200 different bacterial species at a time with great variation within and between individuals and over a lifetime (22). However, the richness of the oral microbiome may even be much greater: new high-throughput sequencing techniques have suggested that the microbiome may be made up of more than 19,000 phylotypes (23). Because of this diversity, it is impossible to define a core microbiome that is associated with oral health.

All intra-oral surfaces are covered with a biofilm formed by microbes embedded in the extracellular polymeric matrix. The composition of this biofilm varies between healthy and diseased oral sites, even within one oral cavity. (24) The bacteria in the biofilm are highly organized and live in symbiosis with the host. They interact with each other in a synergistic or antagonistic manner, for example by providing nutrients, communicating chemically and exchanging genetic information. The oral biofilm is generally non-pathogenic, i.e. it does not cause disease in the host. (25) However, the biofilm is also essential in the development of oral infections. The balance in the biofilm may be disturbed, e.g. by poor oral hygiene, too-frequent carbohydrate intake, weakened immune responses or lack of saliva, which favor the growth of pathogenic bacteria leading to disease development (26).

The oral cavity is the main entry point for bacteria in the human body. The host defense – comprising physical barriers, e.g. enamel on teeth and the mucosal epithelium – and the host response, which includes innate and adaptive immunity, prevent the microbes from entering the body. The innate host response is a local, immediate and non-specific response to microbial challenge, whereas adaptive immunity is an acquired response to specific microbes. In addition, the "healthy"

biofilm prevents non-oral micro-organisms from colonizing the oral cavity and restricts the growth of oral disease-associated microbes. (25)

2.2.3 Dental Caries

The anatomy of a tooth with dental caries and periapical infection is presented in Figure1.

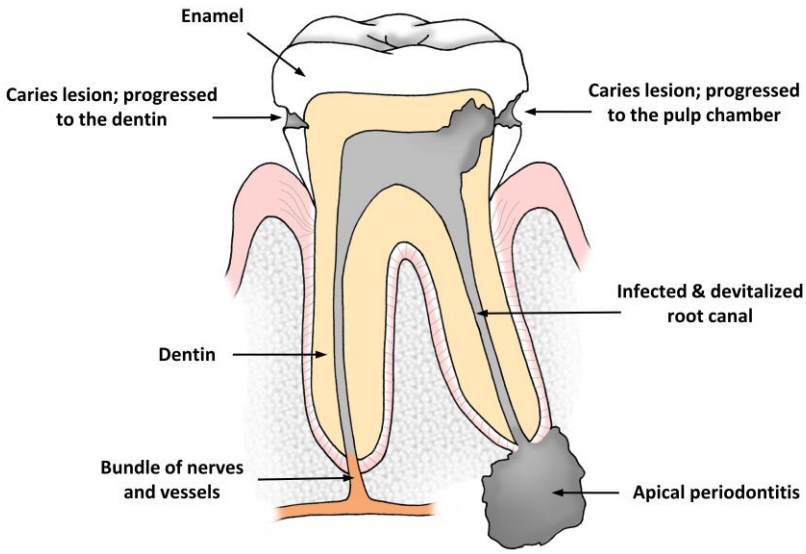


Figure 1. Anatomy of a tooth with dental caries and periapical infection (apical periodontitis) (27). Reprinted with permission from J. Liljestrand.

Caries is a contagious chronic disease that destroys the hard tissues of teeth. It is usually transmitted from parents to young children when their first teeth have erupted (28). Caries is a multifactorial disease whose development entails three prevailing factors: host (teeth and saliva), microflora and favorable diet (29). If dietary sugars are frequently present, the biofilm becomes cariogenic and has an overrepresentation of saccharolytic, acidogenic (acid-producing) and aciduric (acid-tolerant) species. The two major groups of bacteria traditionally associated with caries development are the lactobacilli and streptococci, especially *Streptococcus mutans* and *Streptococcus sorbinus* (24). However, the current understanding is that bacterial complexes rather than individual species are responsible for caries development (24) and many other bacteria are involved, e.g. some strains of non-mutans streptococci,

actinomyces, bifidobacteria and yeasts (23). The acidogenic bacteria metabolize the dietary carbohydrates and excrete acids that dissolve calcium, phosphate and carbonate from the tooth surfaces. Saliva buffers the acids, prevents demineralization and facilitates remineralization with its "supersaturated" calcium and phosphates, and also has antibacterial effects. If the periods of acidity are too frequent, the extent of demineralization exceeds that of remineralization, and a cavity begins to develop. The process is usually slow and takes anywhere from several months to years. (30-32)

Caries development can be restricted and even reversed to some extent by limiting the use of dietary sugars, by proper removal of the biofilm (dental plaque), and through adequate use of fluoride, which hardens the tooth surface and makes it resilient to acids (30-32). Caries causes only mild symptoms until it reaches and infects the pulp. Pulpitis is extremely painful until the pulp becomes necrotic and the pain eases. An untreated caries will progress until the tooth is completely destroyed. Caries is usually diagnosed by visual inspection combined with radiographs (33). The cavity can be treated by professional removal of the carious tissue and replacement with a filling. If the cavity reaches the dental pulp, either a root canal treatment or tooth extraction must be performed (33). The key protective and pathological factors that determine whether or not caries develop are presented in Figure 2.

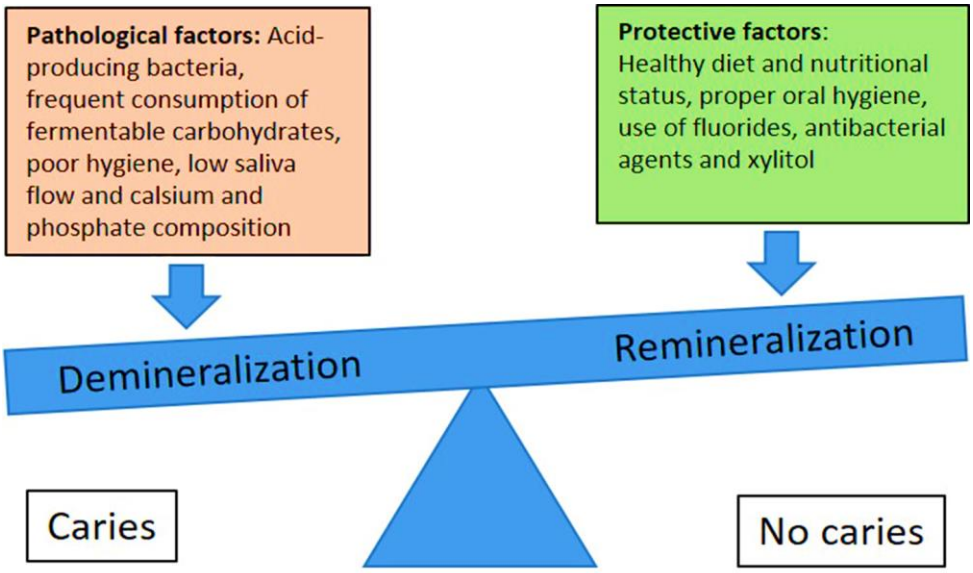


Figure 2. The caries balance. The key factors that influence the development of caries. Modified from Featherstone et al. (34).

2.2.4 Periapical Infection

Periapical infection (PAI), called also apical periodontitis, is an inflammatory response of the periapical tissues to endodontic infection, i.e. infection in the dental pulp and root canals (35). Healthy and intact pulp is sterile. A deep caries lesion or crack in the tooth allows commensal oral bacteria to enter the pulp chamber, after which a polymicrobial community that thrives in the endodontic environment establishes itself (36). The host defense in the apex surrounding tissues is not capable of eliminating the infection from the root canal, but only forming a barrier between the bacteria and the host tissues that manifests as a periapical lesion (37). The reaction is two-faceted: inflammatory cells accumulating in the periapical lesion prevent the bacterial invasion, but they also destroy the tissue, resulting in bone abrasion (38). The progression of a periapical lesion is characterized by long inactive and spontaneous active periods. During the acute phase, macrophages appear at the infection site and produce mediators, of which pro-inflammatory cytokines interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), as well as chemotactic cytokine interleukin-8 (IL-8), are among the most important. The cytokines intensify local immune responses and bone and extracellular matrix degradation and may also be disseminated systematically (39). The dominant defense cells in active PAI lesions are polymorphonuclear leukocytes (PMNs). PMNs are an important source of prostaglandins, hormones that regulate pregnancy and have physiological effects even at very low concentrations (40). During the inactive times, activated T-cells produce cytokines that downregulate the pro-inflammatory cytokines, which in turn suppresses the osteoclastic activity and the bone destruction (38).

More than 400 bacterial species have been detected in infected root canals, 10 to 20 of which are usually found in one individual tooth. PAIs are dominated by Gram-negative and -positive anaerobic bacteria in mixed communities. The bacteria frequently found in infected root canals include *Fusobacterium, Dialister, Porphyromonas, Prevotella, Tannerella, Treponema, Campylobacter, Veillonella, Parvimonas, Filifactor, Pseudoramibacter, Olsenella, Actinomyces, Peptostreptococcus, Streptococcus, Propionibacterium,* and *Eubacterium* species (41). The endodontic bacteria interact synergistically with each other, have the ability to resist the host defense, produce lipopolysaccharides (LPSs) and other bacterial modulins, and synthetize enzymes that can damage the host tissues and advance the spread of the organisms (19). LPSs are released from the cell walls of Gram-negative bacteria during their multiplication and death. They

play important role in the formation of periapical lesions by activating macrophages to produce molecular mediators such as TNF-α ILs. (42)

A tooth with PAI is sometimes painful to bite with, and occasionally swelling and pus develops in the infected apical area (43). In rare cases, the infection may spread through the fascial spaces, leading to a life-threatening generalized infection (19). However, most often a necrotic tooth with PAI is asymptomatic, and the patient is thus not aware of the infection. The diagnosis is typically based on radiographic findings, in which the infection manifests as radiolucent areas surrounding the root tip (44). Development of a periapical lesion is relatively slow (44), but the actual time frame from initiation of an infection and appearance of a detectable lesion is not established in humans (45). PAI is not self-healing, nor can it be eliminated with medication. Adequate treatment of PAI includes chemo-mechanical cleaning and disinfection of the root canals followed by obturation (placing root fillings) or by extraction of the tooth. In cases of severe infection, antibiotics may be needed (43).

2.2.5 Periodontal Diseases

The most common periodontal diseases are gingivitis and periodontitis. Gingivitis is a non-destructive inflammation in the soft tissues of the periodontium (gums), and it is reversible if proper oral hygiene is practiced. Periodontitis is a chronic infection in the periodontium, where a complex interplay of bacterial infection and host response causes destruction in the tooth connective tissues and the alveolar bone. (46) Gingivitis does not always progress to periodontitis, but periodontitis is always preceded by gingivitis (47). Periodontitis has a progressive nature, although the progression rate varies between patients (48). Figure 3 illustrates healthy periodontal tissues on the left side of the tooth and periodontitis on the right.

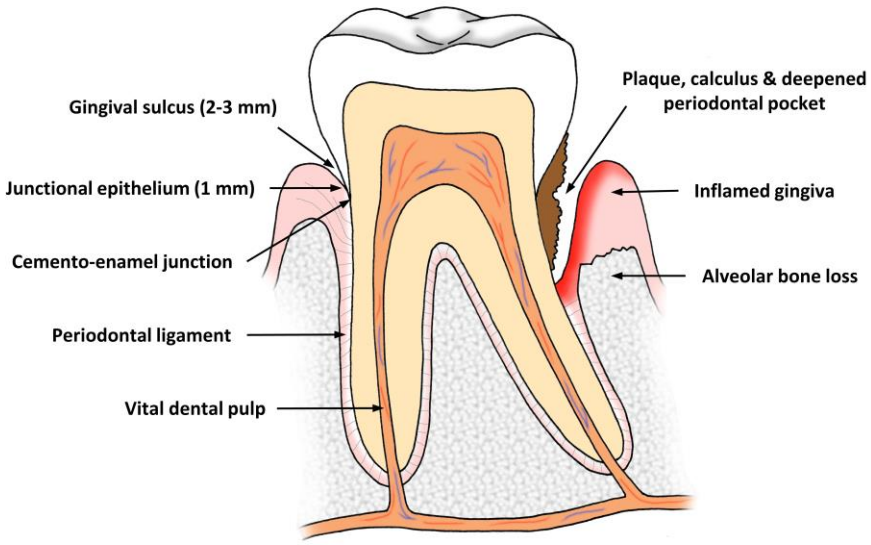


Figure 3. Anatomy of a tooth and periodontal tissues, healthy periodontium on the left and periodontitis on the right (27). Reprinted with permission from J.Liljestrand.

Periodontal diseases are caused by vast variety of pathogens that are, in small amounts, also present in a healthy mouth (49). When periodontitis starts developing, the entire microbial population structure and function in subgingival plaque changes (50). The pathogenic bacteria are usually present in clusters of proteolytic (capable of degrading proteins) and obligately anaerobic and anaerobic species that can live in the deepened gingival pockets (51). LPSs and other virulence factors of the pathogens cause a local inflammatory reaction that activates the innate immunity system. As part of the immune response, pro-inflammatory cytokines such as TNF-α, ILs and prostaglandin E_2 (PGE_2) are produced. (52) This immune response is normally protective, but, in the presence of a constant microbial challenge combined with other risk factors, it leads to hypo- or hyper-responsive defense mechanisms and destruction of the periodontal tissues (53,54). Periodontal bacteria are present in complexes, some of which are associated with disease and some with health. The so-called red complex is most strongly associated with the disease and comprises *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia.* The orange complex, which is also highly pathogenic, consists of *Prevotella intermedia, Prevotella nigrescens, Parvimonas micra* and *Fusobacterium nucleatum* (55). *P. gingivalis*, although usually present only in small numbers, is a key pathogen which is hypothesized to be capable of remodeling a healthy microbiota to a dysbiotic one (56).

The inflamed gingiva is red, swollen and bleeds easily. However, periodontitis as such is usually asymptomatic until its advanced stage, when it causes tooth mobility, an impaired ability to chew, and eventually tooth loss (57). The disease prevalence and progression varies according to teeth and tooth sites: diagnosis requires a thorough clinical and radiological assessment (58). Periodontal diseases can be prevented by targeting and controlling their causal and risk factors, especially oral hygiene (59). Periodontitis is treated by the mechanical removal of calculus and granulation tissue from the deepened periodontal pockets (60). Local antibiotics and systemic low-dose doxycycline are used infrequently (61).

2.2.6 Risk Factors for Dental Caries, Periapical Infection and Periodontal Diseases

The most important risk factors for dental caries and periodontal diseases are presented in Table 1. Caries and periodontal diseases share many risk factors, such as diet and tobacco and alcohol use, which are also risk factors for many other chronic diseases (17,62). Some individuals are more prone to caries; gingivitis progresses to periodontitis in some but not in everyone, which underlines the multifactorial nature of the diseases. Behavioral and environmental factors, genetics and the function of the host defense mechanisms determine a person's susceptibility to these diseases and their progression (63,64) In resource-poor environments, a complex web of interrelationships prevails between oral diseases, poor sanitation and hygiene, poverty, malnutrition, impaired immune functions and many inflammatory diseases (65).

Table 1. Risk factors for dental caries (DC), periapical infection (PAI) and periodontal diseases (PD); "x" indicates a risk factor relating to the disease.

Risk Factor	DC	PAI	PD	Mechanisms	Ref.
Poor oral hygiene	x	x	x	Accumulating plaque prerequisite for the diseases. Differs among populations and genders.	(66,67)
Low fluoride consumption	x	x		Fluoride strengthens the enamel structure and enhances the remineralization process.	(12,68)
Diet and food consumption habits	x	x	x	Too-frequent consumption of fermentable carbohydrates results in demineralization and plaque formation. Malnutrition affects tissue development and immune responses.	(34,69-71)
Smoking	x	x	x	Strong dose-effect relationship. Weakens blood circulation in the gingiva, impairs local immune responses, inhibits growth of periodontal ligament fibroblasts, and alters microflora.	(72,73)
Age	x	x	x	Physiological changes in periodontium cause more rapid development of periodontal inflammation. Altered saliva flow. More time to develop cavities.	(66,74)
Genetic factors	x	x	x	Race and ethnicity. Heritage syndromes, gene polymorphism: defects in host immune responses or production of inflammatory cytokines or matrix metalloproteinases, decreased chemotaxis of neutrophils.	(75-77)
Geographic region	x	x	x	Differences in genotypes and in demographic, environmental and ecologic characteristics.	(15,78,79)
Saliva flow and composition	x		x	Diet, age, diseases and medications affect secretion and composition and its antimicrobial activity, buffer capacity and clearance.	(80)

Risk Factor	DC	PAI	PD	Mechanisms	Ref.
Presence of specific bacteria in biofilm	x	x	x	Prevalence of certain pathogens in the biofilm, e.g. *P. gingivalis, A. actinomycetemcomitans* and *B. forsythus.*	(81,82)
Host immune response		x	x	Hypo- or hyperactive response promotes periodontal tissue destruction. Deficits in response affect pathogenesis of periodontitis, e.g. HIV associated with suppression.	(83,84)
Viral infections			x	Cytomegalo- and other herpesviruses release cytokines, promote growth of periodontal pathogens, and initiate cytotoxic and immunopathogenic reactions.	(85)
Local conditions in oral cavity	x	x	x	Tooth morphology, quality of restorations, calculus formation, and occlusion may affect disease initiation and development.	(86,87)
Diabetes	x	x	x	Stimulates phagocytes and release of cytokines that promote tissue destruction, altered pathogenesis, and exaggerated inflammatory responses, which induce caries development.	(88,89)
Osteoporosis			x	Reduces bone density, which predisposes to alveolar bone loss.	(90)
Medications	x	x	x	Medications that weaken saliva flow affect its buffer capacity, anti-infective agents and sugar clearance.	(91,92)
Socio-economic factors	x	x	x	Low education and income, rural residence, poor housing and sanitation, poor access to healthcare, limited ability for proper self-care, and general health issues.	(17,93)
Psychological factors	x	x	x	Stress downregulates cellular immune response, promoting tissue destruction. Depression affects self-care behavior and medication weakens saliva flow.	(91,94)

2.2.7 Global Epidemiology and Consequences of the Oral Diseases

Oral diseases are among the most prevalent diseases globally. According to the 2010 Global Burden of Disease (GBD) study (15), 3.9 billion people suffer from untreated dental caries, severe periodontitis and severe tooth loss. Every year, 15 billion healthy life years, measured in disability-adjusted life-years (DALYs), are lost due to these diseases. Between 1990 and 2010, the global burden of these diseases increased by 21%, mostly due to population growth and aging. The highest increase was in Eastern (52%) and Central (51%) Sub-Saharan Africa and Oceania (47%). The distribution and severity of the diseases vary within the same region and between populations and sub-groups of the same population. (15)

Of the 291 conditions assessed in the GBD study, dental caries was the most prevalent, affecting 35.3% (2.4 billion) of the total global population and 36.2% of women (15). The age-standardized prevalence varied globally between 19.9% in Australasia and 47.3% in Central Europe. Prevalence in Africa varied between 31.0% in Western Africa and 36.9% in Southern Sub-Saharan Africa. With an increase in developing countries and decrease in developed countries, the global prevalence remained stable between 1990 and 2010. The incidence of caries in 2010 was noticeably lower in all African regions (variation between 16.2 in Western Africa and 29.0 in Southern Africa) than it was, for example, in high-income areas in North America (62.6), Western Europe (49.3) or the Asia Pacific region (50.2) (95). Severe periodontitis was in turn was the sixth-most prevalent condition, globally affecting 10.8% (743 million) people (15). Prevalence peaked at 40 years of age and remained stable at older ages. Prevalence in Africa varied between 9.2% in Southern Africa and 20.1% in Eastern Sub-Saharan Africa. As with caries, the global age-standardized prevalence of severe periodontitis remained static between 1990 and 2010. (96) In addition to a lack of data, heterogeneity in periodontitis definition and questionable reliability in the disease measures hinder estimations of global prevalence (97).

The lack of PAI prevalence data is striking. According to some cross-sectional studies conducted in high- and middle-income countries, the prevalence of primary PAI (at least one lesion, no root canal treatment applied) in adults at an individual level varied by country and population, between 10% in a Finnish population and 56% in a Spanish population, with a median of 20.5%. The prevalence at tooth level varied from 1% to 13%, with a median of 3.5%. (98)

Oral diseases cause not only discomfort and pain, but also nutritional, functional and psycho-social problems, lower quality of life, heavy burden on the healthcare system and even premature death (99,100). Huge amounts of school and working days are lost every year, leading to lowered productivity and adverse economic consequences (101).

2.2.8 Oral Healthcare in Developing Countries

Caries is globally the fourth-most expensive disease to treat. The direct treatment costs attributed to caries, periodontitis and tooth loss have been estimated to be USD 298 billion annually, which accounts for 4.6% of total global health expenditure. With indirect costs, the total global economic impact of the diseases reaches USD 442 billion. Of the total direct treatment expenditure, 82% occurs in developed countries and only 1% in Sub-Saharan Africa. (102)

In addition to funding, most developing countries lack national oral health policies, implementation strategies and training. As a result, they also lack oral healthcare personnel, facilities, equipment and materials. The dentist-to-population ratio in Africa is approximately 1:150,000 compared to 1:2000 in developed countries (103). Most personnel are located in big cities, and in rural areas only emergency care such as pain relief and tooth extraction is available. In addition, the general level of oral health knowledge is low, especially in rural areas, and thus oral self-care is not regularly or adequately performed (104).

2.3 Role of Nutrition in Oral Health and Diseases

Micronutrients such as vitamins, minerals, trace elements, amino acids and poly-unsaturated fatty acids are inevitable for health and metabolism. Nutrition also plays a significant role in oral health, influencing the development and homeostasis of the oral cavity. For instance, vitamins A and C are important components in mucosal tissue formation; protein, calcium and phosphorus are needed for development of the teeth and the alveolar bone; and omega fatty acids, magnesium and many vitamins participate in immune functions (105). Malnutrition may in turn affect the oral microbiota, saliva volume and composition, inflammatory and immune responses in the oral cavity, and the healing capacity of the tissues, all of which

contribute to the development of dental caries and periodontal diseases (11,12,106,107).

The development of caries is directly related to the ingested diet of the host. Except for the well-known adverse effect of sugars (69), mostly protective effects on caries development have been reported with optimal levels of fluorides and various other micronutrients such as calcium, phosphate, vitamin A, vitamin B complex, vitamin D, iron, magnesium and biotin (108-114). Moreover, zinc has been found to decrease tooth predisposition to caries by increasing enamel remineralization (115) and affecting parotid salivary gland function (116). Intervention studies have been published on multiple micronutrient intake on dental caries, but only in children with primary or mixed dentition. A meta-analysis on the effects of vitamin D supplementation concluded that the risk of caries decreases by half if given before 13 years of age, but is ineffective after that, especially in girls. The results also suggest that there was a significant publication bias towards studies that found vitamin D was not effective (110). A cross-sectional questionnaire-based study on nutrition intake in South-African children aged five years suggests that calcium, vitamin B12, vitamin B6, vitamin A, riboflavin, magnesium, and biotin intake is significantly and negatively associated with caries prevalence (117). Another questionnaire-based study that assessed nutrient intake in junior high school students in Seoul, however, indicated that a high niacin intake may enhance caries development (118).

The health of the periodontium is essentially related to nutrition (10) through its effects on the tissue formation and on the complex immune system (119). Several earlier studies, conducted mostly in high-income countries, suggest various degrees of associations between micronutrient intake and periodontal status. For instance, periodontitis is associated with low plasma levels of vitamin D, vitamin C, and other antioxidants (120), and low vitamin B complex, vitamin C, and calcium intake is associated with the progression of periodontal diseases (10,121). Similar effects have also been detected with Omega-3 fatty acids (122,123) and magnesium (124). Despite the large number of studies published, the results regarding the effects of single nutrients on periodontal health remain elusive (107). This discrepancy could be explained by the fact that the association between single nutrient and disease may also be interlinked with the level of another nutrient or even multiple nutrients (125).

Malnutrition affects oral health in many ways. During tooth development, malnutrition can lead to enamel hypoplasia, which predisposes the teeth to dental caries in later life (70). The effects of malnutrition on permanent dentition and periodontal tissues are related to altered microbiota, modulated inflammatory and

immune responses, and diminished salivary gland function, which in turn affects the composition, buffering capacity and volume of saliva (11,106). Especially protein-energy malnutrition diminishes the production and actions of cytokines and weakens acute-phase immune responses to infections (65). The salivary gland functions are affected particularly by a lack of proteins, vitamins, zinc and iron (70), which contribute to the decreased levels of lysozyme, lactoperoxidase and immunoglobulins, which in turn diminish salivary defense mechanisms (126). Malnutrition also alters the oral microbiota, enhancing the growth of several oral pathogens. One suggested mechanism behind the shift may be the increased availability of arginine in saliva in malnourished individuals, which favors an overgrowth of pathogens (127). Nevertheless, an increased micronutrient intake does not always have purely beneficial effects on health. For example, iron treatment has been associated with exacerbation of infectious diseases, particularly malaria (128)

2.4 Pregnancy and Its Adverse Outcomes

2.4.1 Normal Pregnancy

Normal pregnancy duration from conception to delivery is 38-42 gestational weeks, with a mean of 280 days calculated from the first day of the last menstrual period (129). Pregnancy is divided into three trimesters, and each have their unique characteristics and risks. The first trimester includes conception and ends after 12 weeks, during which the fertilized egg attaches to the uterus, and the placenta and umbilical cord form. After eight weeks, the developing embryo is called a fetus, and it has a beating heart, primitive brain activity and the ability to move. The highest risk of miscarriage is during the first trimester: 30-40% of conceptions end in pregnancy loss.(130) The second trimester covers weeks 13 to 28 and is characterized by the development of the organs and physiological body functions. More than 90% of infants can survive if born after 28 weeks and provided with intensive medical care.(131) The third trimester covers weeks 29 to delivery, during which the organs mature and the fetus gains length and most of its weight (132).

Due to the increased secretion of the main female sex hormones, estrogen and progesterone, from the placenta and the ovaries, several functional changes occur in a pregnant woman's body. Insulin resistance and enhanced lipogenesis develop to

allow sufficient glucose transfer to the fetus, and the mother becomes hypercoagulable to prepare for hemostasis after delivery.(133,134) Estrogen maintains the pregnancy and fetal development and regulates progesterone production, while progesterone guides the development of the endometrium and prevents uterine contractions by restricting its estrogen sensitivity (135). The sex hormones also modulate an immunological sift to a more tolerant state, ensuring that a genetically incompatible fetus is not rejected by the maternal immune system (133,134). As an adverse consequence, limitations in T-cell, natural killer cell and B-cell activity; antibody production (136); and neutrophil functions (137) increase susceptibility to infection and change the disease pathogenesis (138).

The actual factors that initiate labor remain partly unknown. It has been proposed, however, that decreased levels of progesterone, increased levels of estrogen, and oxytocin production stimulate uterine contractions, and decidual activation initiates a cascade of events that lead to cervical dilatation, chorio-amniotic membrane rupture and delivery (135,139,140). Fetal cortisol – the secretion of which increases as labor approaches due to the activation of the fetal-adrenal axis – plays an important role in the process by stimulating changes in sex hormone secretion (140). In addition, an increased expression of inflammatory cytokines such as TNF-α and IL-1, proteinases such as MMP-8 and MMP-9, and chemokines participate in the process (141,142).

In 2012, approximately 213 million recognized pregnancies were initiated, 89% of them in developing countries and 40% unintended. Of all pregnancies, 120 million occurred in Asia, 54 million in Africa, 19 million in Europe, 18 million in Latin America and Caribbean and 1 million in Oceania. (143)

2.4.2 Anthropometry in Postnatal Health Assessment

Birth size is a reflection of the conditions in which a fetus has developed in the uterus, and it is therefore used as a proxy for the health of the newborn. Fetuses from healthy and well-nourished mothers who have no environmental constraints grow at a similar rate all over the world. (144) Subnormal anthropometric measurements at birth thus predict mortality, morbidity and development outcomes of the infant. Birth weight data is usually collected after delivery at health facilities worldwide. Birth length and infant head circumference are, however, rarely measured, although especially birth length would be a better indicator of overall health and a better predictor of morbidity risk. (145-147)

2.4.3 Epidemiology, Risk Factors and Consequences of Adverse Pregnancy Outcomes

Preterm Birth

Preterm birth (PTB) is defined as birth before 37 completed gestational weeks. The major risk factors associated with PTB are listed in Table 2. Both term and preterm labor are believed to have common pathways but, in spontaneous PTB, a combination of pathological stimuli activates some components of the pathway, causing the uterus to shift from quiescence to contractions and too-early labor. (148,149) During the second trimester, pregnancy loss is usually related to fetal abnormalities, maternal anatomic and immunologic factors, and infections (131). Although several PTB risks have been identified, many contributing factors are still unclear (5,150). Only intra-amniotic infection has been shown to have a causal relationship with spontaneous PTB, due to mechanisms related to activation of the innate immune system. Such infection has been detected in 25% of mothers who deliver too early, but it may be involved in more than 40% of cases. (149,151)

Table 2. Major risk factors with preterm delivery (5,150).

• Previous preterm delivery	• Multiple gestation	• Low and high maternal age
• Low maternal body-mass index	• Short proximity to previous delivery	• Black race
• Micronutrient deficiencies (iron, folate, zinc)	• Anatomical factors in the uterus	• Vaginal bleeding
• Chronic diseases (e.g. diabetes, hypertension, asthma, thyroid disease)	• Maternal infections (e.g. malaria, syphilis, bacterial vaginosis and periodontitis)	• Male fetus
• Low socioeconomic status	• Psychological and social stress	• Depression
• Smoking	• Drug and alcohol use	• Genetic factors

The annual global incidence of PTB is 15 million, of which 60% occurs in Asia and Sub-Saharan Africa. The incidence is lowest (5%) in many Northern European countries and highest (18%) in some African countries, such as Malawi. (152)

Approximately 30-35% of preterm deliveries emerge spontaneously and the rest are by induction or by cesarean section. About 5% are extremely premature, occurring before 28 weeks; 15% are severely premature at 28-31 weeks; 20% are moderately premature at 32-33 weeks; and 60-70% occur near term at 34-36 weeks. The PTB rate is increasing in many countries, despite the advances in knowledge on its causes and the use of several public health and medical interventions. (5)

Infants born preterm have an increased risk of mortality, serious morbidity, growth failure and other adverse developmental consequences. Prematurity is the leading cause of death in newborns and the second-most common cause in children under five years. Out of the annual 3.1 million neonatal deaths, more than 1 million are caused by PTB complications. (153) In high-income countries, 50% of infants born at 24 weeks may survive, often with disabilities. In many low-income countries where no intensive care is available, only 30% of infants born at 28–32 weeks survive, and almost all of those born earlier die. (154) Premature birth contributes to 75% of all perinatal morbidity and 50% of long-term morbidity (2,3). Common consequences related to immature organ development and physiological functions in premature infants include cerebral palsy; respiratory and gastrointestinal complications; neurodevelopmental problems such as learning difficulties, cognition and developmental delays; emotional and social problems; visual and hearing impairment; and an increased risk of chronic diseases and socioeconomic problems in adulthood (155). The economic costs of preterm birth are high due to the long-term need for medical care, special education and other support, and this imposes a significant public health burden in all countries (4). The annual costs related to preterm birth in the United States alone is estimated to be at least USD 26.2 billion a year and rising (150).

Low Birth Weight, Small for Gestational Age, and Stunting

Low weight and stunting (short stature for age) that develop during the fetal period have their own underlying mechanisms and risk factors although they share some determinants (145). Timing, duration and severity of the insult in the fetal period determine the type of the IUGR that develops. Length gain retardation relates to the first and second trimesters of pregnancy while low weight develops during the last months when the fat stores deposit. (156) The global incidence of impaired fetal growth is approximately 10% of all live births (157).

Low birth weight (LBW) is defined as birth weight below 2500g, very low birth weight as weight below 1500g and severely low birth weight as weight below 1000g. LBW can be a consequence of PTB, IUGR or both. These two causes of LBW have

different underlying mechanisms and health risks. Small for gestational age (SGA) is a proxy measure of IUGR. It is defined as infants <10th centile of a birthweight for gestational age, compared to the gender-specific reference population. Infants who are SGA can be born either at term or preterm. (1) According to a study that evaluates the situation in 138 low- and middle-income countries in 2010, 32.4 million (27%) live births were SGA. A total of 18 million infants were born with LBW: 59% were term SGA, 16% preterm SGA and 25% preterm but appropriate size for gestational age. The highest prevalence of SGA births was in south Asia (44.5%), sub-Saharan Africa (25.5%) and south-east Asia (24.3%). These estimates are only directional, since less than 50% of infants born in low- or middle-income countries are weighed at birth, and the available data is biased because it is collected only from infants born alive in health facilities. (1)

Stunted stature is another manifestation of IUGR, and it is defined as length below -2SD of the growth standard median of the same sex. Global estimates of newborn stunting are missing because birth length is not routinely measured in low-income countries, where stunting is common. Nonetheless, it is estimated that there are approximately 161 million stunted children in the world: 50% in Asia and 30% in Africa. Linear growth faltering usually begins during the fetal period and continues for the first two years of the child's life. (158)

Risks Factors for IUGR

Some major risk factors for IUGR are presented in Table 3. Although many risk factors have been identified, its etiology and the pathologic mechanisms that inhibit the growth of the fetus are not fully understood. The strong association between maternal nutritional status and fetal growth is mediated partly by hormonal and metabolic changes during pregnancy. Impaired utero-placental blood flow and increased levels of maternal stress hormone cortisol lead to placental insufficiency; a reduced nutrient and oxygen supply to the fetus are among other suggested mechanisms. (159)

Table 3. Major maternal and fetal risk factors associated with intra-uterine growth restriction (145,159,160).

Maternal Risk Factors

- Short height
- Placental insufficiency
- Low BMI or weight, low weight gain in pregnancy
- Pre-eclampsia
- Primiparity
- Low birth weight or preterm delivery in previous pregnancy
- Short interpregnancy interval
- Poor nutritional status, malnutrition
- Low education, low socioeconomic status and poverty
- Smoking, alcohol and illicit drug use
- Hypertension
- Hypoglycemia
- Epilepsy
- Cardiopulmonary diseases, hypoxia,
- TORCH (toxoplasmosis, syphilis, varicella zoster, parvovirus, rubella, cytomegalovirus and herpes infections)
- Periodontitis
- Other infections and disorders (?)
- Stress
- Race

Fetal Risk Factors

- Multiple pregnancy
- Genetic disease
- Congenital malformations
- Fetal infections

IUGR is a major risk factor for perinatal mortality, short and long-term morbidity, growth failure, and cognitive and neurodevelopmental disorders (158). Newborns with a low weight have a higher risk of short-term mortality than those with a short stature, probably relating to a lower level of fat deposits (145). Stunting, however, is a marker of multiple pathological disorders that have irreversible long-term consequences. Early life stunting is associated with poorer cognitive and socioeconomic outcomes even later in life: outcomes such as weaker academic performance, lower wages, increased probability of living in poverty, and lower age at first pregnancy. (161)

2.5 Oral Infections and Adverse Pregnancy Outcomes

2.5.1 Effects of Hormonal Changes in Pregnancy on Oral Health

The higher sex hormone levels occurring during gestation also affect the oral cavity. Estrogen and progesterone receptors have been detected in gingiva (162,163): thus their increased levels may affect the periodontium directly, for instance by enhancing vascular permeability and the growth of gingival fibroblasts (164). In addition, sex hormones may affect oral health indirectly by stimulating the production of PGE_2 in monocytes, which enhances inflammatory reaction (165), and by downregulating IL-6 production in gingiva, thus restricting immune responses (166).

Some periodontal pathogens can metabolize these sex hormones, which may favor their growth (167). As an example of such pathogens, increased levels of P*revotella intermedia, Prevotella nigrescens* (168) and *Porphyromonas gingivalis* (169) have been associated with gingivitis in pregnancy. Although pregnancy as such does not cause gingivitis, pregnant women have an increased susceptibility to gingival bleeding and deepened periodontal pockets if bacterial plaque is present. However, these changes reverse after delivery and do not predispose to the development of periodontitis. (170) This phenomenon may be related to the immunological shift, which includes reduced expression and activity of several tissue-degrading proteinases such as MMPs in the periodontium and saliva during pregnancy, which in turn hinders the destruction of tissue (171).

2.5.2 Results from Previous Studies Associating Oral Infections and Adverse Pregnancy Outcomes

Most previous studies assessing the association between oral diseases and birth outcomes have concentrated on periodontitis, PTB and LBW. The first study suggesting an association was conducted by Offenbacher et al. in 1996 (172), and an extensive number of studies have been published since. Most of the studies were conducted in high-income countries among affluent populations (173-176), but some also in low-resource settings such as the African countries of Uganda and Madagascar (177-179). In 2012, a review identified 62 relevant studies suggesting that periodontitis may be an independent risk factor for poor pregnancy outcomes (180). Due to the accumulative evidence, a consensus statement was issued in 2013

by an international working group of experts concluding that periodontitis is modestly associated with PTB and LBW, although the strength of the association seems to vary between populations and to depend on the periodontal disease assessment methods and classification (181). Underlining the uncertainty, several studies have also failed to find the association (182-186), indicating that the underlying mechanisms are not yet fully understood. Nevertheless, periodontitis has been associated independently also with several other diseases and conditions (187), e.g. infective endocarditis (188), diabetes (189), metabolic syndrome and cardiovascular diseases (190), chronic kidney disease (191), respiratory infections (192,193) and obesity (194).

Regarding dental caries, four papers were identified that reported results of studies designed to assess the association between maternal dental caries and PTB or low birth weight (182,195-197). Two other studies assessed the association between decayed missing and filled teeth (DMFT) index score and preterm birth or low birth weight (198,199). Two additional papers reported results on studies designed to assess periodontitis and preterm delivery, but also provided some results on dental caries (183,200). All studies based their assessment solely on clinical examination without x-rays and only one, a study from Tanzania, considered carious pulpal exposure based on clinical symptoms. A study conducted in Jordan among 100 mothers who had delivered recently found a significantly higher DMF score in preterm mothers compared to those who delivered full term (199). The other studies found no significant associations between caries variables and birth outcomes.

Although an association between PAI and systemic diseases such as diabetes (201) and coronary heart disease (202) has been suggested, no studies investigating the association between PAI and birth outcomes in humans had been published at the time this study was conducted. An association between periapical infection and the health of developing offspring was, however, discovered by Bain et al. in 2012 when they found a significant association between induced periapical abscesses in pregnant rats and brain inflammation in their pups (203).

2.5.3 Suggested Mechanisms Linking Oral Infections and Birth Outcomes

Two major pathways, direct and indirect, have been suggested to mediate the association between periodontitis and pregnancy outcomes, although the pathways are not mutually exclusive (204,205). These two pathways are presented in Figure 4.

In the direct pathway, oral bacteria or their byproducts are disseminated through the blood circulation to placental tissues, chorioamniotic membranes, amniotic fluid or fetal circulation, where they induce local inflammation, suppress local growth factors, or cause tissue damage or changes in placental function. In addition to hematogenous dissemination, the bacteria may access the fetoplacental unit from the genitourinary tract. (204) Increased vascular permeability facilitates the transmission of pathogens and their byproducts from the periodontium in pregnancy, and it has been unequivocally shown that bacteria can translocate from mouth to placenta (206). As good evidence, *Fusobacterium nucleatum* is the most commonly found bacterium in the amniotic fluid in preterm labor and its antibodies have also been found in cord blood (207,208). *F. nucleatum* is known to stimulate decidual necrosis that may lead even to the death of the fetus (209). Another periodontal bacterium, *Campylobacter rectus*, has been shown to induce major alterations in the parts of placenta that are responsible for the exchange of nutrients between mother and fetus. These structural alterations may lead to restricted nutrient transmission, but also to disrupted blood flow between the mother and the fetus, predisposing to IUGR. (210) Other periodontal bacteria often associated with poor pregnancy outcomes are *Tannerella forsythia, Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans* and *Treponema denticola* (172). *P. gingivalis*, *F. nucleatum* and *A. actinomycetemcomitans* have been shown to elevate cytokine levels such as IL-2, IL-6 and TNF-α in the placenta in cases of IUGR (211).

In the indirect pathway, bacterial infection in the oral tissues releases inflammatory mediators such as cytokines and TNF-α that circulate to the liver, inducing acute-phase protein production and a systemic inflammatory response, which affect the pregnancy (204,205). Increased concentrations of the major acute-phase proteins CRP and AGP have been associated with both periodontitis and shortened duration of gestation and IUGR (212-214). CRP and AGP differ from each other: serum concentration of CRP increases within hours after the onset of an infection and also falls rapidly after the infection has been resolved, whereas AGP concentration rises more slowly, over 24h, and continues to be detectable for weeks (215,216). The inflammatory mediators can be transmitted to the fetoplacental unit also directly from the periodontium or from the vagina (205,208,217,218). In addition, production of the prostaglandins that participate in the induction of labor is stimulated in infected oral tissues by microbial endotoxins and pro-inflammatory cytokines (149). Another biomarker that could link oral infections and adverse birth outcomes is cortisol. Cortisol is a steroid hormone that is released from the adrenal glands in response to emotional, physical or immunological stress, e.g. infections,

and it also regulates the maintenance of pregnancy and the timing of parturition (219-221).

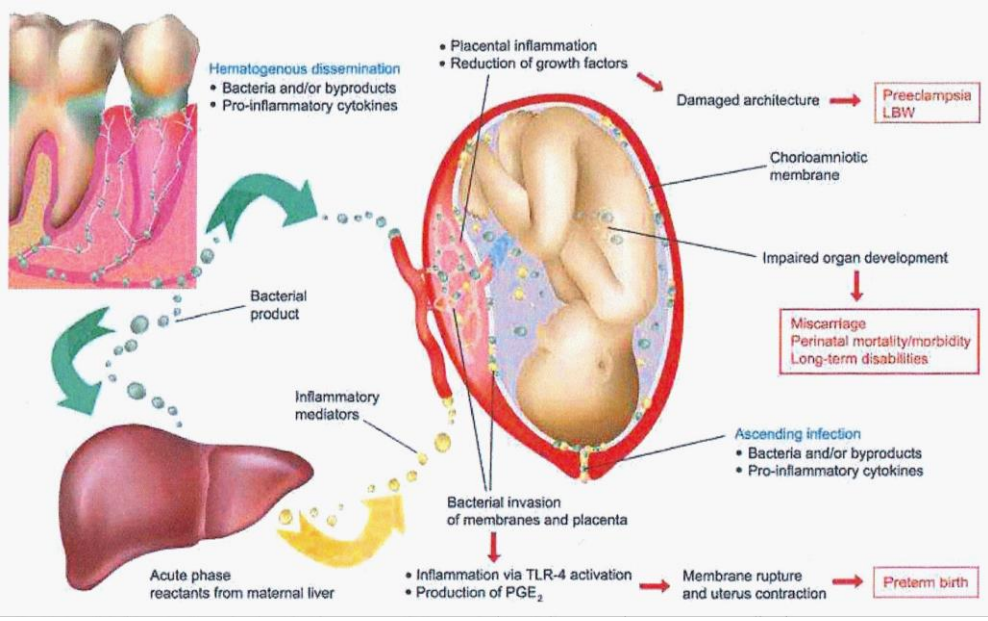


Figure 4. Possible biological pathways associating periodontitis and pregnancy complications (205). Reprinted with permission from John Wiley and Sons.

2.6 Justification for the Present Study

As the literature review has shown, data on the effect of nutrient interventions on the oral health of adults are generally scarce, and there are no published trials that enrolled pregnant women in developing countries. Because multiple micronutrient interventions are increasingly recommended for pregnant mothers in low-resource settings, their impact on oral health must be studied so that their possible adverse effects can be prevented. In addition to poor nutrition, maternal infections such as periodontitis are among the most important identified risk factors for PTB and IUGR, whose prevention is a global priority. Despite of large number of studies, the risk factors and underlying mechanism are not yet completely understood, which hinders the development of preventive interventions. Previously unidentified and modifiable risk factors may include dental caries and its sequela PAI, which are very

common, especially in the areas where most adverse pregnancy outcomes occur. Therefore, the present study is warranted because it fills in some gaps in the evidence related to the effects of micronutrient intervention on oral health in low-resource settings and in the association between oral diseases and adverse pregnancy outcomes and the possible underlying mechanisms.

3 Aims of the Study

The main aim of this research was to assess whether poor maternal oral health is a risk factor for adverse pregnancy outcomes in a food-insecure, rural sub-Saharan African setting where oral diseases and poor pregnancy outcomes are common.

The specific aims were:

1. To examine whether food supplementation with multiple micronutrients or small-quantity lipid-based nutrients affects oral health, especially the development of dental caries or periodontal diseases, in pregnant women in low-resource conditions (Study I).

2. To assess whether maternal dental caries, periapical infections and periodontal diseases are associated with duration of pregnancy or infant birth size (Study II).

3. To investigate whether the associations between periapical infection and shortened duration of pregnancy, lower birth weight and smaller neonatal size are mediated through spread of periapical bacteria to the fetoplacental unit (direct pathway) or systemic inflammation (indirect pathway) (Study III).

The hypotheses of the research were that supplementation of the maternal diet with micronutrients affects oral health and that maternal oral infections affect pregnancy outcomes.

4 Materials and Methods

4.1 Approach to the Study

The oral health study that underlies this thesis was nested in a larger nutrition intervention trial, iLiNS-DYAD, which tested the effect of nutrient supplements on the health of pregnant women and the health and growth of their infants in rural Malawi. In total, 1391 pregnant women were enrolled in the iLiNS-DYAD trial before 20 gestational weeks and randomized into three nutrition supplement groups. The mothers were followed throughout pregnancy and the mother-infant dyads after delivery. For this study, the mothers underwent an oral health examination soon after delivery, aimed at investigating whether the nutritional intervention provided during pregnancy affected the participants' oral health and whether their oral infections contributed to the birth outcomes. This thesis consists of three sub-studies referred to as Study I, Study II and Study III (Figure 5). All three studies used data from the same 1024 included participants.

The overall study approach is presented in Figure 5. Study I was a randomized controlled trial assessing the oral self-care habits, self-reported oral health problems and clinical oral health status of the participants after delivery, by nutrition intervention group. Study II investigated cross-sectionally whether the maternal oral diseases assessed in Study I were associated with the duration of pregnancy or the birth size of the infants. Study III investigated whether there was evidence of two biologically plausible pathways, spread of the periapical bacteria to the fetoplacental unit (direct pathway) or systemic inflammation (indirect pathway), linking periapical infections (PAI) and adverse pregnancy outcomes. Study III combined oral health data from Study I and pregnancy outcome results from Study II with data from several biological samples collected during pregnancy and after delivery.

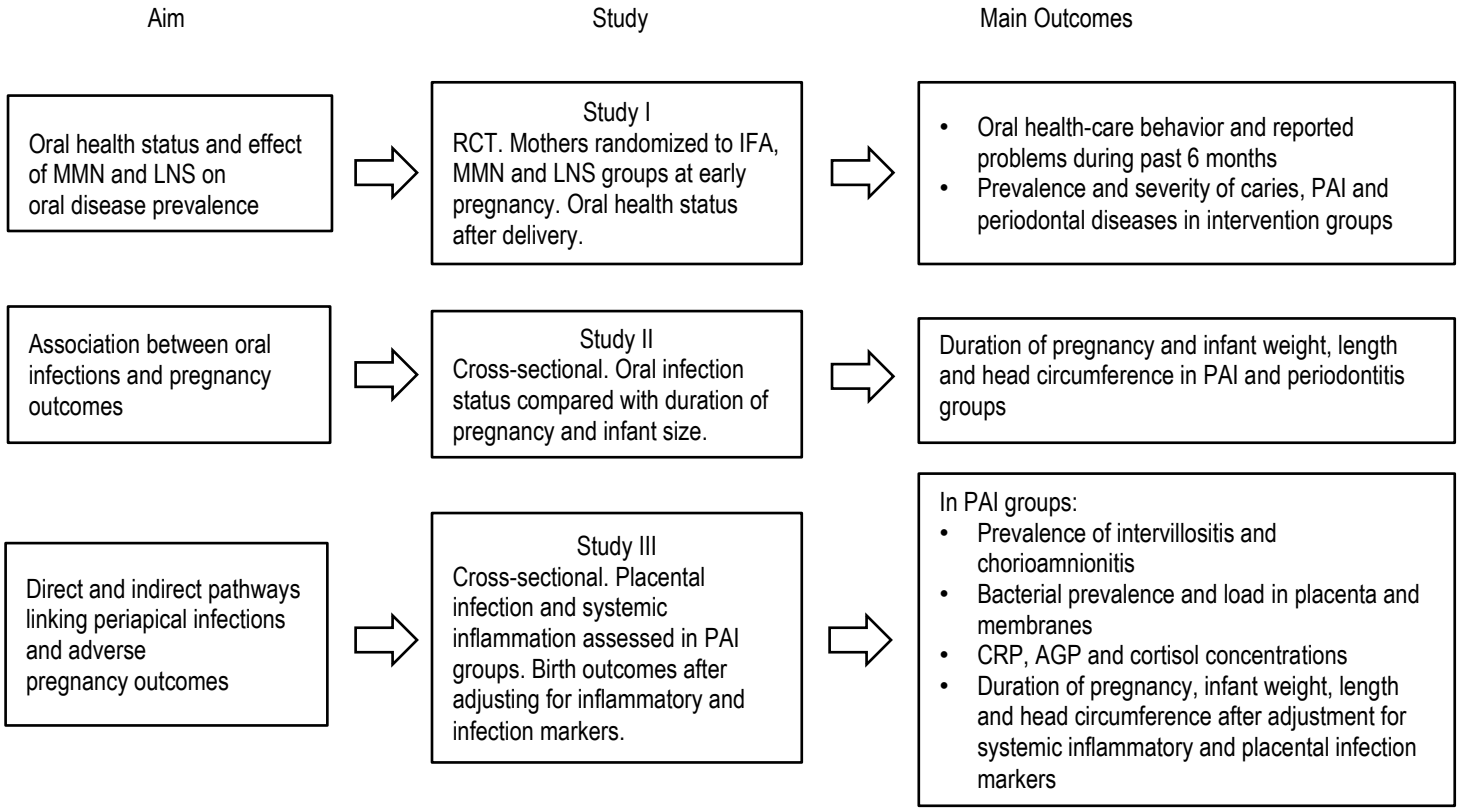


Figure 5. Overall study design of the thesis research.

4.2 Study Area

The study was conducted in the Mangochi district in Southern Malawi. Malawi is a land-locked country located in southeast Africa with area of 118,484 km², of which 24 000 km² is covered with water, mainly Lake Malawi (222). The estimated population of Malawi is 17.2 million (223), with an annual population growth rate of 2.8%, and 48% of the population is below 15 years of age. Of the population, 85% inhabit rural areas, earning their living mainly by rain-fed subsistence farming and fishing. In 2014, life expectancy at birth for women was 56.6 years and the literacy rate 64%. Approximately 51% of the population live below the poverty line, and 60% of households suffer from moderate to severe hunger. (224) Although Malawi has high child and maternal mortality rates, the situation has been steadily improving. As an example, infant mortality decreased from 135 deaths per 1000 live births in 1992 to 42 in 2015-16 and under-five mortality from 234 to 63 deaths per 1000 live births during the same period. Yet, one in every 16 children do not survive to their fifth birthday. (224) More demographic and health indicators are shown in Table 4.

Table 4. Key socioeconomic and health indicators for Malawi.

Indicator	Measure
Households with improved sanitation	52%
Households with electricity	11%
Proportion of women aged 15-49 with primary education	62%
Proportion of women who are thin (BMI<18.5)	7%
Proportion of women who are overweight (BMI>25.0)	21%
HIV prevalence among women aged 15-49	11%
Prevalence of anemia in pregnant women	45%
Total fertility rate (average number of children per woman)	4.4
Maternal mortality ratio (deaths per 100,000 live births)	439
Median age at first delivery, in years	19

Indicator	Measure
Antenatal care coverage, at least one visit	95%
Proportion of births attended by skilled health personnel	90%
Cesarean section rate (% of live births)	6%
Neonatal mortality rate (per 1000 live births)	27
Infant mortality rate (per 1000 live births)	42
Under-5 mortality rate (per 1000 live births)	63
Proportion of infants with low birth weight (<2.5kg)	12%
Percentage of children under 5 stunted	37%
Percentage of children under 5 wasted	3%
Percentage of children under 5 underweight	12%

Source: Malawi demographic and health survey 2015-16 (224).

The study area is presented in Figure 6. The data collection was conducted in the catchment areas of four health facilities in Mangochi district, namely the Mangochi district hospital and the health centers of Malindi, Lungwena and Namwera. The Mangochi district hospital is located in the town of Mangochi, which is semi-urban, and the health centers are located in rural areas. The estimated population of Mangochi district was 797 000 according to the latest census, which was conducted in 2008 (222). The Mangochi district hospital outpatient clinic served a population of 100 000 people and the health centers populations of 30 000 each. The main languages spoken in the study area are Chichewa and Chiyao.

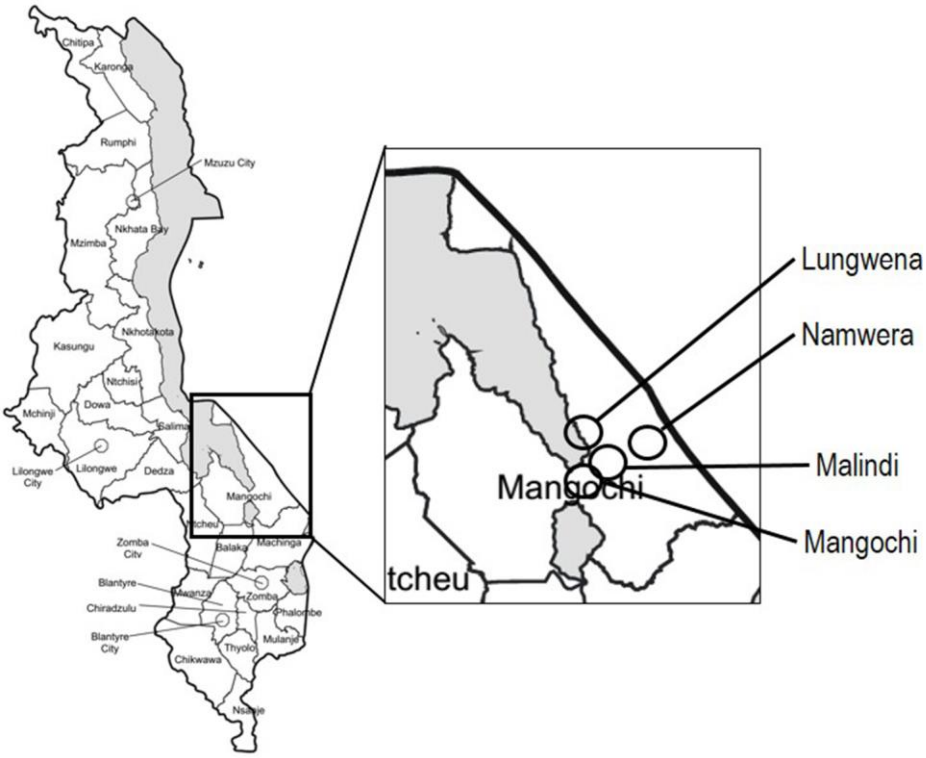


Figure 6. Map of Malawi with the study sites. Modified from National Statistical Office of Malawi, 2016 (222).

4.3 Study Participants

All pregnant women who visited antenatal clinics in the four study sites during the enrollment period were approached, and those who met the enrollment criteria of the iLiNS-DYAD trial and were interested in participating were recruited. The inclusion criteria were ultrasound-confirmed pregnancy of no more than 20 gestational weeks (gw), resident of the catchment area of the study clinic, availability during the study period, and signed or thumb-printed informed consent. The exclusion criteria were age less than 15 years, need for frequent medical care due to a chronic health condition, severe illness requiring hospital referral, allergy to peanuts or serious allergic reaction to any other substance, pregnancy complications evident at enrollment visit (edema, blood hemoglobin of <50 g/L, systolic blood pressure of >160 mmHg or diastolic of >100 mmHg), earlier participation in the iLiNS-

DYAD trial or concurrent participation in another clinical trial. Participants who had a singleton pregnancy were eligible for the oral health studies.

4.4 Nutritional Intervention

Study I investigated the effect of the nutrient supplements on participants' oral health and analyzed the outcomes by intervention group. In Studies II and III, the intervention group was used as a co-variate in multivariate analyses.

The iLiNS-DYAD trial had three arms. Participants in the iron-folic acid (IFA) group received capsules that contained 60 mg of iron and 400 mg of folic acid, similar to normal Malawian antenatal care. Participants in the multiple-micronutrient (MMN) group received capsules that contained IFA and 16 additional micronutrients. Participants in the lipid-based nutrient supplement (LNS) group received 20 g sachets of paste that contained the same nutrients as the MMN capsules plus four additional minerals, protein, fat and 1.2 g of sucrose. The raw ingredients of the LNS were soybean oil, dried skimmed milk, peanut paste, a mineral and vitamin mix, and sugar (225). The iron content in the MMN and LNS supplements was lower (20 mg) than in the IFA ones (60 mg) because the MMN and LNS supplements were provided also six months postpartum, when the recommended dose of iron is lower than during pregnancy. One IFA or MMN capsule or LNS sachet mixed with a small amount of any food was consumed as one daily morning dose. All participants received also one dose of intermittent preventive malaria treatment with sulfadoxine pyrimethamine at enrollment and another dose between 28 and 34 gw as per standard Malawian antenatal care. The detailed nutrient and energy content of the supplements are provided in Supplementary Table 1 of Article I.

4.5 Data Collection

4.5.1 Enrollment and Follow-Up

Participant enrollment for the iLiNS-DYAD trial took place between February 2011 and August 2012. A randomizer allocated the eligible participants into control and intervention groups by asking them to choose one envelope from a set of identical-

looking opaque envelopes that contained a participant number and a group allocation code. Details of the enrollment and randomization are published in Ashorn et al. 2015 (226). The code linking the participant numbers and intervention groups was not disclosed to the outcome assessors or researchers until the data collection, entry and cleaning was completed.

The participants were provided with mobile phones so that they could immediately inform the study personnel about the child´s birth. The oral health visit took place at Mangochi Hospital study clinic where the participants were transported from other sites in the research group vehicles. The follow up and data collection plan regarding the data that was used in this thesis is presented in table 5.

Table 5. Follow-up and data collection plan.

Visit	Time	Place	Collected Data
Enrollment	14-20 gw	Clinic	Health and obstetric history, weight, height, MUAC, HIV and malaria status, saliva for cortisol, blood sample for AGP and CRP, duration of pregnancy
Post enrollment	2 wks after enrollment	Home	Demographic, social and economic background
Supplement follow-up	Bi-weekly	Home	Supplement usage, possible adverse events
28 gw	at 28 gw	Home	Saliva for cortisol
36 gw	at 36 gw	Clinic	Obstetric examination, saliva for cortisol and blood sample for AGP and CRP
Delivery	Within 48 h after delivery	At delivery site	Delivery time and events, placental tissue samples, infant birth weight
Postnatal	1-2 wks post delivery	Clinic	Neonatal weight, length and head circumference
Oral health	At postnatal visit or soon afterwards	Mangochi clinic	Questionnaire, clinical oral health status, panoramic x-ray

4.5.2 Hematological and Salivary Measurements

Hemoglobin concentration was measured with cuvettes and readers and peripheral blood malaria parasitemia was checked using rapid tests from a finger prick on site. HIV infection was tested by using a whole-blood antibody rapid test, and if positive, by another rapid test on site. C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) were measured from venous blood samples at the laboratory by immunoturbidimetric assay (Study III). Cortisol concentration was measured from saliva at the laboratory using the ELISA method. Saliva was collected after a 30-minute fast with a polymer swab placed under the tongue (Study III).

4.5.3 Placental Tissue Collection and Analysis

Placenta and membranes were placed in a sterile container at delivery and collected and transported in a timely fashion to the study clinic for sample preparation. Two chorionic and amniotic membrane samples were taken from the edge of the rupture site and two placental tissue samples from near the umbilical cord insertion. One placental and one membrane sample were prepared and analyzed for histology by microscopy. The other two samples were analyzed for bacterial DNA using a 16S rDNA broad-range SYBR green quantitative PCR assay (Study III).

4.5.4 Anthropometric Measurements

The anthropometric measurements collected from the mothers were weight, height and MUAC, and those collected from infants were birth weight, neonatal weight, length, and head circumference. The weights were recorded to the nearest 0.05 kg in mothers and 0.01 kg in infants, and the height, length, MUAC and head circumference to the nearest 0.1 cm. All measurements were taken and recorded in triplicate. Sex-age standardized anthropometric indices, weight-for-age (WAZ), weight-for-length (WHZ), length-for-age (LAZ) and head circumference-for-age were calculated for the infants using WHO Child Growth Standards (227) (Studies II and III).

4.5.5 Oral Health Examination

The oral health examination was conducted within the six-week puerperal period after delivery between June 2011 and August 2013 by three experienced dental therapists who spoke the local languages. The collected data are presented in Table 6 below.

Table 6. Collected oral health data.

Data Collection Method	Collected Data	Additional Information
Questionnaire with multiple choice questions	• Oral healthcare habits • Perceived oral health problems • Received treatment	Self-reported, during the past 6 months
Clinical examination	• Gingival bleeding on probing	By dental arch sextants
	• Periodontal pocket depth	At deepest point from six sites of each tooth excluding third molars, in mm
	• Dental caries status	full-mouth status
Panoramic X-ray	• Number of teeth • Caries extending to dentine or pulp • Periapical lesions	
	• Horizontal and vertical alveolar bone level	Measured from dento-enamel junction

4.5.6 Training and Quality Control

The data were collected according to standard operating procedures (SOPs) and visit guides, using standardized data collection forms. Data collection forms were translated into the local languages, Chichewa and Chiyao, from English and back-translated to identify translation errors. The staff members involved in the data collection were trained and their measurement reliability verified at the beginning of the study and at four- to six-month intervals thereafter. The equipment used for the anthropometric measurements were calibrated daily using standard weights and

length rods. The study coordinator and researchers made regular site monitoring visits, and an external monitor conducted one visit during the data collection.

4.6 Outcome Variable Definitions

Definitions used in the analysis are presented in Table 7. For periodontitis, several different definitions were tested, but they did not change the results markedly. Radiographs were diagnosed jointly with an experienced oral radiologist based on pre-planned criteria and using structured forms, and disagreements were mutually settled. A radiographic view of periapical infection and vertical bone loss related to periodontitis is shown in Figure 7.

Table 7. Outcome variable definitions.

Variable	Definition	Study
Caries lesion	Caries extending to dentine in clinical or radiological analysis	I, II
Deep caries lesion	Caries exposing dental pulp in radiological analysis	I, II
Periapical infection	Osteolytic finding surrounding the apex (tip) of the dental root	I, II, III
Mild to moderate periapical infection	1-3 periapical lesions	III
Severe periapical infection	4 or more periapical lesions	III
Gingivitis	Profound bleeding from at least one dental arch sextant after probing	I, II
Tooth with periodontitis	>3mm pocket measured clinically or alveolar bone at cervical root level or lower in radiological analysis	I
Participant with periodontitis	At least three teeth with periodontitis or one dental arch sextant with horizontal alveolar bone loss at least at root cervical level, and gingivitis present	I, II
Participant with caries	At least one caries lesion diagnosed	I, II
Participant with deep caries	At least one deep caries lesion diagnosed	I, II

Variable	Definition	Study
Participant with periapical infection	At least one periapical infectious lesion diagnosed	I, II, III
Preterm birth	Birth before 37 gestational weeks	II
Low birth weight	Birth weight < 2500g	II
Underweight	WAZ< -2	II
Stunting	LAZ<-2	II
Small head circumference	Head circumference for-age z-score <-2	II
High CRP	CRP > 5 mg/L	III
High AGP	AGP>1 g/L	III
High cortisol	Cortisol concentration >75th centile of all cortisol concentrations at that time point	III
Chorioamnionitis	≥ 5 neutrophil granulocytes on average per 10 high-power fields present in either the chorionic plate or the amniotic membrane	III
Severe chorioamnionitis	≥ 25 neutrophil granulocytes on average per 10 high-power fields present in either the chorionic plate or the amniotic membrane	III
Acute intervillositis	≥ 5 neutrophils on average per 10 high-power fields in the placental intervillous space	III
Chronic intervillositis	≥ 5 lymphocytes or monocytes on average per 10 high-power fields in the placental intervillous space	III
Positive for bacterial DNA	Ct value lower than 28 ± 3 cycles, depending on variation between runs	III

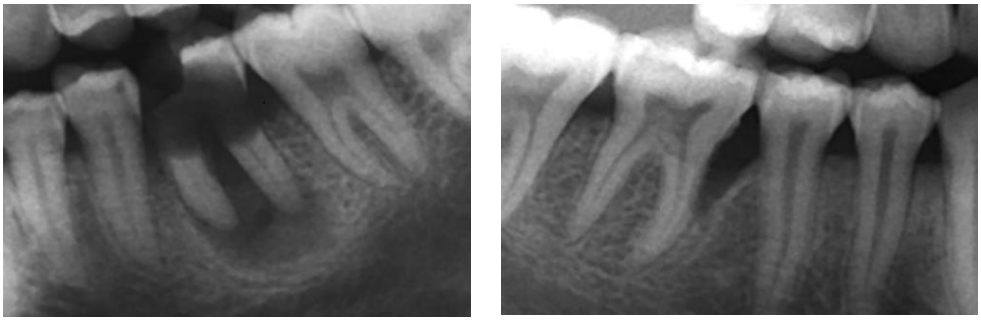


Figure 7. On left: Radiographic view of periapical lesion related to carious left lower first molar. On right: Deep periodontal pocket caused by periodontitis in lower right first molar (adapted from Article II).

4.7 Statistical Approach

4.7.1 Sample Size Calculation

A target sample size of 1400 participants was originally calculated for the iLiNS-DYAD trial. The calculation was based on the trial's primary outcomes, birth weight and newborn length, aiming to detect differences between the three intervention groups and to study the interaction between the intervention and potential effect modifiers (226).

In the oral health studies, participants who delivered a singleton infant and completed the oral health examination within the six weeks puerperal period after delivery were included in the analysis. For Study I, the acquired sample size of approximately 340 participants per intervention group provided 90% power to detect differences between the groups in the continuous oral disease outcomes at a 5% two-sided type I error rate, assuming an effect size of 0.25 (mean difference between the groups divided by the pooled SD). For Studies II and III, the attained sample size of 1024 participants provided 93% power to detect differences between the continuous birth outcome groups at a 5% two-sided type I error rate, expecting an oral infection prevalence of 25% and an effect size of 0.25.

4.7.2 Data Management and Analysis

All data were collected on paper forms and entered into a tailormade database using the scanning and electronic character recognition of a Cardiff-Teleform system, version 10.5 (Digital Vision, Highland Park, IL, USA). Pre-defined critical variables and all suspicious and missing values were verified manually on entry and by logic checking during the cleaning processes. The statistical analysis was conducted using Stata software version 12.1 (Stata-Corp, College Station, TX, USA) according to written statistical analysis plans. For Study I, the analysis plan was published at the trial website (www.ilins.org) before the intervention code was opened.

In Study I, the global null hypothesis of the supplement groups being identical in relation to the oral diseases was tested using Fisher's exact test for binary outcomes such as the prevalence of caries, and using ANOVA for continuous outcomes such as the number of caries lesions. After that, pairwise comparisons between the supplement groups were made with log-binomial regression models for binary outcomes and ANOVA for continuous outcomes. Risk ratios (RR) were calculated for the comparison of binary outcomes, and risk differences and differences in means for the comparison of continuous outcomes to investigate whether nutrient supplementation increased or decreased the risk of oral diseases.

In Study II, pairwise comparisons between binary birth outcomes such as preterm birth by oral infection group were conducted by Fisher's exact test and continuous birth outcomes such as duration of pregnancy by Student's t-test. Risk ratios, risk differences and differences in means were calculated to investigate whether oral diseases increased or decreased the risk of adverse birth outcomes.

In Study III, all analysis, except the bacterial load and prevalence analysis, was conducted using multivariable models based on the Study II results. Missing data in each variable, except 16S rRNA results, were replaced with multiple imputed (50 times) values. The mean bacterial loads (number of 16S rRNA copies) were compared using the Mann-Whitney U test and prevalence using Fisher's exact test by PAI group. Twenty-one commonly found periapical pathogen genera (228-230) were selected for the analysis: *Actinomyces, Anaeroglobus, Atobobium, Bacteroides, Dialister, Enterococcus, Eubacterium, Filifactor, Fusobacterium, Mogibacterium, Olsenella, Parvimonas, Peptostreptococcus, Porphyromonas, Prevotella, Propionibacterium, Pseudoramibacter, Streptococcus, Tannerella, Treponema* and *Veillonella.* To test whether the associations between periapical infection and birth outcomes were mediated by an intermediate variable, i.e. if that variable was on a causal pathway between the infection and birth

outcome, each potential intermediate variable was added to regression models one by one.

In all three studies, log-binomial or log-poisson models were used for binary outcomes and linear regression models for continuous outcomes to determine whether the exposure variables were associated with the primary outcomes. In the multivariate analysis, the following relevant and available covariates were applied using the forced-entry method: maternal age, height, BMI, socioeconomic score, parity, time between delivery and oral health examination, number of teeth, study site, nutrition intervention group, anemia status, HIV status, malaria status, periodontitis (when analyzing periapical infection) and periapical infection status (when analyzing periodontitis). There were some exceptions: 1) the nutrition intervention was used as a predictor variable in the first study but as a covariate in Studies II and III; 2) in the stratified analysis by HIV and malaria status in Study II, the variable with which the analysis was stratified was removed from the model; 3) in Study III, the time between wake-up and saliva collection and the time between the latest meal and the saliva collection were used as covariates in cortisol analysis models. Smoking was not used as a covariate, since all participants reported that they had never smoked. Before conducting the multivariate analysis, interactions between the intervention, oral infection outcomes and selected covariates were tested using the likelihood ratio test, and collinearity between the covariates was tested using variance inflation factor analysis.

In Study II, the covariate-adjusted population-attributable risk fraction (PAR%) was calculated for the binary outcomes. PAR% represents the reduction in the incidence of an outcome if the whole population was unexposed, e.g. how many preterm births could theoretically be avoided if there was no PAI in the population.

4.8 Ethical Aspects

The studies were conducted according to International Conference of Harmonization-Good Clinical Practice guidelines and the Ethical Guidelines of the 2008 World Health Association's Declaration of Helsinki (231). The College of Medicine Research and the Ethics Committee of the University of Malawi and the Regional Ethics Committee of the Pirkanmaa Hospital District in Finland reviewed and approved the iLiNS-DYAD study protocol with the oral health study amendment. Key details of the iLiNS-DYAD protocol were published at ClinicalTrials.gov with the identifier NCT01239693. Participating women signed or

thumb-printed informed consent forms before they were enrolled in the study. The participants received small incentives such as two bars of soap or one kilogram of rice after each data collection visit, subsidized healthcare and complimentary dental treatment after the oral health examination.

4.9 Author's Role

The author designed the iLiNS-DYAD oral health study entity, planned the data collection methods considering oral health, drafted the data collection forms and guides, purchased the data collection and dental treatment equipment and organized their shipment from Finland to Malawi. She also trained the research assistants who collected the data, and she organized regular standardization sessions throughout the data collection period, followed the data collection quality and completeness, maintained the data quality manual for the iLiNS-DYAD trial, cleaned the data and reported progress regularly to the study funders. Panoramic x-rays taken at the study site were sent electronically to the author, who analyzed them at the Tampere University Hospital department of radiology together with an oral and maxillofacial radiologist. The author also equipped the Mangochi district hospital dental clinic to provide good-quality dental treatment for those study participants who were diagnosed with oral diseases. The same services were provided also for other patients approaching the clinic during the study period. The author was present at the study site at the onset of the study and regularly throughout the data collection and cleaning period. While not at the research site, she was overseeing the data collection remotely on daily basis. The author analyzed the data and drafted all the three articles as first author, with the assistance of comments from the co-authors.

5 Summary of the Results

5.1 Enrollment and Follow-Up

The flow of participants in the studies included in this thesis is presented in Figure 8. A total of 9310 women were approached in the iLiNS-DYAD trial enrollment process. A total of 1391 participants were enrolled in the trial and randomized into IFA, MMN and LNS supplement groups. The enrolled mothers gave birth between May 2011 and February 2013, after which 1229 (88.4%) of them completed the oral health examination. After exclusions, 1024 participants were included in the oral health study analysis. Of those, 335 belonged to the IFA group, 346 to the MMN group and 343 to the LNS group. The reasons for exclusion were maternal death (n=8), twin pregnancy (n=14), oral health visit completed after the six-week puerperal period (n=193), and loss to follow-up (n=155).

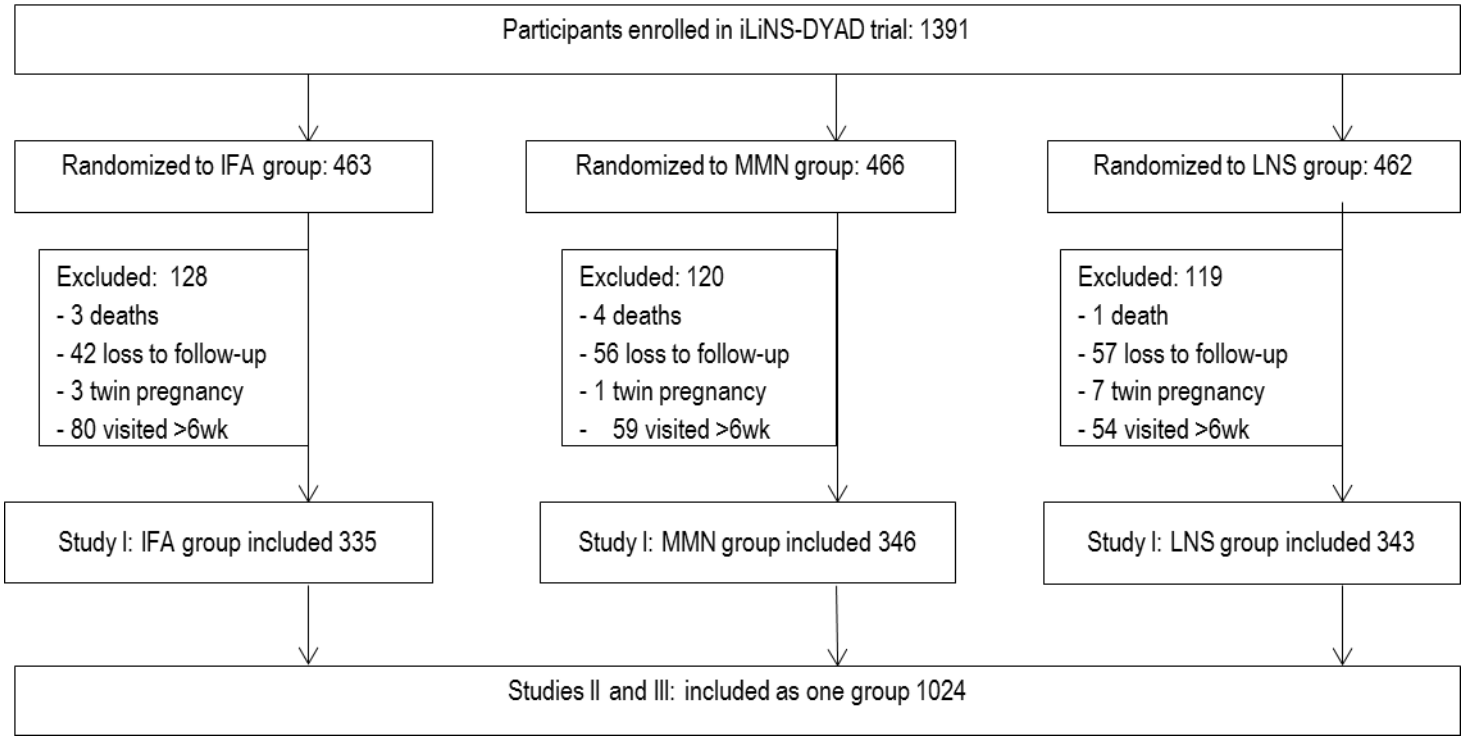


Figure 8. Participant flow in the three studies.

5.2 Background Characteristics of the Participants

At enrollment, the included participants had a mean (SD) age of 25 years (6.2), weight of 53.9 (7.7) kg and BMI of 22.1 (2.7) kg/m^2 and they had completed an average of 3.9 (3.4) years of education. The proportion of primiparous women was 17.8% and HIV positive women 14.1%. The prevalence of anemia was 19.8% and malaria 21.4%. There were slightly fewer participants in the IFA group than in the MMN or LNS groups, but their baseline characteristics were well in balance. The first column in Table 8 shows the background characteristics for all the included study participants combined, and the three following columns show the values by intervention groups

Table 8. Background characteristics of the included participants.

Characteristic	All combined	IFA	MMN	LNS
Number of participants	1024	335	346	343
Mean (SD) age, years	25 (6.2)	25 (6)	25 (6)	26 (6)
Mean (SD) education, completed years	3.9 (3.4)	3.9 (3.4)	4.0 (3.4)	3.9 (3.4)
Mean (SD) score for socio-economic status*	-0.13 (1.68)	-0.22 (1.59)	-0.07 (1.73)	-0.11 (1.72)
Primiparous women, % of total	17.8	18.0	18.6	16.9
Mean (SD) weight, kg	53.9 (7.7)	54.1 (7.2)	54.0 (8.1)	53.7 (7.8)
Mean (SD) BMI (kg/m^2)	22.1 (2.7)	22.1 (2.5)	22.1 (2.9)	22.0 (2.7)
Percentage of anemic women (Hb < 110 g/l)	19.8	21.5	18.2	19.8
Percentage with positive HIV test	14.1	15.0	11.6	15.7
Percentage with positive malaria test (RDT)	21.4	21.2	20.9	22.2

*Combining information on building material of home, source of water, sanitary facility, source of electricity, and type of cooking fuel. Table modified from Table 1 of Article I and Table 1 of Article II.

5.3 Oral Health Characteristics

Among the participants, the mean (SD) time between delivery and the oral health data collection visit was 16.0 (8.0) days. The mean (SD) number of teeth was 31.3 (1.6) and the prevalence (n) of caries 63.1% (646), deep caries exposing the pulp 27.8% (285), gingivitis 85.5% (876), periapical infection 23.5% (241) and periodontitis 31.9% (400) (Supplementary Table 1 of Article II). Only five participants had fillings in their teeth.

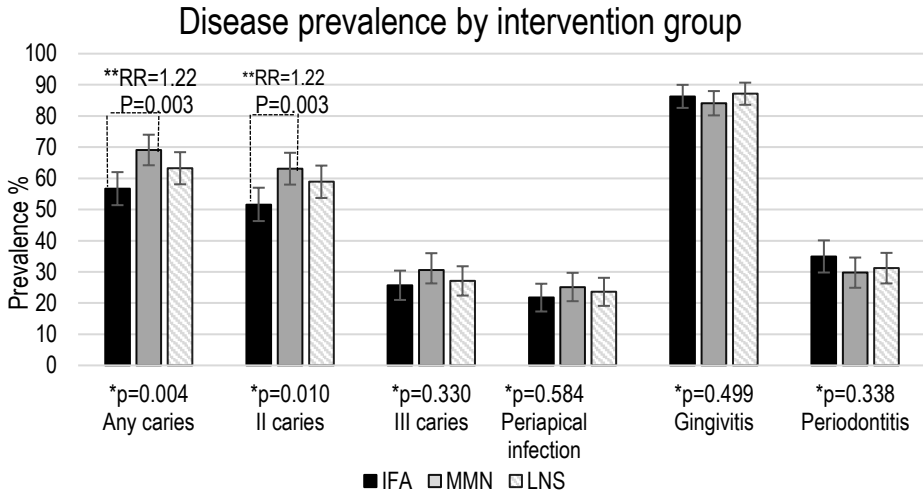
5.4 Oral Healthcare Habits and Self-Reported Oral Health Problems (Study I)

Most of the women (90.0%, n=922) reported that they used a toothbrush to clean their teeth, but less than half of them (43.0%, n=440) used toothpaste. Other cleaning devices mentioned were a finger (8.8%, n=90), stick (0.1%, n=1), sponge (0.1%, n=1) or nothing (0.8%, n=8), and cleaning materials were water only (41.2%, n=422), soap (6.2%, n=64), sand (5.1%, n=52), salt (1.7%, n=17), ash (1.7%, n=17) or nothing (0.8%, n=8). Of the women, 20.1% (n=205) indicated that they had experienced toothache, and 17.3% (n=176) had limited either the type or amount of food consumed due to oral health problems during the past six months. However, only 5.2% (n=53) had visited any healthcare provider to seek treatment, and only 11.0% (n=113) had received any treatment, including painkillers. The self-care habits or perceived oral health problems were similar in all the intervention groups (Table 4 in Article I).

5.5 Effect of the Nutrition Intervention on Maternal Oral Health (Study I)

At the end of the nutrition intervention period (after delivery), there was a statistically significant difference between the supplement groups in the prevalence of caries lesions. Grade II and Grade III caries lesions combined were 56.7% (n=190) in the IFA group, 69.1% (n=239) in the MMN group and 63.3% (n=217) in the LNS group, p=0.004). Grade II caries prevalence when the lesions were analyzed separately was 51.6% (n=173) in the IFA group, 63.1% (n=218) in the MMN group and 58.9% (n=202) in the LNS group (p=0.010). In contrast to the caries prevalence,

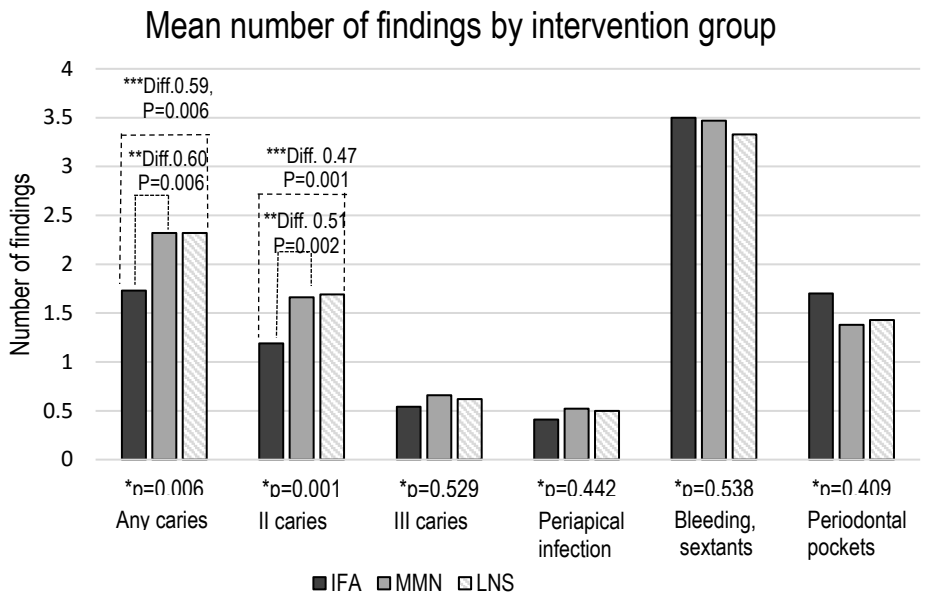
the prevalence of periodontitis was slightly higher but statistically non-significant among the IFA group women, compared with the MMN or LNS group women (34.9%, n=117; 29.8%, n=103; and 31.2%, n=107 respectively; p=0.338). In two group comparisons, the relative risk (95% CI) of having any caries at all was 1.22 (1.08-1.37, p=0.001) and of having Grade II caries was 1.22 (1.06-1.39, p=0.003) in the MMN group when compared with the IFA group. No other significant differences were found in any of the two group comparisons in any of the dichotomous disease outcomes. The disease prevalence and the significant risk ratios are shown by intervention group in Figure 9 below and in more detail in Table 2 of Article I.



* The p-value for three-group comparison with Fisher's exact test.
** Risk ratio, comparison between MMN and IFA groups. Figure based on Table 2 of Article I.

Figure 9. Prevalence (95% confidence interval) of oral diseases by intervention group.

Similar to disease prevalence, the mean number of caries-related continuous disease outcomes were higher and periodontal-disease-related outcomes lower in women in the MMN and LNS groups compared with those in the IFA group. The differences in pairwise comparisons were, however, statistically significant only in the number of any caries lesions when comparing the MMN and IFA groups or the LNS and IFA groups, and only in the number of Grade II caries lesions when comparing the MMN and IFA groups or the LNS and IFA groups. The means and significant differences are presented in Figure 10 and in greater detail in Table 3 of Article I.



* The p-value for three group comparisons with ANOVA.
** Difference in means between the MMN and IFA groups.
*** Difference in means between the LNS and IFA groups.
Figure based on Table 3 of Article I.

Figure 10. Mean number of oral disease findings by intervention group.

5.6 Pregnancy and Birth Outcomes (Studies II and III)

Data on the duration of pregnancy was obtained from all the participants, and birth weight from 956 (93.3%) and neonatal size – including weight, length and head circumference – from 957 (94.4%) of their infants (Figure 2 of Article II). Mean pregnancy duration, infant birth weight, neonatal length and neonatal head circumference are presented in Table 9. The incidence (n) of preterm birth was 7.9% (81) and low birth weight 12.0% (115), and prevalence of stunting 15.7% (152) and small head circumference 3.8% (37) among the participants.

Table 9. Summary statistics of the pregnancy and birth outcomes of the 1024 participants.

Birth Outcome	Mean (SD)	Min	Max
Duration of pregnancy, wks	39.4 (2.3)	17.7	45.0
Birth weight, g	2979 (430)	1200	4300
Neonatal length, cm	49.7 (2.2)	40.8	56.8
Head circumference, cm	35.2 (1.4)	29.8	39.2
WAZ	-0.54 (0.99)	-5.65	2.42
LAZ	-0.99 (1.09)	-6.23	2.13
Head circumference-for-age z-score	-0.13 (1.07)	-6.25	3.13

5.7 Associations between Oral Infections and Adverse Pregnancy and Birth Outcomes (Study II)

When analyzed by disease group, the continuous birth outcomes were essentially similar, with no statistically significant differences in means for participants without caries, with shallow caries or with deep caries when periapical infection was not present. Therefore, these three groups were combined into one "no periapical infection" group and, in subsequent analysis, compared with the group of participants who had at least one periapical lesion (PAI group). Continuous birth outcomes are organized into caries-related disease groups in Table 10.

Table 10. Continuous birth outcomes by caries-related disease group.

Birth Outcome	No Caries n=370	Grade II Caries n=354	Grade III Caries n=59	p-value*
Mean duration of pregnancy, wks (SD)	39.5 (2.1)	39.5 (2.2)	39.3 (2.2)	0.758
Mean birth weight, g (SD)	2977 (436)	3009 (428)	3029 (500)	0.534
Mean neonatal length, cm (SD)	49.8 (2.1)	49.8 (2.2)	49.8 (2.7)	0.975

* The p-value from ANOVA for comparison of groups of participants with no caries, Grade II caries and Grade III caries, all without periapical infection (PAI).

When analyzing continuous birth outcomes by PAI group, there were significant differences in all other analyzed outcomes except in mean neonatal WAZ and head circumference-for-age z-score. After adjusting the analysis with the selected covariates, the differences were significant in each birth outcome: the mean (95% CI) pregnancy duration for women with PAI was 0.4 weeks (0.1 to 0.8) shorter, and their infants had a 79 g (13 to 145) lower birth weight, 0.5 cm (0.2 to 0.9) shorter neonatal length, 0.27 units (0.11 to 0.44) lower LAZ and 0.18 units (0.01 to 0.35) smaller head circumference-for-age z-score than women without periapical infection. No statistically significant differences were seen between participants with or without periodontitis in any of the assessed continuous birth outcomes in non-adjusted or covariate adjusted analysis. The covariate-adjusted differences in mean birth outcomes are presented in Table 11 by infection group.

Table 11. Adjusted comparison of mean birth outcomes between groups of participants with or without periapical infection (PAI) and with or without periodontitis.

Birth Outcome	**Difference in Means** (95% CI), **PAI vs. No PAI**	**p-value**	**Difference in Means** (95% CI), **Periodontitis vs. No Periodontitis**	**p-value**
Duration of pregnancy, wks	-0.4 (-0.8 to -0.1)	0.014	0.2 (-0.1 to 0.5)	0.174
Birth weight, g	-79 (-145 to -13)	0.019	28 (-32 to 88)	0.360
Neonatal length, cm	-0.5 (-0.9 to -0.2)	0.002	0.1 (-0.2 to 0.4)	0.525
Neonatal WAZ	-0.14 (-0.29 to -0.00)	0.057	0.01 (-0.12 to 0.15)	0.839
Neonatal LAZ	-0.27 (-0.44 to -0.11)	0.001	0.06 (-0.08 to 0.21)	0.396
Neonatal Head-Z	-0.18 (-0.35 to -0.01)	0.033	-0.08 (-0.23 to 0.07)	0.310

Analysis adjusted for maternal age, maternal height, BMI, HIV status, malaria status and anemia at enrollment, number of previous pregnancies, study site, socioeconomic score, number of teeth, time between delivery and examination, nutrition intervention group, and periodontitis status when analyzing PAI and PAI status when analyzing periodontitis.

Table modified from Table 2 and Supplementary Table 2 of Article II.

The prevalence of adverse binary birth outcomes was also higher in women with PAI than in women without PAI, and lower in women with periodontitis than in

women without periodontitis. However, the only statistically significant associations were between PAI groups in stunting and small head circumference. The non-adjusted prevalence is presented in Table 3 and Supplementary Table 3 in Article II.

The results were essentially similar after adjusting for the selected covariates. After adjustment, the risk (95% CI) of stunting was 9.0% (2.7-15.2, p=0.007) and risk of small head circumference 4.5% (0.3-8.6, p=0.012) higher in women with PAI compared with those without. The population-attributable risk fraction (PAR%), i.e. the fraction of the adverse birth outcomes that would theoretically be avoided if the infection was eliminated from the target population, varied from 1.8% for low birth weight to 26.3% for small head circumference. The covariate-adjusted risk ratios and risk differences between the infection groups are presented in Table 12.

Table 12. Risk of adverse birth outcomes in women with periapical infection or periodontitis.

Birth Outcome	PAI Group Comparison[a]				Periodontitis Group Comparison[a]			
	RR (95%CI)	**Risk Difference[b]** (95% CI)	**p-value**	**PAR%**	**RR** (95%CI)	**Risk Difference[b]** (95% CI)	**p-value**	**PAR%**
Incidence of preterm birth	1.52 (0.93-2.47)	3.5% (-1.1 to 8.1)	0.092	9.7%	0.74 (0.45 to 1.22)	2.4% (-0.6 to 0.1)	0.236	-8.9%
Incidence of low birth weight	1.08 (0.67-1.72)	- 0.5% (-6.1 to 5.2)	0.785	1.8%	0.94 (0.60 to 1.46)	1.0% (-5.9 to 3.8)	0.786	-1.8%
Prevalence of neonatal underweight	1.52 (0.83-2.75)	2.9% (-1.5 to 7.3)	0.166	10.5%	1.02 (0.58 to 1.81)	0.1% (-3.5 to 3.3)	0.936	0.8%
Prevalence of neonatal stunting	1.68 (1.15-2.46)	9.0% (2.7 to 15.2)	0.007	12.8%	1.03 (0.71 to 1.48)	0.0% (-5.2 to 5.2)	0.895	0.8%
Prevalence of small head circumference	2.52 (1.23-5.16)	4.5% (0.3 to 8.6)	0.012	26.3%	1.95 (0.99 to 3.83)	2.5% (-0.4 to 5.4)	0.053	23.2%

[a] Adjusted for maternal age, maternal height, BMI, HIV status, malaria status and anemia at enrollment, number of previous pregnancies, study site, socioeconomic score, periodontitis, number of teeth, time between delivery and examination, and nutrition intervention group.
[b] The difference is expressed in percentage points.
PAR%= population attributable risk fraction.

Table modified from Table 3 and Supplementary Table 3 of Article II.

5.8 Placental Infection and Systemic Inflammation Markers (Study III)

Placental tissue samples were collected from 819 participants and chorioamniotic membrane samples from 807 participants. CRP and AGP concentrations were analyzed from 1017 participants at enrollment and 888 participants at 36 gw, and cortisol from 999 participants at enrollment and 863 at 36 gw (see Supplementary Figure 1 of Article III). The percentage (n) of women with evidence of histological inflammation or bacterial infection in the placenta or chorioamniotic membranes was as follows: chronic placental intervillositis 1.9% (19), acute placental intervillositis 21.2% (217), chorioamnionitis 19.9% (204), bacterial DNA in chorioamniotic membranes 59.2% (606) and bacterial DNA in placental tissues 38.5% (394). Thirteen of the selected, commonly mentioned PAI bacteria genera were found in both the placental tissues and membranes. The percentage (n) of women with evidence of systemic inflammation was as follows: elevated serum CRP concentration at 14-20 gw 39.1% (400) or 36 gw 29.7% (304), elevated serum AGP concentration at 14-20 gw 8.2% (84) or 36 gw 4.5% (46) and elevated salivary cortisol concentration at 14-20 gw 23.8% (244) or 36 gw 23.6% (242) (see Table 1 of Article III).

5.9 Interrelations Between Periapical Infection, Systemic Immune Response, Bacteremia and Adverse Pregnancy Outcomes (Study III)

Two biologically plausible pathways that could mediate the association between PAI and adverse pregnancy outcomes were investigated: the spread of periapical bacteria to the fetoplacental unit (direct pathway) and systemic inflammation (indirect pathway). Since periodontitis was found not to be associated with birth outcomes in Study II, the effects of periodontitis were not explored further.

5.9.1 Direct Pathway Markers

There were no statistically significant differences ($p>0.05$) in the prevalence of intervillositis or chorioamnionitis between participants with no PAI (n=783), mild-to-moderate PAI (n=215) or severe PAI (n=26) in the covariate-adjusted analysis (Table 2 of Article III). Similarly, the prevalence of any bacteria (bacterial 16S rRNA copies) in the placenta or membrane did not differ between the PAI categories (healthy 66.7%, n=523; mild-to-moderate 74.0%, n=159; severe 58.5%, n=15; and p=0.242; and healthy 44.8%, n=351; mild-to-moderate 53.7%, n=115; severe 38.6%, n=10; and p=0.470). Consistently, no statistically significant differences were detected between the groups of women with or without PAI in the mean bacterial loads (mean number of 16S rRNA copies) (Table 3 of Article III) or in the prevalence of different genera of bacteria in the placental tissues or membrane (Supplementary Table 3 of Article III). The findings were similar when analyzing the selected PAI genera of bacteria only or any bacteria genera that were found in at least ten participants.

5.9.2 Indirect Pathway Markers

When the mean CRP, AGP and cortisol concentrations were analyzed by PAI severity category, a positive dose-dependent association was seen at 36 gw with each of the inflammation markers. No such association was seen at 14-20 gw (Figure 11). The patterns of elevated concentrations of CRP, AGP or cortisol in the various PAI categories were similar to the mean concentrations, but the only statistically significant difference was seen in high cortisol at 36 gw (healthy 21.3%, n=168; mild-to-moderate 28.7%, n=62; severe 45.8%, n=12; and p=0.033).

5.9.3 Mediation of PAI: Birth Outcome Association by Systemic Markers of Inflammation and Cortisol

In the primary analysis (Study II), the mean duration of pregnancy was 0.4 weeks shorter (p=0.005), mean infant birth weight 90 g lower (p=0.009) and mean neonatal length 0.56 cm shorter (p=0.002) in women with PAI compared to those without. When the regression model was adjusted for AGP concentration at 36 gw, the difference in means was reduced by 0.1 weeks in the duration of pregnancy, 15 g in birth weight and 0.08 cm in neonatal length. Adjustment for CRP at 36 gw did not

affect the mean duration of pregnancy but it reduced the difference in mean birth weight by 5 g and neonatal length by 0.02 cm. When adjusting for cortisol concentration at 36 gw, the difference in means was reduced by 0.1 weeks in duration of pregnancy, 10 g in birth weight and 0.02 in neonatal length. The p-values of each of the models increased after each adjustment, i.e. the association weakened, indicating that the inflammation marker was at least partly on the pathway between PAI and the birth outcome (Table 5 of Article III). The results after adjustment by combinations of AGP, CRP and cortisol did not differ substantially from adjustment only by AGP, i.e. the effects were not additive.

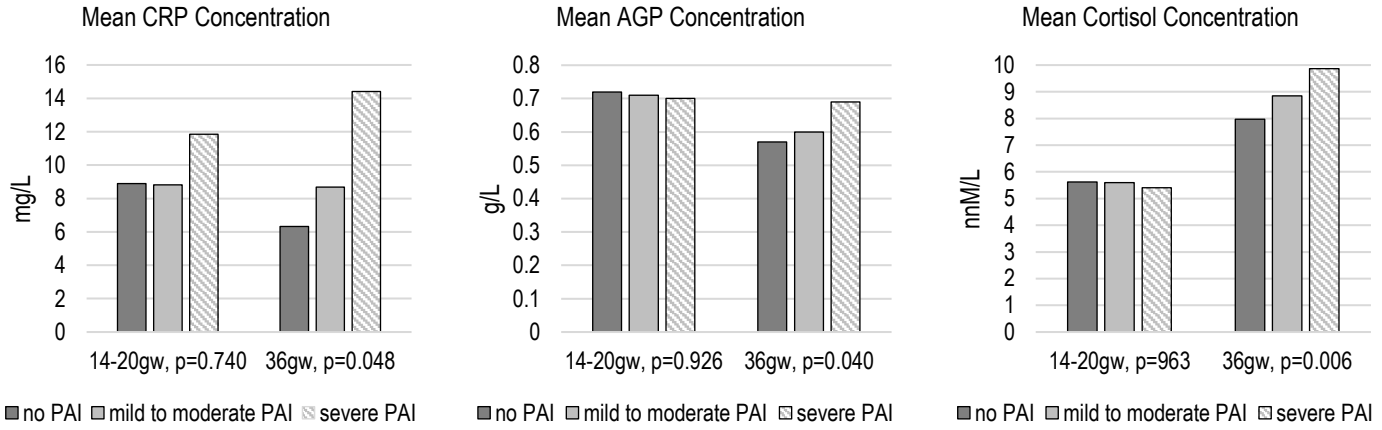


Figure 11. Mean CRP, AGP and cortisol concentrations at 14-20 weeks and at 36 weeks of pregnancy by maternal periapical infection severity.

Models adjusted for HIV status, malaria status, height, body-mass index, age, study site, number of teeth, nutritional intervention group, anemia, periodontitis status, household assets, and oral health examination visit time. Cortisol adjusted also for saliva collection time and time since last meal. Figures based on Table 4 of Article III.

6 Discussion

The aim of Studies I-III, which underlie this thesis, was to evaluate the effect of supplementing pregnant women's diets with MMNs or LNS on their oral disease prevalence, and the association between the oral diseases and birth and pregnancy outcomes, as well as the possible pathways linking PAI and adverse pregnancy outcomes. The hypotheses were that supplementation of the maternal diet during pregnancy affects oral health and that oral infections affect pregnancy outcomes. The results suggest that maternal diet supplementation with multiple micronutrients during pregnancy in resource-poor environments may enhance caries development. PAI, the common sequela of untreated caries, may in turn affect pregnancy adversely through elevated systemic inflammation.

In this chapter, the strengths, weaknesses and limitations of the studies in meeting their aims are reviewed. After that, the findings of the three studies are discussed separately in context of other published studies, and, lastly, general conclusions based on the findings and earlier literature are presented.

6.1 Strengths and Weaknesses of this Research

The three studies had several common strengths. Firstly, data collection and quality assurance were conducted rigorously according to structured forms, user guides, and standard operating procedures during the visits, and by performing regular reliability checks and retraining. Secondly, gestational age was measured by ultrasound at enrollment, which allowed prompt calculation of duration of pregnancy. Also, the anthropometric measurements were collected in triplicate by two experienced anthropometrists. Thirdly, the oral disease diagnostics were based on both a comprehensive clinical examination and a radiological assessment that permitted precise diagnostics.

The individual studies had also specific strengths. In Study I, a major strength was the participants' random allocation to the three supplement groups that led to similarity in a wide range of baseline characteristics, even after the oral health analysis exclusions were made. Furthermore, the data collectors and researchers were blinded

to the group allocation until after the data collection was finished and the database was cleaned. In Study III, one specific strength was the various biological samples that were collected during pregnancy and after delivery, which made it possible to investigate the biological pathways in several ways.

There were also limitations that should be considered when interpreting the results. The main limitation of all the studies was the lack of baseline oral health data and the varying length of time between delivery and the oral health examination. The reason for not collecting baseline data was the radiological examination; such examination is not ethical for asymptomatic pregnant women. What is more, diagnosing and treating the diseases at the beginning of the study would have thwarted the aims of the study. The time gap between delivery and the examination was due to the difficulties that the mothers would encounter in having to travel from their villages to Mangochi study site along rough roads immediately after delivery. Another limitation was the 26.4% loss in follow-up between enrollment and the oral health examination, some differences between the baseline characteristics of the included and excluded participants, and some missing biological sample data. In addition, there might have been some underestimation in the prevalence of PAI due to lesions being obstructed by other anatomical structures in the x-rays. A further limitation in Study III was that placental tissue was mostly not collected from mothers who delivered very preterm, nor were biological samples collected at 36 weeks if the delivery took place before 36 gw. In addition, because the biological sample collection was planned with the iLiNS-DYAD study entity in mind, the biological samples were not collected at the same time as the oral health examination took place.

To overcome these limitations, several adjusted analyses were conducted that included the baseline characteristics that differed between the included and excluded participants together with the time between delivery and examination. Because the results of the adjusted and unadjusted analyses were similar, it is unlikely that there was much selection or follow-up bias involved. The limitation of missing biological sample data in Study III was handled by imputing the missing data points with multiple imputations that led to full datasets. Finally, it is unlikely that the prevalence of periapical lesions changed markedly between enrollment and the examination, since the lesions were almost entirely related to deep caries lesions, which take many months to years to develop (34). Sensitivity analyses that included missing teeth in the number of deep caries lesions confirmed this assumption, since the only available treatment for dental infections in this environment was tooth extraction.

6.2 Prevalence of Caries and Periodontitis

The overall prevalence of untreated caries, 63.1%, was very high in this study population compared with the global prevalence of 36.2% in women and the prevalence in Eastern Sub-Saharan Africa of 36.4% that combined both genders in the GBD study (15). The differences may be at least partly explained by the varying diagnostic methods. Caries diagnosis in epidemiological studies is typically based only on clinical examination, whereas the present study also utilized x-rays to detect invisible lesions between teeth and under thick layers of enamel, which improved the accuracy.

Another explanation for the high caries prevalence might be high fluoride levels in drinking water which is seen sporadically in this study area located in the Rift Valley (232). Although optimal fluoride intake is beneficial in caries prevention, excess amounts during tooth development cause fluorosis, which changes the structure of the enamel, predisposing it to hypomineralization (233). As an example, the fluoride level in the groundwater in the Mangochi hospital area is reported to be 2.45 mg/L, which is considered toxic and far above the safe limit of 1.50 mg/L (232). In addition, malnutrition during the infancy and childhood of these mothers may have affected the development of their tooth and salivary gland structures (70). As presented in Table 4 above, the prevalence of stunting and underweight, which are measures of chronic undernutrition, is high in childhood in Malawi. Also, malnutrition in later life may have altered their salivary gland functions (70) or caused the oral microbiota to become more pathogenic, predisposing these mothers to caries development (127).

Because of the high prevalence of caries, it is not surprising that one in every five women had experienced toothache and limited their food intake due to the problems. The finding is important, since a reduced ability to eat could possibly contribute to malnutrition in areas where the availability and variety of food is limited. The fact that only about 5% of the mothers had visited any healthcare provider supports the assumptions that treatment is not available when needed, as seen also in many other African countries where oral diseases are very common (16).

Regarding periodontitis, comparison between disease prevalences is rather difficult due to different methods and definitions used in various studies, including the GBD study, which reported only data on severe periodontitis (96). In a comprehensive review of studies from many African countries conducted in 2002, the periodontitis prevalence varied between 2% and 9% in Malawi, depending on the age group, and reached almost 100% in some sub-Saharan African countries

(234). The prevalence of periodontitis 31.9% in the present study was thus much higher than in the other Malawian studies, but much lower than the average in the other African countries.

6.3 Effects of Nutrient Interventions on Oral Health

The purpose of Study I was to explore whether the nutrient interventions during pregnancy had any effect on the oral health of the mothers. Considering the usually slow development of dental caries, it is somewhat unexpected that the prevalence of caries and the mean number of caries lesions were found to be significantly higher in mothers who had received MMNs or LNS compared to those who had received IFA. In contrast, those who had received IFA had a slightly higher prevalence of gingivitis and periodontitis and mean number of periodontal pockets compared with the other groups, but these differences were not statistically significant. The self-care behavior or other background characteristics could not explain the differences in the oral disease parameters because they were similar in all groups.

Earlier studies exploring the relationship between nutrients and oral health concentrate on non-pregnant populations mostly in high-income countries, assessing intake of single nutrients using food frequency questionnaires or measuring serum or plasma nutrient concentrations cross-sectionally (105,120). Because no other trials assessing the effect of multiple micronutrient supplementation on caries levels or periodontal health in pregnant women could be identified, straight comparisons with other studies cannot be made as such.

Since sugar intake is the most important risk factor for caries (69), it is possible that the small amount of sucrose in LNS enhances caries development. However, the prevalence of caries was the highest in the MMN group, which did not contain any sugar, indicating that the difference is not likely to be related to sugar intake. Explanations for the differing caries levels must then be sought from the systemic effects of micronutrient intake and from the potential effects of certain nutrients on the oral microbiota. One explanation could relate to iron, whose concentration was higher in the IFA supplement than in the MMN and LNS supplements. It is possible that the higher level of iron intake suppressed caries development in the IFA-supplemented mothers by reducing their enamel demineralization, which has also been seen in other studies (111,235,236).

Another possible explanation for the differing caries prevalence relates to the nonlinear relationship between micronutrient availability and the growth of

cariogenic bacteria (125). The pattern in which an increasing nutrient supply exacerbates the growth of *S. mutans* to a certain threshold, only after which the effect is inhibitory, has been demonstrated in some in vitro studies on iron, manganese, magnesium, zinc, cobalt, tin, copper and calcium (237-239). Although not confirmed, the same pattern may apply to other acidogenic bacteria as well. In addition, a higher dietary protein content has been shown to have direct effects on caries-related oral microbiota in a murine model (240).

Based on these findings, it seems plausible that, in low-resource conditions with a high prevalence of both malnutrition and untreated caries lesions, nutrient supplementation with MMNs or LNS might increase the pathogenicity and amounts of cariogenic bacteria, thus enhancing caries development. Altered salivary gland functions, hypoplastic enamel structure and pregnancy-related immune suppression may further reinforce the effects (241). Whether the effect of the supplementation would be protective after a longer intervention period remains to be confirmed.

In the case of periodontitis, the only multiple-micronutrient supplement intervention study conducted on adults that could be identified has a very small sample size. In that study, two groups comprising respectively 31 and 32 adults with moderate periodontitis received either a tablet containing seven vitamins or placebo, and their periodontal status was assessed at baseline and again after 60 days. In that study, a significant reduction was seen in the periodontal pocket depth and gingival index in the intervention group compared with the controls. (242)

Also, many other studies assessing single nutrients such as vitamins B, C, or D or calcium, magnesium or fatty acids have concluded that their low intake or low plasma concentration is associated with the progression of periodontal diseases or improved periodontal health (10,120,121,124,243). These beneficial effects most probably relate to enhanced immune functions and tissue formation and repair in the periodontium. Although not confirmatory, the findings of the present study somewhat support the results of these studies, suggesting that an increased micronutrient intake may have beneficial effects on periodontal health.

One explanation for the small, non-significant intergroup differences in this study between the supplementation groups in periodontal parameters could be in the pregnancy-related hormonal changes in the periodontium. Those changes, which include increased bleeding and swelling (170), may to some extent have masked the effects of the interventions. Another potential explanation is the rather short intervention period compared with the slow appearance of x-ray-detectable changes in alveolar bone (244). With a longer intervention period, the changes might have been more pronounced. In addition, differences between the periodontal-health-

related findings in this study and in the previous studies might be explained by the differing baseline levels of nutrients and the diseases, which were not recorded.

Based on the findings presented above and from the literature review, the somewhat contradictory findings of enhanced caries development and improved periodontal health, even in one individual mouth, that followed MMN and LNS intake most probably relates to the differing nutrient intake mechanisms of the cariogenic and periodontal bacteria and different immune mechanisms related to the diseases.

6.4 Association Between Oral Diseases and Birth Outcomes

Study II concluded that women with PAI had a shorter pregnancy duration and delivered infants with a lower birth weight, shorter stature and smaller head circumference than mothers without PAI. In addition, the prevalence of PTB and stunting were significantly higher in mothers with PAI compared with those without PAI. No difference was found in parameters of caries or periodontitis between the women with and without PAI.

A recent review and meta-analysis summarized all the published studies of sufficiently high quality on dental caries and PTB (245). In addition to the present study, the review included nine studies conducted in Finland, France, India, Jordan and Mexico. Some of them were presented above in the literature review. All the individual studies and the meta-analysis reached the conclusion that there is no association between uncomplicated caries (no pulp exposure) and preterm birth. The meta-analysis on the risk for PTB yielded an OR of 1.16 (95% CI, 0.90 to 1.49, p=0.25) and a number of decayed (carious), missing or filled teeth (DMFT index) that was no different in women with PTB than in those with a normal pregnancy duration.

Since publication of the results of the present study, two other studies have also been published that examine PAI and pregnancy outcomes and support this association. In 2015, Leal et al. conducted a study among 63 postpartum mothers in Brazil using periapical radiographs. They concluded that mothers with PAI had 3.52 times higher odds for delivering preterm and LBW than mothers who did not have PAI. They also found a dose-dependent association with their PAI score and duration of pregnancy and birth weight (246). The other study, conducted by Khalighinejad and co-authors in 2017 (247), investigated the association between maternal PAI and pre-eclampsia among 100 pregnant women, utilizing panoramic

radiographs taken prior to pregnancy. They found a significantly higher prevalence of PAI in mothers who had pre-eclampsia, with 2.23 times higher odds for development of the condition. The explanation for why caries does not seem to associate with birth outcomes but PAI does relates most probably to systemic dissemination: bacteria in uncomplicated caries do not have access to the systemic circulation, but, in the case of PAI, the pathway exists.

Interestingly, the differences in birth weight and in prevalence of stunting and wasting between the PAI groups in the present study were very similar to what was detected in a study that tested the effect of maternal azithromycin and sulfadoxine-pyrimethamine (SP) treatment during pregnancy on improvement of birth size of Malawian infants (248). Azithromycin is a broad-spectrum antibiotic used to treat sexually transmitted and other reproductive tract infections, but is also effective against many oral pathogens (249), whereas SP is an antimalarial drug. It is therefore possible that at least part of the improvement in birth size in that study was accounted for by the elimination of the oral-pathogen-mediated inflammation. A recently published five-year follow-up on those children showed that the positive effects of azithromycin and SP treatment were sustained and may have also reduced mortality and improved the cognitive development of the children (250).

The seemingly small differences between the PAI groups in birth outcome variables are remarkable at a population level. According to the results, if PAI was eliminated from this population, it would reduce the incidence of PTB by 9.7%, underweight by 10.5%, stunting by 12.8% and small head circumference by 26.3%. Although based only on mathematical calculations, the figures indicate that maternal PAI poses a marked risk to infant health. A pathway analysis that was conducted in this same study population reinforced these conclusions (251). The analysis – which included several maternal constitutional, nutrition status, and infection status variables – concluded that PAI was one of the most important risk factors of all the studied factors for shortened duration of pregnancy and lower LAZ of the infant.

The contradiction that periodontitis was not associated with any birth outcomes in this population, but PAI was, most probably stems from the multifactorial nature of the diseases. Mechanisms of periodontitis and PAI resemble each other in many ways: both are chronic polymicrobial infections with a similar Gram-negative-dominated microbiota (26,252) that can be disseminated through circulation, causing transient infections (208). Both diseases also activate the innate immunity that upregulates the production of inflammatory mediators that elevate systemic inflammatory responses (253). Nevertheless, there is an array of factors in the processes with a range of effects and significance that may contribute and modulate

the association between the infections and the birth outcomes, either potentiating or decreasing it. These factors include local conditions in the mouth, systemic factors related to the host, and external (environmental) factors (62).

An important difference between the diseases is the epithelial barrier, which is present in the periodontium but missing between the infected root canal and the highly vascular granulomatous tissue in the periapical lesions. This missing barrier could allow the bacteria and their byproducts to disseminate more easily into the systemic circulation in mothers with PAI (254). Hypothetically, in this kind of a low-resource environment, in which other infections are also common, the effects of low-grade periodontal infection may be "masked" by the cumulative infection burden, while the association between the more "aggressive" PAI and adverse birth outcomes may be present. Pregnancy, nutrition deficiencies and the nutrition supplements may also contribute to the processes modulating the oral microbiota and the immune functions. Other population characteristics such as race and gene polymorphism may also contribute to the susceptibility and adverse effects of these diseases (64,255,256).

6.5 Interrelations Between Dental Periapical Infections, Systemic Immune Response and Adverse Pregnancy Outcomes

Study III was designed to explore the possible causal mechanisms underlying the associations between periapical infection and adverse birth outcomes that were detected in Study II. Because periodontitis was found not to be associated with birth outcomes in this population, periodontal status was used in Study III only as a covariate in the multivariate analyses. The study concluded that the association was at least partly mediated through the indirect mechanism of elevated systemic inflammation as measured by CRP, AGP and cortisol levels. No evidence of the direct mechanism of bacterial dissemination into the fetoplacental unit was found.

Direct Mechanism

Although several pathogens that are commonly found in infected root canals were detected in the placental tissues of the mothers in this study, no difference was observed between the mothers with or without PAI in any of the histological inflammation or bacterial DNA variables. One explanation for this may be the

missed very early preterm births and miscarriages, in which oral pathogens have been shown to be commonly involved (208). Without exemptions, those births occurred at home, where the fetal and placental tissues were immediately discarded.

Furthermore, it is possible that bacterial presence differed in locations that were not analyzed in this study, such as in other parts of the placenta or membranes, in amniotic fluid, in cord blood or even in the fetus itself. For example, it has been shown that oral bacteria can enter the fetus, whose immune response against the pathogens induces local production of pro-inflammatory cytokines, resulting in placental inflammation, which in turn may lead to IUGR (205).

Indirect Mechanism

The present study's findings that PAI elevates the acute phase protein CRP and AGP concentrations in a dose-dependent manner are supported by several earlier studies (253,257). The induction of a systemic immune response relates to the release of inflammatory modulators such as pro-inflammatory cytokines and MMPs from the infected periapical tissues (37,253,258), which induces the production of CRP and AGP in the liver (259,260). Although other studies associating PAI and salivary concentrations of cortisol were not found, the findings that cortisol concentrations rise in a dose-dependent manner in response to other infections such as periodontitis are in line with the results of the present study (221,261).

The fact that the elevated inflammatory response was seen only in the 36th-week samples and not earlier is most likely connected with the pregnancy-related down-regulation of the immune responses. At early gestation, innate immunity can restrict the effects of chronic low-grade infections, but the susceptibility increases in later pregnancy, leading to a systemic response (262).

In the present study, the association between PAI and shortened duration of pregnancy and smaller infant size was found to be mediated partly by CRP, AGP and cortisol. It has been shown earlier that maternal infections during pregnancy can lead to preterm birth and IUGR through various inflammatory pathways that are also active in the presence of PAI (263,264). Elevated levels of CRP and AGP can amplify the inflammatory response in the fetoplacental unit through complement activation, tissue damage and induction of pro-inflammatory cytokines, affecting fetal growth and the continuity of pregnancy (212-214). Placental inflammation also downregulates the expression of genes related to the growth and development of the placenta and fetus, possibly leading to IUGR (205). In addition, cytokines such as

IL-6 and TNF-α regulate the nutrient transfer between mother and fetus by altering the expression and activity of placental nutrient transporters (265).

Elevated salivary cortisol concentrations have in turn been associated with increased growth and virulence of oral pathogens (266) and with poor pregnancy outcomes (220). Suggested mechanisms include inhibition of nutrient transport through the placenta, resulting in IUGR (160,267), and stimulation of placental corticotropin-releasing-hormone production, whose release into the maternal and fetal circulations contributes to the induction of labor (268).

The results of the present study explain the mediation between PAI and birth outcomes only partly: there are clearly also other inflammation-related mechanisms involved that could not be investigated due to limited data. One of the possible mechanisms relates to PMNs that are the dominant defense cells in active PAI lesions (40). PMNs are an important source of PGE_2, which has been found in high levels in PAI lesions. In addition, IL-1, IL-6 and TNF-α produced in PAI lesions can further stimulate the production of PGE_2 (269-271). In normal pregnancy, amniotic fluid concentrations of PGE_2, TNF-α, IL-1 and IL-6 rise until they reach a threshold level that is believed to induce labor (272). Hence, elevated levels of these biomarkers in maternal serum have been associated with early ripening of the cervix, premature rupture of the membranes, and uterine contractions, leading to PTB and LBW (269-271).

Another possible "missed" mechanism relates to insulin-like growth factors (IGFs) that the placenta and the fetus secrete and that participate in fetal length growth regulation. Maternal systemic inflammation and placental infection have been associated with downregulation of IGFs and increased concentrations of their binding and inactivating proteins in the fetus (160,273).

7 Conclusions

The main conclusions of this study can be summarized as follows:

1. Complementary food supplementation with MMNs or LNS may enhance dental caries development in pregnant women in low-resource conditions. It may have some beneficial effects on periodontal health, but the differences between the intervention groups were statistically non-significant in this study.

2. Maternal periapical infection is associated with a shortened duration of pregnancy and intra-uterine growth restriction in low-resource conditions. No evidence of association between dental caries or periodontitis and adverse pregnancy outcomes was detected in this study population.

3. Periapical infection activates systemic inflammation and may affect pregnancy adversely through this indirect pathway. No evidence was found on a direct pathway in which periapical bacteria cause a local infection in the placenta or amniotic membranes that affects the pregnancy.

8 Public Health Implications and Future Research Needs

The main findings of this PhD research – increased caries prevalence due to micronutrient intervention and an association between PAI and adverse birth outcomes – have not previously been widely studied and are thus novel. For this reason, the findings should be approached with caution and investigated further to determine whether these associations are seen also in other populations and environments and did not occur simply by chance. However, if the findings are confirmed in other studies, they will have important public health impacts.

Given the high overall prevalence of oral diseases and their direct and indirect effects on general health and well-being, the seemingly small impact of micronutrient intervention on oral diseases may actually be quite considerable at a public-health level. This is especially important in low-income settings, where dental care resources are scarce and other risk factors prevalent. Thus, this risk should be taken into account when planning a nutrition intervention program: preventive oral health measures such as self-care education, toothbrush and toothpaste distribution, or fluoride supplementation in deficient areas (274) should be included in such a program.

To investigate this issue further, a follow-up study with oral health examination conducted at baseline and after intervention is warranted. In such a study, initial caries lesions should also be recorded to see whether the intervention enhances the development of new caries lesions or whether it simply advances the existing ones. One innovative idea would be to add xylitol to the LNS supplements. Xylitol is a polyol sweetener that increases saliva flow and reduces *S. Mutans* in the dental biofilm, thus preventing caries development (275).

Regarding adverse birth outcomes, the prevention of PTB and IUGR is a global priority, especially in low-resource settings, which is where most of the cases and their consequences occur (5). An important target in such prevention efforts is infections, since it has been suggested that the health consequences of PTB and LBW are even more severe when they relate to maternal infections during pregnancy (276).

Preventing dental caries, which usually precedes PAI, is relatively easy and inexpensive, even in low-resource environments, because it is based mostly on knowledge and correct self-care habits. In addition, preventive programs that incorporate these factors have also been found to be effective (277,278). Furthermore, it can be predicted that the incidence of the already "omnipresent" caries may increase exponentially in the future due to changing dietary patterns from traditional to cariogenic "Western" diets in low-income countries (14). It would thus be beneficial to target especially girls and young women as part of other public health programs and health services. The outreach should also involve professionals other than dentists, for example at antenatal clinics or at schools, since this would reach an even wider audience and would be cost-effective as well.

In addition to reaching out to other populations and environments, further studies would also be needed to see whether the suggested effects of PAI would be more pronounced when early preterm deliveries are included. Furthermore, other inflammatory pathways possibly linking PAI and adverse birth outcomes, as well as the impact of PAI elimination on pregnancy outcomes, should be investigated in future studies to achieve a greater understanding in the field and the ability to target the preventive and therapeutic measures to the correct points in the cycle.

9 Acknowledgements

The study was carried out at the Tampere Center for Child Health Research, Global Health group, at the University of Tampere's Faculty of Medicine and Life Sciences as part of the iLiNS Project. The work was supported in part by a grant from the Office of Health, Infectious Diseases and Nutrition, Bureau for Global Health of the U.S. Agency for International Development (USAID), through the Food and Nutrition Technical Assistance III Project (FANTA), managed by FHI 360. Additional funding was provided by the Bill & Melinda Gates Foundation through a grant to the University of California, Davis. I also received personal grants from the Finnish Cultural Foundation, the joint fund of the Finnish Dental Society Apollonia and the Finnish Dental Association, and the Hilda Kauhanen Memorial Fund, as well as travel grants from the University of Tampere Foundation, the Finnish Dental Society Apollonia and the Finnish Female Dentists' Society.

There are many people who have helped and supported me during this PhD process. It is impossible to thank all of you individually, but there are some who deserve to be specifically mentioned.

First is my supervisor, Professor Per Ashorn, who provided me the opportunity to join this amazing journey – one that has changed me and the way I think forever. He introduced me not only to the world of science, but also to global health education and many wonderful, likeminded people. He often believed in me much more than I did myself, helped me cross an endless number of boundaries and achieve levels of success far beyond my expectations. His wisdom and ability to guide me to the best solutions is phenomenal. Thank you for always being in reach whenever you were needed.

I would also like to thank my advisory board members Professor Mika Rämet, Professor Heikki Murtomaa and Dr. Helena Forss for guiding me in the right direction and giving me inspiration; preliminary examiners Professor Jukka Meurman and Dr. Tuula Pelkonen for the enlightening conversations and comments that helped to improve the thesis; Dee Shields for editing the language; and Dr. Matthew Chico for consenting to examine my work in a public defense.

My gratitude and thanks also go to the entire international ILiNS team for their wonderful collaboration, especially Professor Kay Dewey for her endless support

and encouragement, invaluable comments on the manuscripts, and hospitality during my short visit to the University of California at Davis; to Dr. Mary Arimond for her iLiNS project coordination and all her advice; and to Professor Ken Maleta for his outstanding management of the iLiNS-Malawi team and his collaboration in Malawi.

I would also like to thank Professor Yin Bun Cheung and Lotta Alho, who taught me most that I know about statistics and who patiently guided me through the almost impossible challenges of selecting and mastering the correct methods, creating functioning do-files and understanding the results. My special thanks go, too, to Dr. Anna Pulakka, my fellow student and dear friend who, being a few steps ahead, kindly and wisely advised and encouraged me at every stage of the study and pulled me across the finish line. Lotta, Anna and I spent months together in Malawi and travelled in other African and European countries, sharing serious work, joyous adventures and true friendship that I will miss for the rest of my life. I am also grateful to Dr. Kirsi-Maarit Lehto, my technical and mental support and a great friend who knows how to chase the sorrows away. I am utterly certain that, without you ladies, I would never have made it.

I thank Jorma Järnsted, with whom we spent hundreds of hours in the darkroom of the Tampere University Hospital assessing x-rays and sharing thoughts about science and life. Without you, the x-ray data quality would be far removed from the excellence that it is now and the work would have been much more arduous.

Additional thanks go to Professor Nigel Klaine and Ronan Doyle, who analyzed the bacterial data, provided their expertise and endured Finnish sauna and swim in an icy lake. Your hilarious company made me forget all my worries.

I would like to thank the skillful, dedicated and persevering iLiNS-Malawi team in Mangochi, Lungwena, Malindi, and Namwera who collected and entered the data, overcoming endless numbers of challenges without hesitation. Deserving of special mention are dental therapists Simeon Mulewa and Davie Charlie for their precise and tireless work on the oral health data collection and treatment of the participants, as well as for giving me insight to Malawian life. I am also grateful to all those who supervised or participated in the collection or entry of iLiNS data and who made my stay and work in Malawi so enjoyable: John P, John S, Enita, Harmony, Eletina, Thoko, Nozga, Misonzie, Brains, Joy, Mathias, Mofolo, Shaibu, Bridget, Harriet, Martin, Lifiness, Vera and Jimmy, to name but a few.

My heartfelt gratitude goes to the study participants, whose commitment to the iLiNS study with dozens of visits was tremendous and without whom there would naturally be no results.

I am also grateful to have had the rest of the amazing DIH and iLiNS families, fellow PhD students and co-authors as good friends, co-workers and supporters who made this journey enjoyable and memorable: Yuemei, Juha, Ulla A, Basho, Tiina, André, Chiza, Minyanga, Austrida, Jaden, Charles, Chrissie, Josh, Mike, Marjorie, Lindsay, Steve, Stephen, Christine, Brietta, Megan, Liam, Emma, Valerie, Beth, Jaimie, Mihir, Mikko, Outi, Johanna, Matti, Hanna, Saara, Fida, Pauliina, Mari, Heli and many others.

Many thanks to Mikko Piippo and Eeva Torppa-Saarinen for understanding the challenges that this research and thesis writing posed for my other work, and for always being flexible. I am also grateful for Mamane Zeilani, Dr. Laura Tarkkila, Dr. Taneli Puumalainen and Dr. Satu Lahti, who in critical moments, when I was about to lose my hope, made me believe in myself again and finish this thesis.

My other friends and relatives who have walked this journey with me from various starting points also deserve my thanks. You always reminded me that there are also other important and joyful things in life outside science.

To my parents, who have appreciated education and wisdom, set an example, encouraged me in my studies and always believed in me, heartfelt thanks for your support, which has been invaluable. Lastly, my deepest gratitude belongs to my husband and children. Antti, you never questioned my decisions and always made it possible for me to fulfill my dreams. Your mental and practical support has been amazing. You have tirelessly listened to my worries and borne the responsibility of our home and kids when I was away. This would have never been possible without you. Samuel and Eelis, my precious, beloved sons, you have patiently endured the travelling laptop-mom who has been working towards a PhD for most of your lives. Believe or not, it is now over and done!

Tampere, August 2018
Ulla Harjunmaa

10 References

(1) Lee AC, Katz J, Blencowe H, Cousens S, Kozuki N, Vogel JP, et al. National and regional estimates of term and preterm babies born small for gestational age in 138 low-income and middle-income countries in 2010. Lancet Glob Health 2013 Jul;1(1):e26-36.

(2) Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet 2008 Jan 19;371(9608):261-269.

(3) Katz J, Lee AC, Kozuki N, Lawn JE, Cousens S, Blencowe H, et al. Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis. Lancet 2013 Aug 3;382(9890):417-425.

(4) Petrou S, Henderson J, Bracewell M, Hockley C, Wolke D, Marlow N, et al. Pushing the boundaries of viability: the economic impact of extreme preterm birth. Early Hum Dev 2006 Feb;82(2):77-84.

(5) Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008 Jan 5;371(9606):75-84.

(6) WHO. Guideline: Daily iron and folic acid supplementation in pregnant women. Geneva: World Health Organization; 2012.

(7) Haider BA, Yakoob MY, Bhutta ZA. Effect of multiple micronutrient supplementation during pregnancy on maternal and birth outcomes. BMC Public Health 2011 Apr 13;11 Suppl 3:S19-2458-11-S3-S19.

(8) Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, et al. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Lancet 2013 Aug 3;382(9890):452-477.

(9) Ramakrishnan U, Grant FK, Imdad A, Bhutta ZA, Martorell R. Effect of multiple micronutrient versus iron-folate supplementation during pregnancy on intrauterine growth. Nestle Nutr Inst Workshop Ser 2013;74:53-62.

(10) Neiva RF, Steigenga J, Al-Shammari KF, Wang HL. Effects of specific nutrients on periodontal disease onset, progression and treatment. J Clin Periodontol 2003 Jul;30(7):579-589.

(11) Enwonwu CO. Interface of malnutrition and periodontal diseases. Am J Clin Nutr 1995 Feb;61(2):430S-436S.

(12) Stephen M. The role of diet, fluoride and saliva in caries prevention. J Indian Soc Pedod Prev Dent 1997 Dec;15(4):109-113.

(13) Barak S, Oettinger-Barak O, Oettinger M, Machtei EE, Peled M, Ohel G. Common oral manifestations during pregnancy: a review. Obstet Gynecol Surv 2003 Sep;58(9):624-628.

(14) Petersen P, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ 2005;83(9):661.

(15) Marcenes W, Kassebaum NJ, Bernabe E, Flaxman A, Naghavi M, Lopez A, et al. Global burden of oral conditions in 1990-2010: a systematic analysis. J Dent Res 2013 Jul;92(7):592-597.

(16) Beaglehole J, Benzian H, Crail J, Mackay J. The Oral Health Atlas. Mapping a neglected global health issue. Cointrin, Swizerland: FDI World Dental Federation; 2009.

(17) Petersen PE. Sociobehavioural risk factors in dental caries - international perspectives. Community Dent Oral Epidemiol 2005 Aug;33(4):274-279.

(18) Ide M, Papapanou PN. Epidemiology of association between maternal periodontal disease and adverse pregnancy outcomes--systematic review. J Periodontol 2013 Apr;84(4 Suppl):S181-94.

(19) Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 2004 Nov 1;15(6):348-381.

(20) Newman M, Takei H, Klokkevold P, Carranza F. Carranza'sclinical periodontology. 11th ed. Philadelphia: W.B.Saunders Co; 2002.

(21) Chen T, Yu WH, Izard J, Baranova OV, Lakshmanan A, Dewhirst FE. The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. Database (Oxford) 2010 Jul 6;2010:baq013.

(22) Paster BJ, Olsen I, Aas JA, Dewhirst FE. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. Periodontol 2000 2006;42:80-87.

(23) Keijser BJ, Zaura E, Huse SM, van der Vossen JM, Schuren FH, Montijn RC, et al. Pyrosequencing analysis of the oral microflora of healthy adults. J Dent Res 2008 Nov;87(11):1016-1020.

(24) Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 2005 Nov;43(11):5721-5732.

(25) Sanz M, Beighton D, Curtis MA, Cury JA, Dige I, Dommisch H, et al. Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. J Clin Periodontol 2017 Mar;44 Suppl 18:S5-S11.

(26) Mira A, Simon-Soro A, Curtis MA. Role of microbial communities in the pathogenesis of periodontal diseases and caries. J Clin Periodontol 2017 Mar;44 Suppl 18:S23-S38.

(27) Liljestrand JM. Systemic exposure to oral infections : a cardiometabolic risk. Helsinki: University of Helsinki; 2017.

(28) Alaluusua S, Matto J, Gronroos L, Innila S, Torkko H, Asikainen S, et al. Oral colonization by more than one clonal type of mutans streptococcus in children with nursing-bottle dental caries. Arch Oral Biol 1996 Feb;41(2):167-173.

(29) Keyes PH. The infectious and transmissible nature of experimental dental caries. Findings and implications. Arch Oral Biol 1960 Mar;1:304-320.

(30) Gupta P, Gupta N, Pawar AP, Birajdar SS, Natt AS, Singh HP. Role of Sugar and Sugar Substitutes in Dental Caries: A Review. ISRN Dent 2013 Dec 29;2013:519421.

(31) Fejerskov O. Concepts of dental caries and their consequences for understanding the disease. Community Dent Oral Epidemiol 1997 Feb;25(1):5-12.

(32) Mandel ID. The role of saliva in maintaining oral homeostasis. J Am Dent Assoc 1989 Aug;119(2):298-304.

(33) Diagnosis and management of dental caries throughout life. National Institutes of Health Consensus Development Conference statement, March 26-28, 2001. J Dent Educ 2001 Oct;65(10):1162-1168.

(34) Featherstone JD. Dental caries: a dynamic disease process. Aust Dent J 2008 Sep;53(3):286-291.

(35) Kakehashi S, Stanley HR, Fitzgerald RJ. The Effects of Surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats. Oral Surg Oral Med Oral Pathol 1965 Sep;20:340-349.

(36) Grossman LI. Origin of microorganisms in traumatized, pulpless, sound teeth. J Dent Res 1967 May-Jun;46(3):551-553.

(37) Stashenko P, Wang CY, Tani-Ishii N, Yu SM. Pathogenesis of induced rat periapical lesions. Oral Surg Oral Med Oral Pathol 1994 Oct;78(4):494-502.

(38) Nair PN. Apical periodontitis: a dynamic encounter between root canal infection and host response. Periodontol 2000 1997 Feb;13:121-148.

(39) Lerner UH. Regulation of bone metabolism by the kallikrein-kinin system, the coagulation cascade, and the acute-phase reactants. Oral Surg Oral Med Oral Pathol 1994 Oct;78(4):481-493.

(40) McNicholas S, Torabinejad M, Blankenship J, Bakland L. The concentration of prostaglandin E2 in human periradicular lesions. J Endod 1991 Mar;17(3):97-100.

(41) Siqueira Jr JF, Rôças IN. Distinctive features of the microbiota associated with different forms of apical periodontitis. Journal of Oral Microbiology 2009 01/01;1(1):2009.

(42) Mizel SB, Farrar JJ. Revised nomenclature for antigen-nonspecific T-cell proliferation and helper factors. Cell Immunol 1979 Dec;48(2):433-436.

(43) Carrotte P. Endodontics: Part 3. Treatment of endodontic emergencies. Br Dent J 2004 Sep 25;197(6):299-305.

(44) Bender IB. Factors influencing the radiographic appearance of bony lesions. J Endod 1997 Jan;23(1):5-14.

(45) Huumonen S, Ørstavik D. Radiological aspects of apical periodontitis. Endodontic Topics 2002;1:3--25.

(46) Flemmig TF. Periodontitis. Ann Periodontol 1999 Dec;4(1):32-38.

(47) Albandar JM, Kingman A, Brown LJ, Loe H. Gingival inflammation and subgingival calculus as determinants of disease progression in early-onset periodontitis. J Clin Periodontol 1998 Mar;25(3):231-237.

(48) Albandar JM, Kingman A. Gingival recession, gingival bleeding, and dental calculus in adults 30 years of age and older in the United States, 1988-1994. J Periodontol 1999 Jan;70(1):30-43.

(49) Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000 2014;64(1):57-80.

(50) Curtis MA. Periodontal microbiology--the lid's off the box again. J Dent Res 2014 Sep;93(9):840-842.

(51) Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, et al. The human oral microbiome. J Bacteriol 2010 Oct;192(19):5002-5017.

(52) Giannobile WV. Host-response therapeutics for periodontal diseases. J Periodontol 2008 Aug;79(8 Suppl):1592-1600.

(53) Preshaw PM, Seymour RA, Heasman PA. Current concepts in periodontal pathogenesis. Dent Update 2004 Dec;31(10):570-2, 574-8.

(54) Yamazaki K, Yoshie H, Seymour GJ. T cell regulation of the immune response to infection in periodontal diseases. Histol Histopathol 2003 Jul;18(3):889-896.

(55) Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL,Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998 Feb;25(2):134-144.

(56) Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nat Rev Microbiol 2012 Oct;10(10):717-725.

(57) Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005 Nov 19;366(9499):1809-1820.

(58) Armitage GC. The complete periodontal examination. Periodontol 2000 2004;34:22-33.

(59) FDI Commission. Mouthrinses and periodontal disease. Int Dent J 2002 Oct;52(5):346-352.

(60) Cobb CM. Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. J Clin Periodontol 2002 May;29 Suppl 2:6-16.

(61) Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy. A systematic review. Ann Periodontol 2003 Dec;8(1):115-181.

(62) Albandar JM. Global risk factors and risk indicators for periodontal diseases. Periodontol 2000 2002;29:177-206.

(63) Seymour GJ. Importance of the host response in the periodontium. J Clin Periodontol 1991;18(6):421-426.

(64) Divaris K, Monda KL, North KE, Olshan AF, Reynolds LM, Hsueh W, et al. Exploring the genetic basis of chronic periodontitis: a genome-wide association study. Human Molecular Genetics 2013 June 01;22(11):2312-2324.

(65) Schaible UE, Kaufmann SH. Malnutrition and infection: complex mechanisms and global impacts. PLoS Med 2007 May;4(5):e115.

(66) Albandar JM. Periodontal diseases in North America. Periodontol 2000 2002;29:31-69.

(67) Lovdal A, Arno A, Waerhaug J. Incidence of clinical manifestations of periodontal disease in light of oral hygiene and calculus formation. J Am Dent Assoc 1958 Jan;56(1):21-33.

(68) ten Cate JM, Featherstone JD. Mechanistic aspects of the interactions between fluoride and dental enamel. Crit Rev Oral Biol Med 1991;2(3):283-296.

(69) Touger-Decker R, van Loveren C. Sugars and dental caries. Am J Clin Nutr 2003 Oct;78(4):881S-892S.

(70) Psoter WJ, Reid BC, Katz RV. Malnutrition and dental caries: a review of the literature. Caries Res 2005 Nov-Dec;39(6):441-447.

(71) Sheetal A, Hiremath VK, Patil AG, Sajjansetty S, Kumar SR. Malnutrition and its oral outcome - a review. J Clin Diagn Res 2013 Jan;7(1):178-180.

(72) Kinane DF, Chestnutt IG. Smoking and periodontal disease. Crit Rev Oral Biol Med 2000;11(3):356-365.

(73) Persic Bukmir R, Jurcevic Grgic M, Brumini G, Spalj S, Pezelj-Ribaric S, Brekalo Prso I. Influence of tobacco smoking on dental periapical condition in a sample of Croatian adults. Wien Klin Wochenschr 2016 Apr;128(7-8):260-265.

(74) Van der Velden U. Effect of age on the periodontium. J Clin Periodontol 1984 May;11(5):281-294.

(75) Laine ML, Crielaard W, Loos BG. Genetic susceptibility to periodontitis. Periodontol 2000 2012 Feb;58(1):37-68.

(76) Menezes-Silva R, Khaliq S, Deeley K, Letra A, Vieira AR. Genetic susceptibility to periapical disease: conditional contribution of MMP2 and MMP3 genes to the development of periapical lesions and healing response. J Endod 2012 May;38(5):604-607.

(77) De Soet JJ, van Gemert-Schriks MC, Laine ML, van Amerongen WE, Morre SA, van Winkelhoff AJ. Host and microbiological factors related to dental caries development. Caries Res 2008;42(5):340-347.

(78) Loe H, Anerud A, Boysen H, Smith M. The natural history of periodontal disease in man. The rate of periodontal destruction before 40 years of age. J Periodontol 1978 Dec;49(12):607-620.

(79) Frencken JE, Sharma P, Stenhouse L, Green D, Laverty D, Dietrich T. Global epidemiology of dental caries and severe periodontitis - a comprehensive review. J Clin Periodontol 2017 Mar;44 Suppl 18:S94-S105.

(80) Greabu M, Battino M, Mohora M, Totan A, Didilescu A, Spinu T, et al. Saliva--a diagnostic window to the body, both in health and in disease. J Med Life 2009 Apr-Jun;2(2):124-132.

(81) Wolff L, Dahlen G, Aeppli D. Bacteria as risk markers for periodontitis. J Periodontol 1994 May;65(5 Suppl):498-510.

(82) Grossi SG, Zambon JJ, Ho AW, Koch G, Dunford RG, Machtei EE, et al. Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. J Periodontol 1994 Mar;65(3):260-267.

(83) Souza AJ, Gomes-Filho IS, Silva CALD, Passos-Soares JS, Cruz SSD, Trindade SC, et al. Factors associated with dental caries, periodontitis and intra-oral lesions in individuals with HIV / AIDS. AIDS Care 2017 Nov 10:1-8.

(84) Schenkein HA, Van Dyke TE. Early-onset periodontitis: systemic aspects of etiology and pathogenesis. Periodontol 2000 1994 Oct;6:7-25.

(85) Slots J, Contreras A. Herpesviruses: a unifying causative factor in periodontitis? Oral Microbiol Immunol 2000 Oct;15(5):277-280.

(86) Kornman KS, Loe H. The role of local factors in the etiology of periodontal diseases. Periodontol 2000 1993 Jun;2:83-97.

(87) Blieden TM. Tooth-related issues. Ann Periodontol 1999 Dec;4(1):91-97.

(88) Nishimura F, Takahashi K, Kurihara M, Takashiba S, Murayama Y. Periodontal disease as a complication of diabetes mellitus. Ann Periodontol 1998 Jul;3(1):20-29.

(89) Nakahara Y, Ozaki K, Matsuura T. Long-term Hyperglycemia Naturally Induces Dental Caries but Not Periodontal Disease in Type 1 and Type 2 Diabetic Rodents. Diabetes 2017 Nov;66(11):2868-2874.

(90) Straka M, Straka-Trapezanlidis M, Deglovic J, Varga I. Periodontitis and osteoporosis. Neuro Endocrinol Lett 2015;36(5):401-406.

(91) Friedlander AH, Mahler ME. Major depressive disorder. Psychopathology, medical management and dental implications. J Am Dent Assoc 2001 May;132(5):629-638.

(92) Scully C. Drug effects on salivary glands: dry mouth. Oral Dis 2003 Jul;9(4):165-176.

(93) Williams DM, Sheiham A, Honkala E. Addressing Oral Health Inequalities in the Africa and Middle East Region. J Dent Res 2015 Jul;94(7):875-877.

(94) Genco RJ, Ho AW, Grossi SG, Dunford RG, Tedesco LA. Relationship of stress, distress and inadequate coping behaviors to periodontal disease. J Periodontol 1999 Jul;70(7):711-723.

(95) Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of untreated caries: a systematic review and metaregression. J Dent Res 2015 May;94(5):650-658.

(96) Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. J Dent Res 2014 Nov;93(11):1045-1053.

(97) Costa FO, Guimaraes AN, Cota LO, Pataro AL, Segundo TK, Cortelli SC, et al. Impact of different periodontitis case definitions on periodontal research. J Oral Sci 2009 Jun;51(2):199-206.

(98) Persoon IF, Ozok AR. Definitions and Epidemiology of Endodontic Infections. Curr Oral Health Rep 2017;4(4):278-285.

(99) Sheiham A. Oral health, general health and quality of life. Bull World Health Organ 2005 Sep;83(9):644-96862005000900004. Epub 2005 Sep 30.

(100) Beikler T, Flemmig TF. Oral biofilm-associated diseases: trends and implications for quality of life, systemic health and expenditures. Periodontol 2000 2011 Feb;55(1):87-103.

(101) Petersen PE. World Health Organization global policy for improvement of oral health - World Health Assembly 2007. Int Dent J 2008;58(3):115-121.

(102) Listl S, Galloway J, Mossey PA, Marcenes W. Global Economic Impact of Dental Diseases. J Dent Res 2015 Oct;94(10):1355-1361.

(103) Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century--the approach of the WHO Global Oral Health Programme. Community Dent Oral Epidemiol 2003 Dec;31 Suppl 1:3-23.

(104) Varenne B, Petersen PE, Ouattara S. Oral health behaviour of children and adults in urban and rural areas of Burkina Faso, Africa. Int Dent J 2006 Apr;56(2):61-70.

(105) Kaye EK. Nutrition, dietary guidelines and optimal periodontal health. Periodontol 2000 2012 Feb;58(1):93-111.

(106) Boyd LD, Madden TE. Nutrition, infection, and periodontal disease. Dent Clin North Am 2003 Apr;47(2):337-354.

(107) Neiva RF, Steigenga J, Al-Shammari KF, Wang HL. Effects of specific nutrients on periodontal disease onset, progression and treatment. J Clin Periodontol 2003 Jul;30(7):579-589.

(108) MacKeown JM, Cleaton-Jones PE, Fatti P. Caries and micronutrient intake among urban South African children: a cohort study. Community Dent Oral Epidemiol 2003 Jun;31(3):213-220.

(109) Oppermann RV, Rolla G. Effect of some polyvalent cations on the acidogenicity of dental plaque in vivo. Caries Res 1980;14(6):422-427.

(110) Hujoel PP. Vitamin D and dental caries in controlled clinical trials: systematic review and meta-analysis. Nutr Rev 2013 Feb;71(2):88-97.

(111) Eshghi A, Kowsari-Isfahan R, Rezaiefar M, Razavi M, Zeighami S. Effect of iron containing supplements on rats' dental caries progression. J Dent (Tehran) 2012 Winter;9(1):14-19.

(112) Harris SS, Navia JM. Vitamin A deficiency and caries susceptibility of rat molars. Arch Oral Biol 1980;25(6):415-421.

(113) Lin HS, Lin JR, Hu SW, Kuo HC, Yang YH. Association of dietary calcium, phosphorus, and magnesium intake with caries status among schoolchildren. Kaohsiung J Med Sci 2014 Apr;30(4):206-212.

(114) Yen CE, Huang YC, Hu SW. Relationship between dietary intake and dental caries in preschool children. Int J Vitam Nutr Res 2010 Jun;80(3):205-215.

(115) Osorio R, Osorio E, Cabello I, Toledano M. Zinc Induces Apatite and Scholzite Formation during Dentin Remineralization. Caries Res 2014 Feb 7;48(4):276-290.

(116) Johnson DA, Alvares OF. Zinc deficiency-induced changes in rat parotid salivary proteins. J Nutr 1984 Oct;114(10):1955-1964.

(117) MacKeown JM, Cleaton-Jones PE. Dental caries incidence in relation to nutrient intake in urban preschool children. S Afr J Clin Nutr 2001;14:132--136.

(118) Kwon HK, Suh I, Kim YO, Kim HJ, Nam CM, Jun KM, et al. Relationship between nutritional intake and dental caries experience of junior high students. Yonsei Med J 1997 Apr;38(2):101-110.

(119) Dawson DR,3rd, Branch-Mays G, Gonzalez OA, Ebersole JL. Dietary modulation of the inflammatory cascade. Periodontol 2000 2014 Feb;64(1):161-197.

(120) Van der Velden U, Kuzmanova D, Chapple IL. Micronutritional approaches to periodontal therapy. J Clin Periodontol 2011 Mar;38 Suppl 11:142-158.

(121) Amaliya, Timmerman MF, Abbas F, Loos BG, Van der Weijden GA, Van Winkelhoff AJ, et al. Java project on periodontal diseases: the relationship between vitamin C and the severity of periodontitis. J Clin Periodontol 2007 Apr;34(4):299-304.

(122) Naqvi AZ, Buettner C, Phillips RS, Davis RB, Mukamal KJ. n-3 fatty acids and periodontitis in US adults. J Am Diet Assoc 2010 Nov;110(11):1669-1675.

(123) El-Sharkawy H, Aboelsaad N, Eliwa M, Darweesh M, Alshahat M, Kantarci A, et al. Adjunctive treatment of chronic periodontitis with daily dietary supplementation with omega-3 Fatty acids and low-dose aspirin. J Periodontol 2010 Nov;81(11):1635-1643.

(124) Meisel P, Schwahn C, Luedemann J, John U, Kroemer HK, Kocher T. Magnesium deficiency is associated with periodontal disease. J Dent Res 2005 Oct;84(10):937-941.

(125) Coulston A, Boushey C. Nutrition in the prevention and treatment of disease. 2nd ed.: Elsevier Academic Press; 2008.

(126) Johansson I, Ericson T, Bowen W, Cole M. The effect of malnutrition on caries development and saliva composition in the rat. J Dent Res 1985 Jan;64(1):37-43.

(127) Sawyer DR, Nwoku AL, Rotimi VO, Hagen JC. Comparison of oral microflora between well-nourished and malnourished Nigerian children. ASDC J Dent Child 1986 Nov-Dec;53(6):439-443.

(128) Oppenheimer SJ. Iron and its relation to immunity and infectious disease. J Nutr 2001 Feb;131(2S-2):616S-633S; discussion 633S-635S.

(129) Hoffman CS, Messer LC, Mendola P, Savitz DA, Herring AH, Hartmann KE. Comparison of gestational age at birth based on last menstrual period and ultrasound during the first trimester. Paediatr Perinat Epidemiol 2008;22(6):587-596.

(130) Petitti DB. The epidemiology of fetal death. Clin Obstet Gynecol 1987 Jun;30(2):253-258.

(131) Michels TC, Tiu AY. Second trimester pregnancy loss. Am Fam Physician 2007 Nov 1;76(9):1341-1346.

(132) Herrera E. Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. Eur J Clin Nutr 2000 Mar;54 Suppl 1:S47-51.

(133) Zeng Z, Liu F, Li S. Metabolic Adaptations in Pregnancy: A Review. Ann Nutr Metab 2017;70(1):59-65.

(134) Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. Cardiovasc J Afr 2016 Mar-Apr;27(2):89-94.

(135) Kamel RM. The onset of human parturition. Arch Gynecol Obstet 2010 Jun;281(6):975-982.

(136) Pazos M, Sperling RS, Moran TM, Kraus TA. The influence of pregnancy on systemic immunity. Immunol Res 2012 Dec;54(1-3):254-261.

(137) Miyagi M, Aoyama H, Morishita M, Iwamoto Y. Effects of sex hormones on chemotaxis of human peripheral polymorphonuclear leukocytes and monocytes. J Periodontol 1992 Jan;63(1):28-32.

(138) Robinson DP, Klein SL. Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. Horm Behav 2012 Aug;62(3):263-271.

(139) Liggins GC, Fairclough RJ, Grieves SA, Forster CS, Knox BS. Parturition in the sheep. Ciba Found Symp 1977;(47)(47):5-30.

(140) Sfakianaki AK, Norwitz ER. Mechanisms of progesterone action in inhibiting prematurity. J Matern Fetal Neonatal Med 2006 Dec;19(12):763-772.

(141) Menon R, Fortunato SJ. Infection and the role of inflammation in preterm premature rupture of the membranes. Best Pract Res Clin Obstet Gynaecol 2007 Jun;21(3):467-478.

(142) Moore RM, Mansour JM, Redline RW, Mercer BM, Moore JJ. The physiology of fetal membrane rupture: insight gained from the determination of physical properties. Placenta 2006 Nov-Dec;27(11-12):1037-1051.

(143) Sedgh G, Singh S, Hussain R. Intended and Unintended Pregnancies Worldwide in 2012 and Recent Trends. Stud Fam Plann 2014;45(3):301-314.

(144) Villar J, Papageorghiou AT, Pang R, Ohuma EO, Cheikh Ismail L, Barros FC, et al. The likeness of fetal growth and newborn size across non-isolated populations in the INTERGROWTH-21st Project: the Fetal Growth Longitudinal Study and Newborn Cross-Sectional Study. Lancet Diabetes Endocrinol 2014 Oct;2(10):781-792.

(145) Victora CG, Villar J, Barros FC, et a. Anthropometric characterization of impaired fetal growth: Risk factors for and prognosis of newborns with stunting or wasting. JAMA Pediatrics pages = {e151431-}, 2015;169(7).

(146) Barker DJ, Eriksson JG, Forsen T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol 2002 Dec;31(6):1235-1239.

(147) de Onis M, Blossner M, Villar J. Levels and patterns of intrauterine growth retardation in developing countries. Eur J Clin Nutr 1998 Jan;52 Suppl 1:S5-15.

(148) Gravett MG, Rubens CE, Nunes TM, GAPPS Review Group. Global report on preterm birth and stillbirth (2 of 7): discovery science. BMC Pregnancy Childbirth 2010 Feb 23;10 Suppl 1:S2-2393-10-S1-S2.

(149) Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, et al. The preterm parturition syndrome. BJOG 2006 Dec;113 Suppl 3:17-42.

(150) Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science 2014 Aug 15;345(6198):760-765.

(151) Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med 2000 May 18;342(20):1500-1507.

(152) Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010

with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012 Jun 9;379(9832):2162-2172.

(153) Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 2012 Jun 9;379(9832):2151-2161.

(154) Lawn JE, Davidge R, Paul VK, von Xylander S, de Graft Johnson J, Costello A, et al. Born too soon: care for the preterm baby. Reprod Health 2013;10 Suppl 1:S5-4755-10-S1-S5. Epub 2013 Nov 15.

(155) Mwaniki MK, Atieno M, Lawn JE, Newton CR. Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet 2012 Feb 4;379(9814):445-452.

(156) Villar J, Belizan JM. The timing factor in the pathophysiology of the intrauterine growth retardation syndrome. Obstet Gynecol Surv 1982 Aug;37(8):499-506.

(157) Victora CG, de Onis M, Hallal PC, Blossner M, Shrimpton R. Worldwide timing of growth faltering: revisiting implications for interventions. Pediatrics 2010 Mar;125(3):e473-80.

(158) de Onis M, Branca F. Childhood stunting: a global perspective. Matern Child Nutr 2016 May;12 Suppl 1:12-26.

(159) Romo A, Carceller R, Tobajas J. Intrauterine growth retardation (IUGR): epidemiology and etiology. Pediatr Endocrinol Rev 2009 Feb;6 Suppl 3:332-336.

(160) Dimasuay KG, Boeuf P, Powell TL, Jansson T. Placental Responses to Changes in the Maternal Environment Determine Fetal Growth. Front Physiol 2016 Jan 29;7:12.

(161) Hoddinott J, Behrman JR, Maluccio JA, Melgar P, Quisumbing AR, Ramirez-Zea M, et al. Adult consequences of growth failure in early childhood. Am J Clin Nutr 2013 Nov;98(5):1170-1178.

(162) Vittek J, Hernandez MR, Wenk EJ, Rappaport SC, Southren AL. Specific estrogen receptors in human gingiva. J Clin Endocrinol Metab 1982 Mar;54(3):608-612.

(163) Kawahara K, Shimazu A. Expression and intracellular localization of progesterone receptors in cultured human gingival fibroblasts. J Periodontal Res 2003 Jun;38(3):242-246.

(164) Mealey BL, Moritz AJ. Hormonal influences: effects of diabetes mellitus and endogenous female sex steroid hormones on the periodontium. Periodontol 2000 2003;32(1):59-81.

(165) Miyagi M, Morishita M, Iwamoto Y. Effects of sex hormones on production of prostaglandin E2 by human peripheral monocytes. J Periodontol 1993 Nov;64(11):1075-1078.

(166) Lapp CA, Thomas ME, Lewis JB. Modulation by progesterone of interleukin-6 production by gingival fibroblasts. J Periodontol 1995 Apr;66(4):279-284.

(167) Kornman KS, Loesche WJ. The subgingival microbial flora during pregnancy. J Periodontal Res 1980 Mar;15(2):111-122.

(168) Gursoy M, Haraldsson G, Hyvonen M, Sorsa T, Pajukanta R, Kononen E. Does the frequency of Prevotella intermedia increase during pregnancy? Oral Microbiol Immunol 2009 Aug;24(4):299-303.

(169) Carrillo-de-Albornoz A, Figuero E, Herrera D, Bascones-Martínez A. Gingival changes during pregnancy: II. Influence of hormonal variations on the subgingival biofilm. J Clin Periodontol 2010;37(3):230-240.

(170) Gursoy M, Pajukanta R, Sorsa T, Kononen E. Clinical changes in periodontium during pregnancy and post-partum. J Clin Periodontol 2008 Jul;35(7):576-583.

(171) Gursoy M, Kononen E, Tervahartiala T, Gursoy UK, Pajukanta R, Sorsa T. Longitudinal study of salivary proteinases during pregnancy and postpartum. J Periodontal Res 2010 Aug;45(4):496-503.

(172) Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, Maynor G, et al. Periodontal infection as a possible risk factor for preterm low birth weight. J Periodontol 1996 Oct;67(10 Suppl):1103-1113.

(173) Wang YL, Liou JD, Pan WL. Association between maternal periodontal disease and preterm delivery and low birth weight. Taiwan J Obstet Gynecol 2013 Mar;52(1):71-76.

(174) Offenbacher S, Boggess KA, Murtha AP, Jared HL, Lieff S, McKaig RG, et al. Progressive periodontal disease and risk of very preterm delivery. Obstet Gynecol 2006 Jan;107(1):29-36.

(175) Nabet C, Lelong N, Colombier ML, Sixou M, Musset AM, Goffinet F, et al. Maternal periodontitis and the causes of preterm birth: the case-control Epipap study. J Clin Periodontol 2010 Jan;37(1):37-45.

(176) Jeffcoat MK, Geurs NC, Reddy MS, Cliver SP, Goldenberg RL, Hauth JC. Periodontal infection and preterm birth: results of a prospective study. J Am Dent Assoc 2001 Jul;132(7):875-880.

(177) Muwazi L, Rwenyonyi CM, Nkamba M, Kutesa A, Kagawa M, Mugyenyi G, et al. Periodontal conditions, low birth weight and preterm birth among postpartum mothers in two tertiary health facilities in Uganda. BMC Oral Health 2014 Apr 28;14:42-6831-14-42.

(178) Rakoto-Alson S, Tenenbaum H, Davideau JL. Periodontal diseases, preterm births, and low birth weight: findings from a homogeneous cohort of women in Madagascar. J Periodontol 2010 Feb;81(2):205-213.

(179) Jacob PS, Nath S. Periodontitis among poor rural Indian mothers increases the risk of low birth weight babies: a hospital-based case control study. J Periodontal Implant Sci 2014 Apr;44(2):85-93.

(180) Shanthi V, Vanka A, Bhambal A, Saxena V, Saxena S, Kumar SS. Association of pregnant women periodontal status to preterm and low-birth weight babies: A systematic and evidence-based review. Dent Res J (Isfahan) 2012 Jul;9(4):368-380.

(181) Sanz M, Kornman K, Working group 3 of joint EFP/AAP workshop. Periodontitis and adverse pregnancy outcomes: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. J Clin Periodontol 2013 Apr;40 Suppl 14:S164-9.

(182) Mumghamba EG, Manji KP. Maternal oral health status and preterm low birth weight at Muhimbili National Hospital, Tanzania: a case-control study. BMC Oral Health 2007 Jun 26;7:8.

(183) Buduneli N, Baylas H, Buduneli E, Turkoglu O, Kose T, Dahlen G. Periodontal infections and pre-term low birth weight: a case-control study. J Clin Periodontol 2005 Feb;32(2):174-181.

(184) Santa Cruz I, Herrera D, Martin C, Herrero A, Sanz M. Association between periodontal status and pre-term and/or low-birth weight in Spain: clinical and microbiological parameters. J Periodontal Res 2013 Aug;48(4):443-451.

(185) Srinivas SK, Sammel MD, Stamilio DM, Clothier B, Jeffcoat MK, Parry S, et al. Periodontal disease and adverse pregnancy outcomes: is there an association? Am J Obstet Gynecol 2009 May;200(5):497.e1-497.e8.

(186) Abati S, Villa A, Cetin I, Dessole S, Luglie PF, Strohmenger L, et al. Lack of association between maternal periodontal status and adverse pregnancy outcomes: a

multicentric epidemiologic study. J Matern Fetal Neonatal Med 2013 Mar;26(4):369-372.

(187) Linden GJ, Lyons A, Scannapieco FA. Periodontal systemic associations: review of the evidence. J Clin Periodontol 2013 Apr;40 Suppl 14:S8-19.

(188) Barco CT. Prevention of infective endocarditis: a review of the medical and dental literature. J Periodontol 1991 Aug;62(8):510-523.

(189) Llambes F, Arias-Herrera S, Caffesse R. Relationship between diabetes and periodontal infection. World J Diabetes 2015 Jul 10;6(7):927-935.

(190) Carramolino-Cuellar E, Tomas I, Jimenez-Soriano Y. Relationship between the oral cavity and cardiovascular diseases and metabolic syndrome. Med Oral Patol Oral Cir Bucal 2014 May 1;19(3):e289-94.

(191) Fisher MA, Taylor GW, West BT, McCarthy ET. Bidirectional relationship between chronic kidney and periodontal disease: a study using structural equation modeling. Kidney Int 2011 Feb;79(3):347-355.

(192) Azarpazhooh A, Leake JL. Systematic review of the association between respiratory diseases and oral health. J Periodontol 2006 Sep;77(9):1465-1482.

(193) Scannapieco FA, Bush RB, Paju S. Associations between periodontal disease and risk for nosocomial bacterial pneumonia and chronic obstructive pulmonary disease. A systematic review. Ann Periodontol 2003 Dec;8(1):54-69.

(194) Keller A, Rohde JF, Raymond K, Heitmann BL. Association between periodontal disease and overweight and obesity: a systematic review. J Periodontol 2015 Jun;86(6):766-776.

(195) Vergnes JN, Kaminski M, Lelong N, Musset AM, Sixou M, Nabet C, et al. Maternal dental caries and pre-term birth: results from the EPIPAP study. Acta Odontol Scand 2011 Jul;69(4):248-256.

(196) Heimonen A, Rintamaki H, Furuholm J, Janket SJ, Kaaja R, Meurman JH. Postpartum oral health parameters in women with preterm birth. Acta Odontol Scand 2008;66(6):334-341.

(197) Durand R, Gunselman EL, Hodges JS, Diangelis AJ, Michalowicz BS. A pilot study of the association between cariogenic oral bacteria and preterm birth. Oral Dis 2009 Sep;15(6):400-406.

(198) Meurman JH, Furuholm J, Kaaja R, Rintamaki H, Tikkanen U. Oral health in women with pregnancy and delivery complications. Clin Oral Investig 2006 Jun;10(2):96-101.

(199) Ryalat S, Sawair F, Baqain Z, Barghout N, Amin W, Badran D, et al. Effect of oral diseases on mothers giving birth to preterm infants. Med Princ Pract 2011;20(6):556-561.

(200) Dasanayake AP. Poor periodontal health of the pregnant woman as a risk factor for low birth weight. Ann Periodontol 1998 Jul;3(1):206-212.

(201) Lima SM, Grisi DC, Kogawa EM, Franco OL, Peixoto VC, Goncalves-Junior JF, et al. Diabetes mellitus and inflammatory pulpal and periapical disease: a review. Int Endod J 2013 Aug;46(8):700-709.

(202) Caplan DJ, Chasen JB, Krall EA, Cai J, Kang S, Garcia RI, et al. Lesions of endodontic origin and risk of coronary heart disease. J Dent Res 2006 Nov;85(11):996-1000.

(203) Bain JL, Lester SR, Henry WD, Pongetti JL, Blackman ME, Johnson RB. Association between maternal periapical lesions and brain inflammation in rat pups. Arch Oral Biol 2013 Mar;58(3):266-271.

(204) Bobetsis YA, Barros SP, Offenbacher S. Exploring the relationship between periodontal disease and pregnancy complications. J Am Dent Assoc 2006 Oct;137 Suppl:7S-13S.

(205) Madianos PN, Bobetsis YA, Offenbacher S. Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms. J Periodontol 2013 Apr;84(4 Suppl):S170-80.

(206) Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med 2014 May 21;6(237):237ra65.

(207) Doyle RM, Alber DG, Jones HE, Harris K, Fitzgerald F, Peebles D, et al. Term and preterm labour are associated with distinct microbial community structures in placental membranes which are independent of mode of delivery. Placenta 2014 Oct 19;35(12):1099-1101.

(208) Han YW, Wang X. Mobile microbiome: oral bacteria in extra-oral infections and inflammation. J Dent Res 2013 Jun;92(6):485-491.

(209) Liu H, Redline RW, Han YW. Fusobacterium nucleatum induces fetal death in mice via stimulation of TLR4-mediated placental inflammatory response. J Immunol 2007 Aug 15;179(4):2501-2508.

(210) Offenbacher S, Riche EL, Barros SP, Bobetsis YA, Lin D, Beck JD. Effects of maternal Campylobacter rectus infection on murine placenta, fetal and neonatal survival, and brain development. J Periodontol 2005 Nov;76(11 Suppl):2133-2143.

(211) Keelan JA, Wong PM, Bird PS, Mitchell MD. Innate inflammatory responses of human decidual cells to periodontopathic bacteria. Am J Obstet Gynecol 2010 May;202(5):471.e1-471.11.

(212) Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. J Clin Periodontol 2008 Apr;35(4):277-290.

(213) Tjoa ML, van Vugt JM, Go AT, Blankenstein MA, Oudejans CB, van Wijk IJ. Elevated C-reactive protein levels during first trimester of pregnancy are indicative of preeclampsia and intrauterine growth restriction. J Reprod Immunol 2003 Jun;59(1):29-37.

(214) Pitiphat W, Gillman MW, Joshipura KJ, Williams PL, Douglass CW, Rich-Edwards JW. Plasma C-reactive protein in early pregnancy and preterm delivery. Am J Epidemiol 2005 Dec 1;162(11):1108-1113.

(215) Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003 Jun;111(12):1805-1812.

(216) Wieringa FT, Dijkhuizen MA, West CE, Northrop-Clewes CA, Muhilal. Estimation of the effect of the acute phase response on indicators of micronutrient status in Indonesian infants. J Nutr 2002 Oct;132(10):3061-3066.

(217) Boggess KA, Moss K, Madianos P, Murtha AP, Beck J, Offenbacher S. Fetal immune response to oral pathogens and risk of preterm birth. Am J Obstet Gynecol 2005 Sep;193(3 Pt 2):1121-1126.

(218) Van Dyke TE, van Winkelhoff AJ. Infection and inflammatory mechanisms. J Clin Periodontol 2013 Apr;40 Suppl 14:S1-7.

(219) Voltolini C, Petraglia F. Neuroendocrinology of pregnancy and parturition. Handb Clin Neurol 2014;124:17-36.

(220) Giurgescu C. Are maternal cortisol levels related to preterm birth? J Obstet Gynecol Neonatal Nurs 2009 Jul-Aug;38(4):377-390.

(221) Bellavance MA, Rivest S. The HPA - Immune Axis and the Immunomodulatory Actions of Glucocorticoids in the Brain. Front Immunol 2014 Mar 31;5:136.

(222) Government of Malawi. Statistical Yearbook 2015. Zomba: National Statistical Office; [2016].

(223) Watkins K. The state of the world's children 2016: A fair change for every child. New York: United Nations Children's Fund (UNICEF); 2016.

(224) National Statistical Office (NSO) [Malawi], ICF. Malawi demographic and health survey 2015-16. Zomba: National Statistical Office (NSO); 2017.

(225) Arimond M, Zeilani M, Jungjohann S, Brown KH, Ashorn P, Allen LH, et al. Considerations in developing lipid-based nutrient supplements for prevention of undernutrition: experience from the International Lipid-Based Nutrient Supplements (iLiNS) Project. Matern Child Nutr 2013 May 6.

(226) Ashorn P, Alho L, Ashorn U, Cheung Y, Dewey K, Harjunmaa U, et al. The impact of lipid-based nutrient supplement provision to pregnant women on newborn size in rural Malawi: A randomised controlled trial. Am J Clin Nutr 2015 Feb;101:387-97.

(227) WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr Suppl 2006 Apr;450:76-85.

(228) Fujii R, Saito Y, Tokura Y, Nakagawa KI, Okuda K, Ishihara K. Characterization of bacterial flora in persistent apical periodontitis lesions. Oral Microbiol Immunol 2009 Dec;24(6):502-505.

(229) Siqueira JF,Jr, Rocas IN. Diversity of endodontic microbiota revisited. J Dent Res 2009 Nov;88(11):969-981.

(230) Somma F, Castagnola R, Bollino D, Marigo L. Oral inflammatory process and general health. Part 2: How does the periapical inflammatory process compromise general health? Eur Rev Med Pharmacol Sci 2011 Jan;15(1):35-51.

(231) World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013 Nov 27;310(20):2191-2194.

(232) Sajidu S, Masamba W, Thole B, Mwatseteza J. Groundwater fluoride levels in villages of Southern Malawi and removal studies using bauxite. Int J Phys Sci 2008 January;3(1):001-- 011.

(233) Ramesh M, Narasimhan M, Krishnan R, Aruna RM, Kuruvilla S. The effect of fluorosis on human teeth under light microscopy: A cross-sectional study. J Oral Maxillofac Pathol 2017 Sep-Dec;21(3):345-350.

(234) Baelum V, Scheutz F. Periodontal diseases in Africa. Periodontol 2000 2002;29:79-103.

(235) Alves KM, Franco KS, Sassaki KT, Buzalaf MA, Delbem AC. Effect of iron on enamel demineralization and remineralization in vitro. Arch Oral Biol 2011 Nov;56(11):1192-1198.

(236) Torell P. Iron and dental caries. Swed Dent J 1988;12(3):113-124.

(237) Aranha H, Strachan RC, Arceneaux JE, Byers BR. Effect of trace metals on growth of Streptococcus mutans in a teflon chemostat. Infect Immun 1982 Feb;35(2):456-460.

(238) Strachan RC, Aranha H, Lodge JS, Arceneaux JE, Byers BR. Teflon chemostat for studies of trace metal metabolism in Streptococcus mutans and other bacteria. Appl Environ Microbiol 1982 Jan;43(1):257-260.

(239) Aranha H, Evans SL, Arceneaux JE, Byers BR. Calcium modulation of growth of Streptococcus mutans. J Gen Microbiol 1986 Sep;132(9):2661-2663.

(240) Blais JF, Lavoie MC. Effect of dietary components on the indigenous oral bacterial flora of BALB/c mice. J Dent Res 1990 Mar;69(3):868-873.

(241) Laine MA. Effect of pregnancy on periodontal and dental health. Acta Odontol Scand 2002 Oct;60(5):257-264.

(242) Munoz CA, Kiger RD, Stephens JA, Kim J, Wilson AC. Effects of a nutritional supplement on periodontal status. Compend Contin Educ Dent 2001 May;22(5):425-8, 430, 432 passim; quiz 440.

(243) Naqvi AZ, Buettner C, Phillips RS, Davis RB, Mukamal KJ. n-3 fatty acids and periodontitis in US adults. J Am Diet Assoc 2010 Nov;110(11):1669-1675.

(244) Reddy MS, Jeffcoat MK. Methods of assessing periodontal regeneration. Periodontol 2000 1999 Feb;19:87-103.

(245) Wagle M, D'Antonio F, Reierth E, Basnet P, Trovik TA, Orsini G, et al. Dental caries and preterm birth: a systematic review and meta-analysis. BMJ Open 2018 Mar 2;8(3):e018556-2017-018556.

(246) Leal AS, de Oliveira AE, Brito LM, Lopes FF, Rodrigues VP, Lima KF, et al. Association between chronic apical periodontitis and low-birth-weight preterm births. J Endod 2015 Mar;41(3):353-357.

(247) Khalighinejad N, Aminoshariae A, Kulild JC, Mickel A. Apical Periodontitis, a Predictor Variable for Preeclampsia: A Case-control Study. J Endod 2017 Oct;43(10):1611-1614.

(248) Luntamo M, Kulmala T, Cheung YB, Maleta K, Ashorn P. The effect of antenatal monthly sulphadoxine-pyrimethamine, alone or with azithromycin, on foetal and neonatal growth faltering in Malawi: a randomised controlled trial. Trop Med Int Health 2013 Apr;18(4):386-397.

(249) Sasaki J. Azithromycin in dentistry and oral surgery. Jpn J Antibiot 2000 Jun;53 Suppl B:117-124.

(250) Hallamaa L, Cheung YB, Maleta K, Luntamo M, Ashorn U, Gladstone M, et al. Child Health Outcomes After Presumptive Infection Treatment in Pregnant Women: A Randomized Trial. Pediatrics 2018 Feb 22.

(251) Ashorn P, Hallamaa L, Allen LH, Ashorn U, Chandrasiri U, Deitchler M, et al. Co-causation of reduced newborn size by maternal undernutrition, infections, and inflammation. Matern Child Nutr 2018 Jan 8.

(252) Santos AL, Siqueira JF,Jr, Rocas IN, Jesus EC, Rosado AS, Tiedje JM. Comparing the bacterial diversity of acute and chronic dental root canal infections. PLoS One 2011;6(11):e28088.

(253) Gomes MS, Blattner TC, Sant'Ana Filho M, Grecca FS, Hugo FN, Fouad AF, et al. Can apical periodontitis modify systemic levels of inflammatory markers? A systematic review and meta-analysis. J Endod 2013 Oct;39(10):1205-1217.

(254) Olsen I. Update on bacteraemia related to dental procedures. Transfus Apher Sci 2008 Oct;39(2):173-178.

(255) Aminoshariae A, Kulild JC. Association of Functional Gene Polymorphism with Apical Periodontitis. J Endod 2015 Jul;41(7):999-1007.

(256) Marton IJ, Kiss C. Protective and destructive immune reactions in apical periodontitis. Oral Microbiol Immunol 2000 Jun;15(3):139-150.

(257) Vidal F, Fontes TV, Marques TV, Goncalves LS. Association between apical periodontitis lesions and plasmatic levels of C-reactive protein, interleukin 6 and fibrinogen in hypertensive patients. Int Endod J 2015 Oct 24.

(258) Caplan DJ. Epidemiologic issues in studies of association between apical periodontitis and systemic health. Endodontic Topics 2004;8(1):15-35.

(259) de Soet JJ, Schriks MC, Kratz E, Poland DC, van Dijk W, van Amerongen WE. Dental caries related to plasma IgG and alpha1-acid glycoprotein. Caries Res 2003 Mar-Apr;37(2):79-84.

(260) Glurich I, Grossi S, Albini B, Ho A, Shah R, Zeid M, et al. Systemic inflammation in cardiovascular and periodontal disease: comparative study. Clin Diagn Lab Immunol 2002 Mar;9(2):425-432.

(261) Refulio Z, Rocafuerte M, de la Rosa M, Mendoza G, Chambrone L. Association among stress, salivary cortisol levels, and chronic periodontitis. J Periodontal Implant Sci 2013 Apr;43(2):96-100.

(262) Kourtis AP, Read JS, Jamieson DJ. Pregnancy and infection. N Engl J Med 2014 Jun 5;370(23):2211-2218.

(263) Bolton M, Horvath DJ, Li B, Cortado H, Newsom D, White P, et al. Intrauterine Growth Restriction Is a Direct Consequence of Localized Maternal Uropathogenic Escherichia coli Cystitis. PLoS ONE 2012 02/18;7(3):e33897.

(264) Association of Chlamydia trachomatis and Mycoplasma hominis with intrauterine growth retardation and preterm delivery. The John Hopkins Study of Cervicitis and Adverse Pregnancy Outcome. Am J Epidemiol 1989 Jun;129(6):1247-1257.

(265) Jones HN, Jansson T, Powell TL. IL-6 stimulates system A amino acid transporter activity in trophoblast cells through STAT3 and increased expression of SNAT2. Am J Physiol Cell Physiol 2009 Nov;297(5):C1228-35.

(266) Jentsch HF, Marz D, Kruger M. The effects of stress hormones on growth of selected periodontitis related bacteria. Anaerobe 2013 Dec;24:49-54.

(267) Vaughan OR, Sferruzzi-Perri AN, Fowden AL. Maternal corticosterone regulates nutrient allocation to fetal growth in mice. J Physiol 2012 Nov 1;590(21):5529-5540.

(268) Petraglia F, Imperatore A, Challis JR. Neuroendocrine mechanisms in pregnancy and parturition. Endocr Rev 2010 Dec;31(6):783-816.

(269) von Minckwitz G, Grischke EM, Schwab S, Hettinger S, Loibl S, Aulmann M, et al. Predictive value of serum interleukin-6 and -8 levels in preterm labor or rupture of the membranes. Acta Obstet Gynecol Scand 2000 Aug;79(8):667-672.

(270) Hitti J, Tarczy-Hornoch P, Murphy J, Hillier SL, Aura J, Eschenbach DA. Amniotic fluid infection, cytokines, and adverse outcome among infants at 34 weeks' gestation or less. Obstet Gynecol 2001 Dec;98(6):1080-1088.

(271) Gucer F, Balkanli-Kaplan P, Yuksel M, Yuce MA, Ture M, Yardim T. Maternal serum tumor necrosis factor-alpha in patients with preterm labor. J Reprod Med 2001 Mar;46(3):232-236.

(272) Haram K, Mortensen JH, Wollen AL. Preterm delivery: an overview. Acta Obstet Gynecol Scand 2003 Aug;82(8):687-704.

(273) Jansson N, Pettersson J, Haafiz A, Ericsson A, Palmberg I, Tranberg M, et al. Down-regulation of placental transport of amino acids precedes the development of intrauterine growth restriction in rats fed a low protein diet. J Physiol 2006 Nov 1;576(Pt 3):935-946.

(274) Jepsen S, Blanco J, Buchalla W, Carvalho JC, Dietrich T, Dorfer C, et al. Prevention and control of dental caries and periodontal diseases at individual and population level: consensus report of group 3 of joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. J Clin Periodontol 2017 Mar;44 Suppl 18:S85-S93.

(275) Soderling EM, Ekman TC, Taipale TJ. Growth inhibition of Streptococcus mutans with low xylitol concentrations. Curr Microbiol 2008 Apr;56(4):382-385.

(276) Eichenwald EC, Stark AR. Management and outcomes of very low birth weight. N Engl J Med 2008 Apr 17;358(16):1700-1711.

(277) Albandar JM, Buischi YA, Mayer MP, Axelsson P. Long-term effect of two preventive programs on the incidence of plaque and gingivitis in adolescents. J Periodontol 1994 Jun;65(6):605-610.

(278) Axelsson P, Lindhe J, Nystrom B. On the prevention of caries and periodontal disease. Results of a 15-year longitudinal study in adults. J Clin Periodontol 1991 Mar;18(3):182-189.

11 Original Publications

Maternal & Child Nutrition

DOI: 10.1111/mcn.12204

Original Article

Nutrient supplementation may adversely affect maternal oral health – a randomised controlled trial in rural Malawi

Ulla Harjunmaa*, Jorma Järnstedt†, Kathryn G. Dewey‡, Ulla Ashorn*, Kenneth Maleta§, Stephen A. Vosti¶ and Per Ashorn*,**

*Department of International Health, University of Tampere School of Medicine, Tampere, Finland, †Department of Radiology, Medical Imaging Center, Tampere University Hospital, Tampere, Finland, ‡Department of Nutrition, University of California Davis, Davis, California, USA, §Department of Community Health, University of Malawi College of Medicine, Blantyre, Malawi, ¶Department of Agricultural and Resource Economics, University of California Davis, Davis, California, USA and **Department of Paediatrics, Tampere University Hospital, Tampere, Finland*

Abstract

Nutritional supplementation during pregnancy is increasingly recommended especially in low-resource settings, but its oral health impacts have not been studied. Our aim was to examine whether supplementation with multiple micronutrients (MMN) or small-quantity lipid-based nutrient supplements affects dental caries development or periodontal health in a rural Malawian population. The study was embedded in a controlled iLiNS-DYAD trial that enrolled 1391 pregnant women <20 gestation weeks. Women were provided with one daily iron–folic acid capsule (IFA), one capsule with 18 micronutrients (MMN) or one sachet of lipid-based nutrient supplements (LNS) containing protein, carbohydrates, essential fatty acids and 21 micronutrients. Oral examination of 1024 participants was conducted and panoramic X-ray taken within 6 weeks after delivery. The supplement groups were similar at baseline in average socio-economic, nutritional and health status. At the end of the intervention, the prevalence of caries was 56.7%, 69.1% and 63.3% ($P = 0.004$), and periodontitis 34.9%, 29.8% and 31.2% ($P = 0.338$) in the IFA, MMN and LNS groups, respectively. Compared with the IFA group, women in the MMN group had 0.60 (0.18–1.02) and in the LNS group 0.59 (0.17–1.01) higher mean number of caries lesions. In the absence of baseline oral health data, firm conclusions on causality cannot be drawn. However, although not confirmatory, the findings are consistent with a possibility that provision of MMN or LNS may have increased the caries incidence in this target population. Because of the potential public health impacts, further research on the association between gestational nutrient interventions and oral health in low-income settings is needed.

Keywords: oral health, nutritional intervention, micronutrient, pregnancy, dental caries, periodontal diseases.

Correspondence: Dr Ulla Harjunmaa, Department of International Health, University of Tampere School of Medicine, FIN-33014 Tampere, Finland. E-mail: ulla.harjunmaa@uta.fi

Introduction

Dietary supplementation with iron and folic acid (IFA) is recommended to pregnant women all over the world (WHO 2012). The rationale for such a widespread policy is to prevent anaemia in the mother and intrauterine growth restriction in the fetus (Pena-Rosas & Viteri 2009). In areas with high burden of undernutrition, the provision of IFA alone may, however, be insufficient to ensure adequate maternal and fetal nutrition and health. This hypothesis is supported by findings from several controlled trials suggesting reduced maternal mortality, increased fetal growth or improved infant outcomes when pregnant women received supplementation with multiple micronutrients (MMN) rather than IFA (Ramakrishnan *et al.* 2013). Because of these results, several authors have suggested that at least in

low-income settings, IFA supplementation during pregnancy should be substituted by a more comprehensive dietary intervention, consisting of MMNs, possibly coupled with protein, essential fatty acids and other macronutrients (Haider *et al*. 2011; Bhutta *et al*. 2013).

Besides other health effects, nutritional interventions might also affect oral health, most notably the development of periodontitis and dental caries, which are influenced by the host's nutritional status and its impact on oral microflora, saliva flow and composition, and local immunological reactions (Enwonwu 1995; Stephen 1997; Neiva *et al*. 2003). Such an effect might also be important for the fetus, as poor maternal oral health is known to be associated with adverse pregnancy outcomes, such as preterm delivery and low birthweight (Heimonen *et al*. 2008; Ide & Papapanou 2013). Because the development of caries or periodontitis is usually slow, a 3- to 6-month long dietary supplementation would not be likely to have detectable dental effects under most situations. However, the impact might be intensified during pregnancy and especially among women in low-income contexts due to the impaired immune responses (Barak *et al*. 2003), and the high prevalence of both nutritional deficiencies (Black *et al*. 2008) and untreated oral diseases (Petersen *et al*. 2005; Marcenes *et al*. 2013). The scarcity of preventive interventions and available dentistry services (Petersen 2005; Beaglehole *et al*. 2009) in resource-poor settings may further reinforce the effects. The data on the effect of nutrient interventions on oral health of adults are scarce overall and there are no published trials that enrolled pregnant women in low-income settings. Because MMN interventions are increasingly recommended in low-income countries, their impact on oral health needs to be studied.

We conducted a randomised controlled trial in rural Malawi in which we supplemented pregnant women with either IFA, MMN or small-quantity lipid-based nutrient supplements (LNS). The latter (LNS) are a range of macro- and micronutrient-containing products that have been proven effective in the rehabilitation of children with severe acute malnutrition in sub-Saharan Africa (Arimond *et al*. 2013) and may also be useful in the prevention of undernutrition. The main aim of the oral health component of the trial was to evaluate the association between oral diseases and pregnancy outcomes. Because we provided the participants with the nutrient supplements, we found it important to assess also whether the nutrient intervention could affect the oral health of these mothers living in low-resource conditions. We speculated that women provided with LNS might have a lower prevalence of periodontitis but higher prevalence of caries than MMN- or IFA-supplemented women. We based these speculations on the suggested protective effects of dietary fatty acids (Naqvi *et al*. 2010), proteins and micronutrients against periodontitis (Enwonwu 1995; Kaye 2012) and the cariogenic potential of sucrose, which were ingredients in LNS. In this paper we report the prevalence of maternal caries, periapical infections and periodontal diseases in the three supplement groups soon after delivery.

Materials and methods

Study design and outcomes

This study was a sub-study of a randomised, outcome assessor-blinded clinical trial iLiNS-DYAD-M (trial registration: www.clinicaltrials.gov, trial identification NCT01239693). The primary outcomes of the

Key messages

- Supplementation of maternal diet during pregnancy with multiple micronutrients is increasingly recommended due to various health benefits especially in low-income countries.
- Although not confirmatory, the findings of this study suggest that multiple micronutrient supplementation may increase the risk for dental caries development.
- Even slightly increased caries incidence and progression may have significant public health effects, especially in low-resource settings where dental care resources are scarce and other risk factors are highly prevalent.

currently reported study were the prevalence of periodontitis and dental caries, and secondary outcomes were the prevalence of gingivitis and periapical infections.

Study site, participants, enrolment and randomisation

We enrolled women who came to antenatal clinics between February 2011 and August 2012 at two hospitals (Mangochi and Malindi) and two health centres (Lungwena and Namwera) in Mangochi district, southern Malawi. The catchment area of Mangochi district hospital was semi-urban with an estimated population of 100 000 while the other facilities served rural areas with a population of 30 000 each, subsisting mainly on farming and fishing.

At enrolment, research personnel recorded participants' medical, social and obstetric history, and performed anthropometric measurements and health and antenatal examinations. Participants were eligible for enrolment into the iLiNS-DYAD trial if they had ultrasound-confirmed pregnancy <20 weeks, age ≥15 years, no chronic illnesses requiring frequent medical care, no allergies and no evident pregnancy complications.

Enrolled participants were randomly assigned to three groups. The first group received standard Malawian antenatal care including supplementation with one iron–folic acid capsule a day (IFA). The capsules of the second group contained 16 additional micronutrients (MMN). The third group (LNS) received a daily 20 g sachet of LNS containing the same 18 micronutrients and four additional minerals, protein, fat and 1.2 g of sucrose (Supporting Information Table S1). The IFA and MMN interventions were provided using double-masked procedures and LNS using single-masked procedures. The IFA and MMN capsules looked identical, but the field workers could identify the LNS sachets. The outcome assessors and researchers remained blind to the trial code until the data collection and cleaning process was completed. The enrolment, randomisation and intervention procedures have been published in details elsewhere (Ashorn *et al*. 2015).

Participants were eligible for the oral health substudy if they were enrolled in the iLiNS-DYAD trial, had singleton pregnancies and completed the oral health visit after delivery or miscarriage. Participants who completed the visit within 6 weeks after delivery or miscarriage were included in the analysis regardless of the number of teeth they had. Six weeks was selected as the cut point because it represents the end of the puerperal period.

Oral health examination

Participants were invited and transport provided to Mangochi district hospital for oral health examination as soon as possible after delivery. Three experienced and specifically trained dental therapists conducted full-mouth dental and periodontal examinations, took digital panoramic radiographs (Planmega Proline XC, Planmega, Finland) and asked multiple choice questions on the participant's oral health care habits, oral health problems and treatment received during the previous 6 months. The participant sat on a chair with back and arm rests and the examiner used a head lamp for visibility (Pezl Tikka XP^2, Pezl, France). The examiners recorded caries lesions extending unambiguously to the dentin. They did not record superficial caries (restricted to the enamel) because its accurate diagnosis was not feasible in the study environment. They measured periodontal pocket probing depth from six sites of each tooth excluding third molars, using a World Health Organization periodontal probe (LM-Instruments, Parainen, Finland; reading increments at 3.5, 5.5, 8.5 and 11.5 mm) and recorded the deepest measurement of each tooth rounded to the nearest mm. They assessed the presence of gingivitis as profound bleeding from the gums after gentle (20 g weight) probing and recorded it by dental arch sextants (right, mid and left upper and lower). The examiners' measurement reliability was assessed and verified at the beginning and regularly during the study against the measurements of an experienced dentist (U.H.) representing the gold standard.

An oral and maxillofacial radiologist (J.J.) and a dentist (U.H.) analysed the radiographs with digital imaging software (Planmega Romexis™) and a

good-quality computer screen in a darkened room. They calculated the number of teeth, including impacted teeth and root remnants and number of restorations (fillings). They recorded caries as lesions extending to the dentin or to the pulp, and diagnosed periapical infections if an osteolytic finding >1 mm, surrounding the apex (tip) of the dental root, was present. If the finding was questionable, they recorded it as 'not present'. They assessed alveolar bone loss by measuring the bone level of each tooth from the dento-enamel junction (between the crown and the roots) to the deepest point of the bony pocket (if present) and the mean horizontal bone level by arch sextants, and expressed these measurements relative to the full length of the root (normal level, cervical, mid or apical third of root length). The X-ray analysts re-assessed at least two earlier X-rays each week and if any deviance between the two readings was found, they discussed them until agreement was reached.

Definitions

We defined dentine caries (grade II) as a lesion penetrating the enamel and extending to the dentine either in clinical or radiological examination or both. We diagnosed pulpal caries (grade III) from the radiographs if a lesion extended to the pulp (dental nerve chamber) with no bony layer visible in between was confirmed. We defined gingivitis as at least one dental arch sextant with bleeding from the gums after probing. We considered that a tooth had periodontitis if either a ≥4 mm pocket was measured in clinical examination, or a vertical bony pocket was identified at least at the cervical root level on the radiographs. We considered that a participant had periodontitis if she had at least three teeth with periodontitis or one dental arch sextant with horizontal bone loss at least at cervical level, and gingivitis present.

Statistical analysis

The sample size was originally calculated in accordance with the main objective of the iLiNS-DYAD trial (Ashorn *et al*. 2015). The sample size of about 340 per group obtained for this substudy offered about 90% power to detect differences between the three groups, assuming an effect size of at least 0.25 [mean difference between groups, divided by the pooled standard deviation (SD)] for continuous birth outcomes at 5% two-sided type I error rate.

We carried out the statistical analysis with Stata 12.1 (StataCorp, College Station, TX, USA) according to the analysis plan written and published before the intervention code was opened (http://www.ilins.org). We based the analysis on the principle of intention to treat.

We created a proxy for socio-economic status with principal component analysis by combining information on the building material of the house, main source of water and electricity, sanitary facility and main type of cooking fuel used (Filmer & Pritchett 2001).

To prevent inflated type I errors caused by multiple comparisons, we tested global null hypotheses of all three groups being identical before doing pairwise comparisons. We tested the global null hypothesis either with Fisher's exact test (for binary endpoints) or analysis of variance (ANOVA) (for quantitative end points) and the pairwise hypotheses with log-binomial regression model (for binary end points) or ANOVA (for quantitative end points). We estimated risk ratio (RR) for comparison of binary end points and risk difference for comparison of quantitative end points at a single time point. With a large sample size, parametric analysis of means is robust and valid regardless of the shape of outcome distribution as per the central limit theorem (Rice 1995; Cheung 2013).

To control for possible confounding and to maximise power by reducing the variance of the outcomes, we created multivariate models using forced entry method. All relevant and available covariates that could confound the nutrition intervention effect on the oral diseases were included in the model. The covariates selected *a priori* were visit time (days from delivery), maternal age, number of previous pregnancies, socio-economic status, education (completed years at school), body mass index and number of teeth as continuous variables, HIV status, malaria status, anaemia, tooth brush usage and tooth paste usage as dichotomous variables and study site as a categorical variable. Smoking was omitted as no participants reported that they had ever smoked. All the models

were adjusted for the same set of variables by log-Poisson models (for binary end points) and linear regression models (for quantitative end points). Before using covariates in the model, we performed tests for interaction between the intervention, oral infection outcomes and selected variables from the covariate list using likelihood ratio test. Tooth brush and paste usage and number of teeth were excluded from interaction testing because they were recorded after the intervention was finished.

As a sensitivity analysis to explore if extraction of diseased teeth during the intervention period might have biased the results, we repeated the analyses after adding extracted teeth to the number of deep caries lesions. Because none of the participants had received restorative treatment during the intervention period, we did not conduct sensitivity analyses including fillings.

Ethics

The study was conducted according to good clinical practice guidelines and the ethical standards of the Helsinki Declaration. The protocol was approved by the College of Medicine Research and Ethics Committee, University of Malawi, Malawi, and the Ethical Committee of Pirkanmaa Hospital District, Finland. Only participants who signed or thumb printed an informed consent form were enrolled in the study. The participants received transport to the study clinic and small incentives (e.g. 1 kg of rice or two bars of soap) after each visit, subsidised health care and complimentary dental care after the oral health examination.

Results

Deliveries or miscarriages took place between May 2011 and February 2013. Of the 1391 women who were enrolled into the iLiNS-DYAD study, 1229 (88.4%) completed the oral health examination. After excluding twin pregnancies and those whose examination was performed later than 6 weeks after delivery, 1024 (73.6%) participants were included in the analyses. Loss to follow-up was similar in all of the supplement groups ($P = 0.762$) (Fig. 1).

The three study groups of participants included in the analysis were similar at enrolment in terms of their average demographic and socio-economic characteristics and nutritional and health status (Table 1). The 367 enrolled participants who were excluded were similar to the included ones in terms of their anthropometric and nutritional status but they were slightly more often primiparous (33.3% vs. 17.8%, $P < 0.001$) and malaria positive (28.1% vs. 21.4%, $P = 0.009$) and had a somewhat higher mean proxy for socio-economic status (0.46 vs. –0.13, $P < 0.001$) (Supporting Information Table S2).

The mean (SD) time interval between delivery and the dental examination was 15.1 (7.6) days in the IFA, 16.0 (7.7) days in the MMN and 16.8 (8.6) days in the LNS group ($P = 0.020$). All participants combined, 85.8% ($n = 879$) had gingivitis and 31.9% ($n = 327$) periodontitis. The prevalences (n) of caries exposing dentin (grade II + III), pulpal caries (grade III) and periapical infections were 63.1% (646), 27.8% (285) and 23.5% (241), respectively. Overall, the participants had an average of 31.3 teeth (SD 1.6, min. 18, max. 37) and 2.1 (SD 2.8, min. 0, max. 25) carious teeth. Thirty women had supernumerous teeth (one, two or five extra teeth per woman). Only five participants had received restorative treatment (fillings): one in IFA, two in MMN and two in LNS group (one to seven fillings per woman).

Table 2 shows the prevalence of caries, periapical infections and periodontal diseases by intervention group. The prevalence of grade II–III caries lesions was 56.7% in the IFA group, 69.1% in the MMN group and 63.3% in the LNS group ($P = 0.004$). Compared with the IFA control, the relative risk (95% confidence interval) of grade II–III caries was 1.12 (0.99–1.26) in the LNS group ($P = 0.083$) and 1.22 (1.08–1.37) in the MMN group ($P = 0.001$). When analysed separately, there were similar differences in the prevalence of grade II caries while the differences in grade III caries and periapical infections were smaller and statistically non-significant. The prevalence of clinically or radiologically diagnosed periodontitis was slightly higher in the IFA group than in the MMN and LNS groups, but the differences were statistically

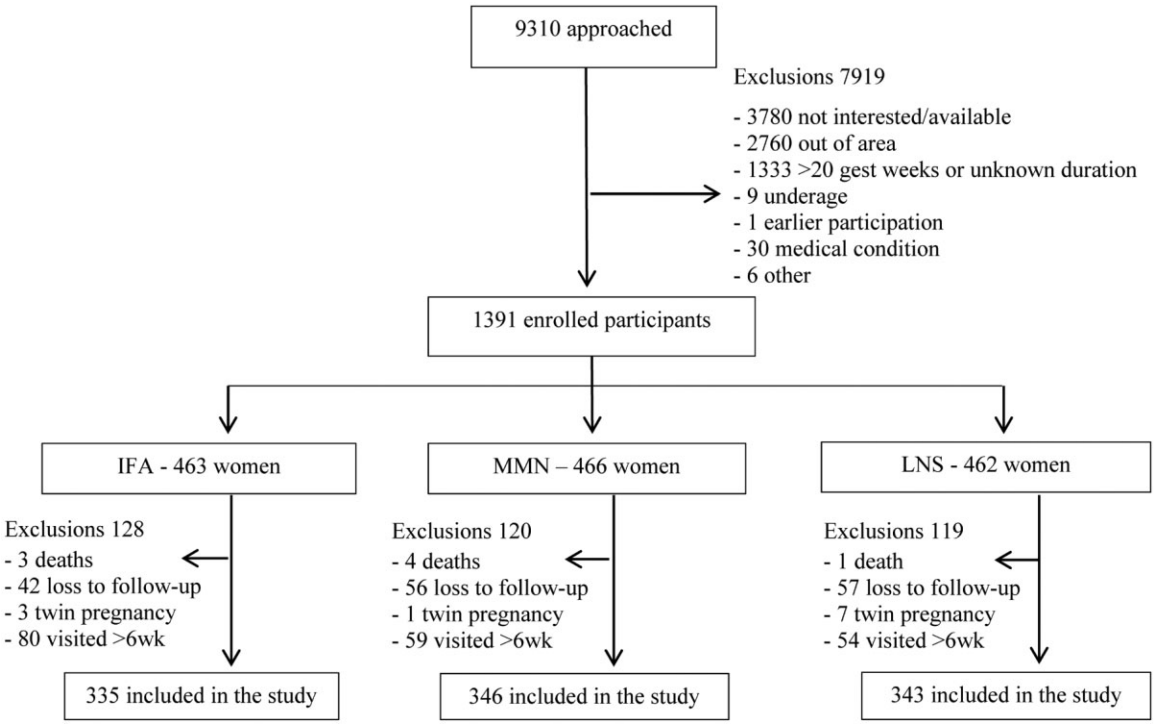


Fig. 1. Participant flow in CONSORT-recommended form.

Table 1. Baseline characteristics of participants at enrolment by intervention group

Characteristic	IFA	MMN	LNS
Mean (range) duration of pregnancy at enrolment (weeks)	16.8 (11.6–20.0)	16.8 (12.0–20.3)	16.8 (12.3–21.0)
Number of participants	335	346	343
Mean (SD) age, years	25 (6)	25 (6)	26 (6)
Mean (SD) education, completed years	3.9 (3.4)	4.0 (3.4)	3.9 (3.4)
Mean (SD) proxy for socio-economic status*	–0.22 (1.59)	–0.07 (1.73)	–0.11 (1.72)
Proportion of primiparous women	18.0%	18.6%	16.9%
Mean (SD) weight, kg	54.1 (7.2)	54.0 (8.1)	53.7 (7.8)
Mean (SD) mid-upper arm circumference, cm	26.4 (2.3)	26.2 (2.7)	26.4 (2.6)
Mean (SD) body mass index, BMI (kg m^{-2})	22.1 (2.5)	22.1 (2.9)	22.0 (2.7)
Proportion of women with a low BMI (<18.5 kg m^{-2})	4.8%	5.0%	6.4%
Proportion of anaemic women (Hb <110 g L^{-1})	21.5%	18.2%	19.8%
Proportion of women with a positive HIV test	15.0%	11.6%	15.7%
Proportion with a positive malaria test (RDT)	21.2%	20.9%	22.2%

Hb, blood haemoglobin concentration; HIV, human immunodeficiency virus; IFA, iron and folic acid supplement; LNS, lipid-based nutrient supplement; MMN, multiple micronutrient supplement; RDT, malaria rapid diagnostic test obtained from a finger prick; SD, standard deviation.
*Combining information on building material of house, source of water, sanitary facility, source of electricity and type of cooking fuel.

non-significant. Adjustment of the analyses for thethree baseline variables that differed between the included and excluded participants, the time between delivery and the dental examination and other potential predictors did not markedly change the association between the intervention and grade II–III caries (Table 2) or other oral health outcomes (Supporting Information Table S3).

Table 2. Oral disease prevalence by intervention groups

Outcome	Number of outcomes/women with outcome data				Comparison between LNS and IFA group		Comparison between LNS and MMN group		Comparison between MMN and IFA group	
	IFA ($n = 335$)	MMN ($n = 346$)	LNS ($n = 343$)	*P*-value	Risk ratio (95 % CI)	*P*-value	Risk ratio (95 % CI)	*P*-value	Risk ratio (95 % CI)	*P*-value
Prevalence of any (grade II–III) caries*	190 (56.7%)	239 (69.1%)	217 (63.3%)	0.004	1.12 (0.99–1.26)	0.083	0.92 (0.82–1.02)	0.108	1.22 (1.08–1.37)	0.001
RR, adjusted model†					1.10 (0.90–1.34)	0.342	0.92 (0.76–1.11)	0.365	1.20 (0.99–1.46)	0.065
Prevalence of grade II caries*	173 (51.6%)	218 (63.1%)	202 (58.9%)	0.010	1.14 (1.00–1.31)	0.059	0.93 (0.83–1.05)	0.269	1.22 (1.06–1.39)	0.003
Prevalence of grade III caries*	86 (25.7%)	106 (30.6%)	93 (27.1%)	0.330	1.06 (0.82–1.36)	0.670	0.89 (0.70–1.12)	0.308	1.19 (0.94–1.52)	0.151
Prevalence of periapical infections*	73 (21.8%)	87 (25.1%)	81 (23.6%)	0.584	1.08 (0.82–1.43)	0.636	0.94 (0.72–1.22)	0.640	1.15 (0.88–1.51)	0.303
Prevalence of gingivitis*	289 (86.3%)	291 (84.1%)	299 (87.2%)	0.499	1.00 (1.00–1.00)	n/a	1.04 (0.98–1.11)	0.211	0.83 (0.80–0.87)	0.312
Prevalence of clinical periodontitis*	68 (20.3%)	62 (17.9%)	58 (16.9%)	0.505	0.83 (0.61–1.14)	0.258	0.94 (0.68–1.31)	0.727	0.88 (0.65–1.20)	0.430
Prevalence of periodontitis (clinical + X-ray)*	117 (34.9%)	103 (29.8%)	107 (31.2%)	0.338	0.89 (0.72–1.11)	0.302	1.05 (0.84–1.31)	0.684	0.85 (0.69–1.06)	0.151

CI, confidence interval; IFA, iron and folic acid supplement; LNS, lipid-based nutrient supplement; MMN, multiple micronutrient supplement; RR, risk ratio. *Crude values. †Adjusted for visit time, age, body mass index at enrolment, number of previous pregnancies, anaemia, human immunodeficiency virus status, malaria status at enrolment, education (completed years), socio-economic status, study site, number of teeth, tooth brush usage and daily tooth paste usage.

Table 3 shows the oral health variables by intervention group, using continuous outcomes. There was a between-group difference in the mean number of grade II–III ($P = 0.006$) and grade II ($P = 0.001$) caries lesions. Compared with women in the IFA group, those in the LNS group had 0.59 (0.17–1.01) and those in the MMN group 0.60 (0.18–1.02) more grade II–III caries lesions. Signs of gingivitis or periodontal disease were roughly equally distributed between groups. Adjustment of the analyses for the selected baseline variables did not markedly change the results (Table 3) (Supporting Information Table S4).

The sensitivity analyses adding extracted teeth to the number of deep caries lesions did not lead to markedly different results (data not shown). Tests for interaction did not indicate a modification of the intervention effect on oral health outcomes by any of the tested baseline characteristics.

The mean number of teeth was equal in all the intervention groups. The groups did not differ in the participant-reported oral health care habits, oral health problems or received treatment during the preceding 6 months. Almost all women (90.0%, $n = 622$) reported using a tooth brush but only 43.0% ($n = 440$) reported using tooth paste daily. Tooth ache was experienced by 20.1% ($n = 205$) of women and 17.3% ($n = 176$) had limited their food consumption due to oral health problems within the past 6 months (Table 4).

Discussion

The purpose of this study was to investigate whether nutrient intervention during pregnancy with MMN or LNS affects oral health, especially dental caries and periodontal disease prevalence, within a deprived population in rural Malawi. The participants who had received either MMN or LNS had higher caries prevalence and mean number of caries lesions but were not significantly different in the periodontal parameters compared with those who were supplemented with IFA.

The probabilities of bias in this study were minimised by the randomised study design, blinding of the examiners to intervention groups, rigorous quality assurance in data collection and the combination of clinical and high-quality X-ray-based assessment that allowed exact diagnostics, especially on dental caries. The main weakness of the study was the lack of baseline oral health data. The other weaknesses and potential sources of bias were the 26.4% loss to follow-up, our inability to document the oral health status immediately after delivery, some inter-group variation in the time between delivery and oral health examination and some differences in baseline characteristics between participants who were included in analyses and those lost to follow-up. Hence, the sample findings may be biased and not adequately representative of the target population and therefore no definitive conclusions on causality can be made from the data. However, allocation to different interventions was performed randomly, the groups were similar to each other in terms of many studied baseline variables, loss to follow-up was balanced between groups and various adjusted analyses gave similar results to the main ones. Therefore, although not confirmatory, the results are consistent with the possibility that provision of MMN or LNS may have increased grade II caries incidence in this target population. Concerning the prevalence of periodontitis and the mean number of periodontal pockets, the data did not support the assumption that the gestational nutrient intervention would have beneficial impacts.

Excluding the well-known detrimental effect of sugars (Touger-Decker & van Loveren 2003) and protective effect of fluorides (Marinho 2009) on dental health, little is known about the effect of other nutrients on dental caries development in adults. With that incomplete knowledge, and also being aware of the slow process of caries development, we assumed that sucrose in LNS might slightly increase the caries incidence in our study population but supplementing with IFA or MMN would not have any effect. Although we were not able to assess superficial caries (grade I), which might have been a more sensitive indicator for the possible impacts of the intervention on enamel demineralisation, we speculate that the intervention may advance the development of superficial caries to grade II lesions. Rather surprisingly, the caries prevalence and the

Table 3. Continuous oral health variables by intervention groups

Outcome	Result by study group				Comparison between LNS and IFA group		Comparison between LNS and MMN group		Comparison between MMN and IFA group	
	IFA (n = 335)	MMN (n = 346)	LNS (n = 343)	P-value	Difference in means (95% CI)	P-value	Difference in means (95% CI)	P-value	Difference in means (95% CI)	P-value
Mean (SD) no. of any (grade II–III) caries lesions*	1.73 (2.32)	2.32 (2.84)	2.32 (3.16)	0.006	0.59 (0.17 to 1.01)	0.006	–0.00 (–0.42 to 0.41)	0.989	0.60 (0.18 to 1.02)	0.006
Difference in mean, adjusted model†					0.53 (0.13 to 0.94)	0.010	–0.04 (–0.44 to 0.36)	0.834	0.57 (0.17 to 0.98)	0.005
Mean (SD) no. of grade II caries lesions*	1.19 (1.65)	1.66 (1.98)	1.69 (2.21)	0.001	0.51 (0.21 to 0.80)	0.001	0.03 (–0.26 to 0.33)	0.815	0.47 (0.18 to 0.77)	0.002
Mean (SD) no. of grade III caries lesions*	0.54 (1.25)	0.66 (1.37)	0.62 (1.65)	0.529	0.08 (–0.13 to 0.30)	0.480	–0.04 (–0.25 to 0.18)	0.689	0.12 (–0.09 to 0.34)	0.269
Mean (SD) no. of periapical lesions*	0.41 (0.98)	0.52 (1.20)	0.50 (1.41)	0.442	0.09 (–0.09 to 0.28)	0.321	–0.02 (–0.20 to 0.16)	0.839	0.11 (–0.07 to 0.29)	0.231
Mean (SD) no. of sextants with bleeding on probing*	3.50 (2.08)	3.47 (2.17)	3.33 (2.12)	0.538	–0.17 (–0.49 to 0.15)	0.293	–0.14 (–0.45 to 0.18)	0.402	–0.04 (–0.36 to 0.28)	0.824
Mean (SD) no. of periodontal pockets ≥4 mm (clinical)*	1.70 (3.54)	1.38 (3.05)	1.43 (3.39)	0.409	–0.27 (–0.77 to 0.23)	0.291	0.04 (–0.45 to 0.54)	0.853	–0.32 (–0.82 to 0.18)	0.214
Mean (SD) periodontal pocket depth, mm*	2.32 (0.53)	2.29 (0.52)	2.32 (0.53)	0.662	–0.00 (–0.08 to 0.08)	0.950	0.03 (–0.05 to 0.11)	0.444	–0.03 (–0.11 to 0.05)	0.410

IFA, iron and folic acid supplement; LNS, lipid-based nutrient supplement; MMN, multiple micronutrient supplement; SD, standard deviation. *Crude values. †Adjusted for visit time, age, body mass index at enrolment, number of previous pregnancies, anaemia, human immunodeficiency virus status, malaria status at enrolment, education (completed years), socio-economic status, study site, number of teeth, tooth brush usage and daily tooth paste usage.

Table 4. Oral health characteristics, oral health-related behaviour and reported oral health problems by intervention group

Characteristic	IFA (n = 335)	MMN (n = 346)	LNS (n = 343)	P-value
Mean (SD) number of teeth	31.4 (1.4)	31.3 (1.6)	31.3 (1.8)	0.493
Proportion of women (n) using tooth brush	90.8% (304)	91.6% (317)	87.8% (301)	0.213
Proportion of women (n) using tooth paste daily	44.2% (148)	43.4% (150)	41.4% (142)	0.756
Proportion of women (n) who experienced toothache*	18.2% (61)	21.2% (73)	20.9% (71)	0.560
Proportion of women (n) who had disturbed daily activities due to oral health problems*	15.2% (51)	16.0% (55)	15.0% (51)	0.940
Proportion of women (n) who limited food consumption due to oral health problems*	18.0% (60)	17.8% (61)	16.2% (55)	0.806
Proportion of women (n) who visited health care provider due to oral health problems*	6.0% (20)	5.5% (19)	5.3% (18)	0.403
Proportion of women (n) who had tooth extracted*	3.3% (11)	2.6% (9)	1.5% (5)	0.244
Proportion of women (n) who had used painkillers due to toothache*	7.8% (26)	9.3% (32)	10.2% (35)	0.395

IFA, iron and folic acid supplement; LNS, lipid-based nutrient supplement; MMN, multiple micronutrient supplement; SD, standard deviation.
*During the past 6 months, based on own report.

mean number of lesions were similar in both MMN and LNS groups and higher than in the IFA group. One possible explanation for this finding is the higher dose of iron in the IFA than in the MMN and LNS supplements that might have suppressed caries development for instance by reducing enamel demineralisation (Torell 1988; Alves *et al.* 2011). Another explanation might be the potential effects of certain nutrients on the oral microbiota (Blais & Lavoie 1990). Hypothetically, in resource-poor settings where the baseline nutrient levels are low and the prevalence of untreated caries lesions high, nutrient supplementation might lead to increasing bacterial amounts or virulence and thus proliferate caries development. The effect may be even more pronounced due to pregnancy-related changes in the oral cavity and suppression in immune responses (Laine 2002).

The development of periodontal diseases is indirectly related to nutrition that affects the tissue formation, the immune system in the periodontium and the host susceptibility to the infections (Dawson *et al.* 2014). Earlier studies have suggested that periodontitis is associated with low plasma micronutrient concentrations, especially with magnesium (Meisel *et al.* 2005), vitamins D and C and other antioxidants (Van der Velden *et al.* 2011), and that low vitamin B complex, vitamin C, calcium (Neiva *et al.* 2003) and omega-3 fatty acid (Naqvi *et al.* 2010) intake may be related to progression of periodontal diseases. Based on that, we expected that the group receiving MMN might have slightly better periodontal health compared with the IFA group and that LNS might have an even more pronounced beneficial effect due to its extra nutrients and fatty acids. A potential explanation for not finding any significant differences between groups may be the rather short intervention period relative to the slow appearance of X-ray detectable changes in alveolar bone level (Reddy & Jeffcoat 1999).

We are aware that the lack of baseline oral health data was a substantial limitation in this study. This was however unavoidable because the disease diagnosis was mainly based on X-rays, which should not be taken routinely during pregnancy. In addition, treating the diagnosed diseases at the beginning of the intervention would have distorted the study itself, and not treating would have been unethical. In that light, it is possible that the findings are spurious and represent the play of chance. Yet, the probability of a causal pathway cannot be excluded. Given the frequency of micronutrient supplementation, the high prevalence of oral diseases, and their impact on general health (Linden *et al.* 2013), even small changes in disease incidence may have significant public health consequences, especially in low-income settings where dental care resources are scarce and other risk factors prevalent (Petersen 2003). Therefore, further studies are warranted in other low-resource populations.

Acknowledgements

We thank the study participants, the local communities, our research personnel at the study sites and the iLiNS extended research team for their positive attitude and support during the study. We particularly thank Simeon Mulewa, Davie Charlie and Mofolo Paundi for the clinical oral health data collection, Lotta Alho for her statistical input and the iLiNS Project Steering Committee members and Project Manager Mary Arimond (http://ilins.org) for technical support.

Source of funding

This publication is based on research funded in part by a grant to the University of California, Davis from the Bill and Melinda Gates Foundation, with additional funding from the Office of Health, Infectious Diseases, and Nutrition, Bureau for Global Health, US Agency for International Development (USAID) under terms of Cooperative Agreement no. AID-OAA-A-12-00005), through the Food and Nutrition Technical Assistance III Project (FANTA), managed by FHI 360. The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of the Bill and Melinda Gates Foundation, USAID, the US government or the other funders. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Conflict of interest

UH received a travel grant from Nutriset S.A.S, a company that produces and sells LNS and that also produced the LNS supplements purchased for the current trial. Other authors declare no conflicts of interest.

Contributions

The authors' responsibilities were as follows: UH, PA, KGD, UA, KM and SAV designed research; UH, JJ and PA conducted research; UH analysed data; UH and PA wrote the paper, with critical input and comments from all other authors. All authors read and approved the final manuscript.

References

Alves K.M., Franco K.S., Sassaki K.T., Buzalaf M.A. & Delbem A.C. (2011) Effect of iron on enamel demineralization and remineralization in vitro. *Archives of Oral Biology* **56**, 1192–1198.

Arimond M., Zeilani M., Jungjohann S., Brown K.H., Ashorn P., Allen L.H. *et al*. (2013) Considerations in developing lipid-based nutrient supplements for prevention of undernutrition: experience from the International Lipid-Based Nutrient Supplements (iLiNS) project. *Maternal & Child Nutrition* **2013** [E-pub ahead of print]. doi: 10.1111/mcn.12049.

Ashorn P., Alho L., Ashorn U., Cheung Y., Dewey K., Harjunmaa U. *et al*. (2015) The impact of lipid-based nutrient supplement provision to pregnant women on newborn size in rural Malawi: a randomised controlled trial. *The American Journal of Clinical Nutrition* **101**, 387–397.

Barak S., Oettinger-Barak O., Oettinger M., Machtei E.E., Peled M. & Ohel G. (2003) Common oral manifestations during pregnancy: a review. *Obstetrical & Gynecological Survey* **58**, 624–628.

Beaglehole J., Benzian H., Crail J. & Mackay J. (2009) *The Oral Health Atlas. Mapping a Neglected Global Health Issue*. FDI World Dental Federation: Cointrin, Switzerland.

Bhutta Z.A., Das J.K., Rizvi A., Gaffey M.F., Walker N., Horton S. *et al*. (2013) Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? *Lancet* **382**, 452–477.

Black R.E., Allen L.H., Bhutta Z.A., Caulfield L.E., de Onis M., Ezzati M. *et al*. (2008) Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* **371**, 243–260.

Blais J.F. & Lavoie M.C. (1990) Effect of dietary components on the indigenous oral bacterial flora of BALB/c mice. *Journal of Dental Research* **69**, 868–873.

Cheung Y. (2013) *Statistical Analysis of Human Growth and Development*, 1st edn. CRC Press: Boca Raton, FL.

Dawson D.R. 3rd, Branch-Mays G., Gonzalez O.A. & Ebersole J.L. (2014) Dietary modulation of the inflammatory cascade. *Periodontology 2000* **64**, 161–197.

Enwonwu C.O. (1995) Interface of malnutrition and periodontal diseases. *The American Journal of Clinical Nutrition* **61**, 430S–436S.

Filmer D. & Pritchett L.H. (2001) Estimating wealth effects without expenditure data – or tears: an application to educational enrollments in states of India. *Demography* **38**, 115–132.

Haider B.A., Yakoob M.Y. & Bhutta Z.A. (2011) Effect of multiple micronutrient supplementation during pregnancy on maternal and birth outcomes. *BMC Public Health* **11** (Suppl. 3), S3–S19.

Heimonen A., Rintamaki H., Furuholm J., Janket S.J., Kaaja R. & Meurman J.H. (2008) Postpartum oral health parameters in women with preterm birth. *Acta Odontologica Scandinavica* **66**, 334–341.

Ide M. & Papapanou P.N. (2013) Epidemiology of association between maternal periodontal disease and adverse pregnancy outcomes – systematic review. *Journal of Periodontology* **84** (Suppl. 4), S181–S194.

Kaye E.K. (2012) Nutrition, dietary guidelines and optimal periodontal health. *Periodontology 2000* **58**, 93–111.

Laine M.A. (2002) Effect of pregnancy on periodontal and dental health. *Acta Odontologica Scandinavica* **60**, 257–264.

Linden G.J., Lyons A. & Scannapieco F.A. (2013) Periodontal systemic associations: review of the evidence. *Journal of Clinical Periodontology* **40** (Suppl. 14), S8–S19.

Marcenes W., Kassebaum N.J., Bernabe E., Flaxman A., Naghavi M., Lopez A. *et al*. (2013) Global burden of oral conditions in 1990–2010: a systematic analysis. *Journal of Dental Research* **92**, 592–597.

Marinho V.C. (2009) Cochrane reviews of randomized trials of fluoride therapies for preventing dental caries. *European Archives of Paediatric Dentistry* **10**, 183–191.

Meisel P., Schwahn C., Luedemann J., John U., Kroemer H.K. & Kocher T. (2005) Magnesium deficiency is associated with periodontal disease. *Journal of Dental Research* **84**, 937–941.

Naqvi A.Z., Buettner C., Phillips R.S., Davis R.B. & Mukamal K.J. (2010) n-3 fatty acids and periodontitis in US adults. *Journal of the American Dietetic Association* **110**, 1669–1675.

Neiva R.F., Steigenga J., Al-Shammari K.F. & Wang H.L. (2003) Effects of specific nutrients on periodontal disease onset, progression and treatment. *Journal of Clinical Periodontology* **30**, 579–589.

Pena-Rosas J.P. & Viteri F.E. (2009) Effects and safety of preventive oral iron or iron+folic acid supplementation for women during pregnancy. *The Cochrane Database of Systematic Reviews* (4), CD004736.

Petersen P., Bourgeois D., Ogawa H., Estupinan-Day S. & Ndiaye C. (2005) The global burden of oral diseases and risks to oral health. *Bulletin of the World Health Organization* **83**, 661–669.

Petersen P.E. (2003) The World Oral Health Report 2003: continuous improvement of oral health in the 21st century – the approach of the WHO Global Oral Health Programme. *Community Dentistry and Oral Epidemiology* **31** (Suppl. 1), 3–23.

Petersen P.E. (2005) Sociobehavioural risk factors in dental caries – international perspectives. *Community Dentistry and Oral Epidemiology* **33**, 274–279.

Ramakrishnan U., Grant F.K., Imdad A., Bhutta Z.A. & Martorell R. (2013) Effect of multiple micronutrient versus iron-folate supplementation during pregnancy on intrauterine growth. *Nestle Nutrition Institute Workshop Series* **74**, 53–62.

Reddy M.S. & Jeffcoat M.K. (1999) Methods of assessing periodontal regeneration. *Periodontology 2000* **19**, 87–103.

Rice J. (1995) *Mathematical Statistics and Data Analysis*, 2nd edn. Duxbury Press: Belmont, CA.

Stephen M. (1997) The role of diet, fluoride and saliva in caries prevention. *Journal of the Indian Society of Pedodontics and Preventive Dentistry* **15**, 109–113.

Torell P. (1988) Iron and dental caries. *Swedish Dental Journal* **12**, 113–124.

Touger-Decker R. & van Loveren C. (2003) Sugars and dental caries. *The American Journal of Clinical Nutrition* **78**, 881S–892S.

Van der Velden U., Kuzmanova D. & Chapple I.L. (2011) Micronutritional approaches to periodontal therapy. *Journal of Clinical Periodontology* **38** (Suppl. 11), 142–158.

World Health Organization (WHO) (2012) *Guideline: Daily Iron and Folic acid Supplementation in Pregnant Women*. World Health Organization: Geneva.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Nutrient and energy contents of the dietary supplements in iLiNS-DYAD trial.

Table S2. Baseline characteristics of participants included into and excluded from the analysis (n = 1391).

Table S3. Oral infection prevalence by intervention groups, adjusted models.

Table S4. Continuous oral health variables by intervention groups, adjusted models.

Supplementary material, Nutrition intervention May Adversely Affect Maternal Oral Health

Supplementary table 1. Nutrient and energy contents of the dietary supplements in iLiNS-DYAD trial

Nutrient	**IFA**	**MMN**	**LNS**
Ration (g/day)	1 tablet	1 tablet	20 g sachet
Total energy (kcal)	0	0	118
Protein (g)	0	0	2.6
Fat (g)	0	0	10
Linoleic acid (g)	0	0	4.59
α-Linolenic acid (g)	0	0	0.59
Vitamin A (µg RE)	0	800	800
Vitamin C (mg)	0	100	100
Vitamin B1(mg)	0	2.8	2.8
Vitamin B2 (mg)	0	2.8	2.8
Niacin (mg)	0	36	36
Folic acid (µg)	400	400	400
Pantothenic acid (mg)	0	7	7
Vitamin B6 (mg)	0	3.8	3.8
Vitamin B12 (µg)	0	5.2	5.2
Vitamin D (IU)	0	400	400
Vitamin E (mg)	0	20	20
Vitamin K (µg)	0	45	45
Iron (mg)	60	20	20
Zinc (mg)	0	30	30
Cu (mg)	0	4	4
Calcium (mg)	0	0	280
Phosphorus (mg)	0	0	190
Potassium (mg)	0	0	200
Magnesium (mg)	0	0	65
Selenium (µg)	0	130	130
Iodine (µg)	0	250	250
Manganese (mg)	0	2.6	2.6

Supplementary material, Nutrition intervention May Adversely Affect Maternal Oral Health

Supplementary table 2. Baseline characteristics of participants included into and excluded from the analysis (n=1391)

Characteristic	**Included into the oral health study**	**Excluded from the oral health study**	**P-value**
Number of participants	1024	367	n/a
Mean (SD) age, years	25 (6.2)	24 (5.7)	<0.001
Mean (SD) education, completed years at school	3.9 (3.4)	4.3 (3.6)	0.087
Mean (SD) proxy for socioeconomic status[a]	-0.13 (1.68)	0.46 (2.12)	<0.001
Proportion of primiparous women (n)	17.8% (182)	33.3% (122)	<0.001
Mean (SD) weight, kg	53.9 (7.7)	54.5 (8.7)	0.224
Mean (SD) mid-upper arm circumference, cm	26.3 (2.5)	26.5 (2.9)	0.284
Mean (SD) body-mass index, BMI (kg/m²)	22.1 (2.7)	22.4 (3.1)	0.046
Proportion of women with a low BMI (< 18.5 kg/m²), (n)	5.4% (55)	5.2% (19)	0.891
Proportion of anemic women (Hb < 110 g/l), (n)	19.8% (203)	23.0% (84)	0.208
Proportion of women with a positive HIV test (n)	14.1% (144)	12.5% (39)	0.460
Proportion with a positive malaria test (RDT), (n)	21.4% (219)	28.1% (103)	0.009

[a]Created with principal components analysis by combining information on the building material of the house, main source of water and electricity, sanitary facility and main type of cooking fuel used

Supplementary material, Nutrition intervention May Adversely Affect Maternal Oral Health

Supplementary table 3. Oral infection prevalence by intervention groups, adjusted models

Outcome	Number of outcomes / women with outcome data[a]				Comparison between LNS and IFA group, adjusted model[b]		Comparison between LNS and MMN group, adjusted model[b]		Comparison between MMN and IFA group, adjusted model[b]	
	IFA (n=335)	MMN (n=346)	LNS (n=343)	P-value	Risk ratio (95 % CI)	P-value	Risk ratio (95 % CI)	P-value	Risk ratio (95 % CI)	P-value
Prevalence of any (grade II-III) caries	190 (56.7%)	239 (69.1%)	217 (63.3%)	0.004	1.10 (0.90-1.34)	0.337	0.92 (0.76-1.11)	0.371	1.20 (0.99-1.46)	0.065
Prevalence of grade II caries	104 (31.0%)	133 (38.4%)	124 (36.2%)	0.121	1.19 (0.92-1.56)	0.188	1.00 (0.78-1.29)	0.992	1.19 (0.92-1.54)	0.186
Prevalence of grade III caries	86 (25.7%)	106 (30.6%)	93 (27.1%)	0.330	0.99 (0.73-1.34)	0.952	0.83 (0.62-1.10)	0.195	1.20 (0.90-1.59)	0.226
Prevalence of periapical infections	73 (21.8%)	87 (25.1%)	81 (23.6%)	0.584	1.01 (0.73-1.39)	0.967	0.87 (0.64-1.19)	0.372	1.16 (0.84-1.59)	0.368
Prevalence of gingivitis	289 (86.3%)	291 (84.1%)	299 (87.2%)	0.499	1.01 (0.86-1.19)	0.884	1.04 (0.88-1.23)	0.635	0.97 (0.82-1.15)	0.744
Prevalence of clinical periodontits	68 (20.3%)	62 (17.9%)	58 (16.9%)	0.505	0.85 (0.60-1.22)	0.379	0.96 (0.67-1.39)	0.839	0.86 (0.62-1.26)	0.499
Prevalence of periodontits clinical+x-ray	117 (34.9%)	103 (29.8%)	107 (31.2%)	0.338	0.93 (0.71-1.21)	0.574	1.05 (0.80-1.38)	0.730	0.88 (0.67-1.16)	0.363

[a] Crude values

[b] Adjusted for age, BMI at enrollment, number of previous pregnancies, anemia, HIV status, malaria status at enrollment, education (completed years at school), socio-economic status, study site, number of teeth, tooth brush usage and daily tooth paste usage

Supplementary material, Nutrition intervention May Adversely Affect Maternal Oral Health

Supplementary table 4. Continuous oral health variables by intervention groups, adjusted models

	Result by study group[a]				Comparison between LNS and IFA group, adjusted model[b]		Comparison between LNS and MMN group, adjusted model[b]		Comparison between MMN and IFA group, adjusted model[b]	
Outcome	**IFA (n=335)**	**MMN (n=346)**	**LNS (n=343)**	**P-value**	**Difference in means (95 % CI)**	**P-value**	**Difference in means (95 % CI)**	**P-value**	**Difference in means (95 % CI)**	**P-value**
Mean (SD) no. of any (grade II-III) caries lesions	1.73 (2.32)	2.32 (2.84)	2.32 (3.16)	0.006	0.53 (0.13-0.94)	0.010	-0.04 (-0.44-0.36)	0.834	0.58 (0.17-0.98)	0.005
Mean (SD) no. of grade II caries lesions	1.19 (1.65)	1.66 (1.98)	1.69 (2.21)	0.001	0.48 (0.19-0.77)	0.001	0.04 (-0.25-0.33)	0.783	0.43 (0.15-0.72)	0.003
Mean (SD) no. of grade III caries lesions	0.54 (1.25)	0.66 (1.37)	0.62 (1.65)	0.529	0.06 (-0.15-0.27)	0.588	-0.08 (-0.29-0.13)	0.433	0.14 (-0.07-0.35)	0.185
Mean (SD) no. of periapical lesions	0.41 (0.98)	0.52 (1.20)	0.50 (1.41)	0.442	0.07 (-0.11-0.25)	0.434	-0.06 (-0.24-0.12)	0.498	0.13 (-0.05-0.31)	0.144
Mean (SD) no. of sextants with BOP	3.50 (2.08)	3.47 (2.17)	3.33 (2.12)	0.538	-0.13 (-0.43-0.18)	0.410	-0.11 (-0.41-0.19)	0.463	-0.01 (-0.32-0.29)	0.924
Mean (SD) no. of periodontal pockets ≥4mm (clinical)	1.70 (3.54)	1.38 (3.05)	1.43 (3.39)	0.409	-0.25 (-0.73-0.24)	0.318	0.08 (-0.41-0.56)	0.753	-0.33 (-0.81-0.16)	0.188
Mean (SD) periodontal pocket depth, mm	2.32 (0.53)	2.29 (0.52)	2.32 (0.53)	0.662	-0.00 (-0.08-0.07)	0.916	0.03 (-0.05-0.10)	0.466	-0.03 (-0.11-0.04)	0.405

[a] Crude values

[b] Adjusted for age, BMI at enrollment, number of previous pregnancies, anemia, HIV status, malaria status at enrollment, education (completed years at school), socio-economic status, study site, number of teeth, tooth brush usage and daily tooth paste usage

Tropical Medicine and International Health doi:10.1111/tmi.12579

VOLUME 20 NO 11 PP 1549–1558 NOVEMBER 2015

Association between maternal dental periapical infections and pregnancy outcomes: results from a cross-sectional study in Malawi

Ulla Harjunmaa[1], Jorma Järnstedt[2], Lotta Alho[1], Kathryn G. Dewey[3], Yin Bun Cheung[4], Megan Deitchler[5], Ulla Ashorn[1], Kenneth Maleta[6], Nigel J. Klein[7] and Per Ashorn[1,8]

1 *Department for International Health, University of Tampere School of Medicine, Tampere, Finland*
2 *Department of Radiology, Medical Imaging Center, Tampere University Hospital, Tampere, Finland*
3 *Department of Nutrition, University of California, Davis, Davis, CA, USA*
4 *Centre for Quantitative Medicine, Duke-National University of Singapore Graduate Medical School, Singapore, Singapore*
5 *FHI360, Food and Nutrition Technical Assistance III Project (FANTA), Washington, DC, USA*
6 *School of Public Health, University of Malawi College of Medicine, Blantyre, Malawi*
7 *Institute of Child Health, University College London, London, UK*
8 *Department of Paediatrics, Tampere University Hospital, Tampere, Finland*

Abstract

OBJECTIVES Maternal infections are associated with intrauterine growth restriction (IUGR) and preterm birth (PTB). Dental infections are common in low-income settings, but their contribution to adverse pregnancy outcomes is unknown. We studied the epidemiology of dental periapical infections among pregnant women and their association to foetal growth restriction and the duration of pregnancy in a rural sub-Saharan African population.

METHODS This was a cross-sectional study on the association between maternal dental periapical infections and birth outcomes, in Malawi, Africa. We assessed oral health clinically and radiologically among recently delivered women with known duration of pregnancy and measured birthweight (BW), length and head circumference of their infants.

RESULTS Of 1024 analysed participants, 23.5% had periapical infections. Mean duration of pregnancy was 39.4 weeks, BW 2979 g and length 49.7 cm. Women with periapical infection had mean (95% CI) pregnancy duration 0.4 weeks (0.1–0.8) shorter and delivered infants with 79 g (13–145) lower BW and 0.5 cm (0.2–0.9) shorter neonatal length than women without periapical infection. The incidence of PTB was 10.0% among women with periapical infection and 7.3% among those without (adjusted difference 3.5%, 95% CI −1.1–8.1%). Corresponding prevalences for stunting were 20.9% and 14.2% (adjusted difference 9.0%, 95% CI 2.7%–15.2%). The population-attributable risk fraction attributable to periapical infection was 9.7% for PTB and 12.8% for stunting.

CONCLUSIONS Periapical infection was associated with shorter pregnancy duration and IUGR in the study area; interventions addressing this risk factor may improve birth outcomes in low-income settings.

keywords pregnant women, intrauterine growth restriction, duration of pregnancy, dental caries, periapical infection, periodontitis

Introduction

Every year, 32 million infants are born too small, either because they are preterm or as a result of intrauterine growth restriction (IUGR). Most of them are born in low- and middle-income countries [1]. These infants have an increased risk for mortality, serious morbidity, growth failure of early onset and different adverse long-term consequences [2, 3]. Although several risk factors have been identified, the actual causes and mechanisms leading to preterm birth (PTB) or IUGR are still unresolved [4]. A recent report identified an 'urgent need for research into underlying mechanisms of PTBs and development of innovative interventions', especially in low-resource settings where the incidence of PTB- and IUGR-related mortality and morbidity is highest [5].

One of the conditions most commonly associated with adverse birth outcomes is maternal infections [4]. In low-income settings, studies have focused mainly on sexually transmitted diseases, bacterial vaginosis, HIV infection and malaria [6]. Another disease that has been frequently studied is periodontitis, an infection in tooth-supporting tissues. Many studies have suggested that periodontitis is associated with PTB and IUGR, although contradictory results have also been found [7]. In contrast to the active research on periodontitis, few data are available on the role of periapical infection, another prominent oral disease. Periapical infection is formed mostly when a deep dental caries exposes the pulp of a tooth allowing access of oral microflora to the dental root canals, from where infection then spreads to the root-surrounding tissues [8]. Considering that dental caries is the most prevalent chronic disease globally [9], and that there is a lack of both personnel and material resources for oral health in low-income countries [10], it is possible that the global health community has missed a substantial and largely modifiable risk factor for adverse pregnancy outcomes.

We explored the influence of maternal oral health on the duration of pregnancy and the size of the newborn infant in low-income settings. Therefore, we embedded a cross-sectional substudy in a controlled trial in rural Malawi that investigated the maternal and infant health effect of a nutritional intervention during pregnancy. We included data from both comprehensive clinical examinations conducted soon after delivery and panoramic oral radiographs in the analysis. We tested the hypothesis that mothers who have dental caries, periapical infection or periodontitis would have shorter pregnancies and deliver smaller infants than mothers who do not have these infections.

Methods

Study design, outcomes and ethics statement

This substudy was nested as a cross-sectional study in controlled iLiNS-DYAD-M trial in Malawi. The hypothesis of the trial was that home fortification of pregnant women's diets with nutrient supplement would increase birth size in an African community. The women were provided with one daily iron-folic acid capsule, one capsule with 18 micronutrients, or one 20 g sachet of lipid-based nutrient supplement containing 118 kcal, protein, carbohydrates, essential fatty acids and 21 micronutrients during pregnancy. Details and key results of the main trial are published elsewhere [11].

The primary outcome measures of the oral health substudy were duration of pregnancy, birthweight (BW) and neonatal length. The secondary outcomes were neonatal weight and head circumference.

We performed the trial according to Good Clinical Practice guidelines and the ethical standards of the Helsinki Declaration. The protocol was approved by the College of Medicine Research and Ethics Committee, University of Malawi and the Ethics Committee of Pirkanmaa Hospital District, Finland. We published key details of the iLiNS-DYAD protocol at ClinicalTrials.gov, identifier NCT01239693. We enrolled only participants who signed or thumb-printed an informed consent and provided them with subsidised health care and complimentary dental care.

Study site, participants and enrolment

Between February 2011 and August 2012, we enrolled 1391 pregnant women who visited antenatal clinics at two hospitals (Mangochi and Malindi) and two health centres (Lungwena and Namwera) in Mangochi district. The Mangochi district hospital was located in a semi-urban area and the other health facilities in rural areas with total catchment population of 160 000 which subsisted mainly on fishing and agriculture.

At enrolment, research personnel recorded participants' medical and obstetric history performed health and antenatal examinations and assessed the duration of pregnancy with ultrasound imagers. Participants were eligible for enrolment into the main iLiNS-DYAD trial if they were pregnant <20 weeks, ≥15 years old, had no chronic illnesses requiring frequent medical care, no allergies, no evident pregnancy complications (oedema, blood haemoglobin <50 g/l, systolic blood pressure >160 mmHg or diastolic >100 mmHg), no earlier participation in this trial and no concurrent participation in any other trial. Enrolled participants who had singleton pregnancies and who completed the oral health examination were eligible for the oral health substudy.

Data collection

Research personnel recorded delivery events within 48 h after delivery at home or clinic and measured infant's BW with an electronic scale with a reading increment of 20 g. BW measured within 48 h after delivery was used as such. If the weight was first measured within 2–5 days after delivery when infants usually lose weight, we estimated BW by applying an age-dependent multiplicative factor to the measured weight [12]. If the weight was first measured between 6 and 13 days after delivery, we back-calculated BW from the measured weight using the WHO *z*-scores. At 1–6 weeks after delivery, anthropometrists measured infant's length in triplicate

with length boards and head circumference with plastic tapes and recorded them to the nearest millimetre, and neonatal weight with the same scales used to measure BW. Weight measured after 13 days, and length, head circumference or neonatal weight measured later than 6 weeks after delivery were considered missing. All anthropometric measurements were taken only at one time point.

Two dental therapists conducted full-mouth examinations of the mothers and took digital panoramic radiographs (Planmeca Proline XC, Planmeca, Finland) as soon as possible after delivery at Mangochi hospital. The examination was conducted after delivery for three reasons: (i) we wanted to assess the oral health situation after the full duration of the pregnancy; (ii) X-rays should not be taken routinely during pregnancy; and (iii) treating the diagnosed diseases before delivery would have distorted the main intervention trial, and not treating would have been unethical. The dental therapists completed calibration and standardisation practices prior to the initiation of the data collection and approximately every 4 months thereafter. They conducted a structured interview on oral health history, recorded caries lesions and measured periodontal pocket probing depth from six sites of each tooth, excluding third molars, using a WHO periodontal probe (LM-Instruments, Parainen, Finland). They recorded the deepest measurement of each tooth rounded to the nearest millimetre and gingival bleeding after probing by dental arch sextants (left, mid and right, upper and lower). An oral and maxillofacial radiologist (JJ) and an experienced dentist (UH) analysed the radiographs with digital imaging software (Planmega Romexis^{TM}, Planmeca, Finland). They recorded number of teeth, caries lesions extending to dentine or pulp, periapical lesions and alveolar bone level at each tooth from the dentino-enamel junction to the bone margin, relative to the full root length (normal, cervical, middle or apical third). As a standardisation practice, the radiological analysts re-assessed at least two earlier X-rays each week and if any deviance between the two readings was found, they discussed them until agreement was reached. The dental therapists and the X-ray analysts were blinded to the birth outcomes and the intervention arms of the main trial during the examinations. However, some of the mothers came for the examination visit with their infants and the dental therapists could sometimes see that particular infant.

Definitions

We defined PTB as delivery before 37 gestation weeks (gw) (259 days) and low birthweight (LBW) as <2500 g. We calculated sex–age-standardised anthropometric indices length-for-age (LAZ), weight-for-age (WAZ) and head circumference-for-age *z*-scores using the WHO Child Growth Standards [13] and considered values below −2.0 indicative of stunting, underweight or small head circumference, respectively. WAZ was calculated using neonatal weight measurements.

We diagnosed dental caries and periodontitis clinically and radiologically, and deep caries (exposing the pulp) and periapical infections radiologically (Figure 1). We defined periapical infection as an osteolytic finding >1 mm surrounding the dental root apex. We considered that a participant had caries or periapical infection if at least one finding was recorded, and gingivitis if bleeding occurred at least at one dental arch sextant. We considered that a tooth had periodontitis if either a ≥4 mm pocket was measured in clinical examination, or a vertical bony pocket was identified at least at the cervical root level on the radiographs. In the absence of a standard periodontitis definition, we considered that a participant had periodontitis if she had at least three teeth with periodontitis or one dental arch sextant with horizontal bone loss at least at cervical level and gingivitis present. Third molars were excluded from the periodontitis assessment because pseudo-pockets ('false pockets') are often found especially in young adults, and these pockets are related to partial eruption of the tooth and not to infection, thus including them would lead to false-positive diagnoses.

Statistical analysis

We carried out statistical analysis with Stata 12.1 (StataCorp, College Station, USA). Participants who completed the oral health visit within 6 weeks puerperal period after delivery were included in the analysis. The sample size was originally calculated in accordance with the main objective of the iLiNS-DYAD-M trial. The sample size of about 1000 participants obtained for this substudy offered approximately 93% power to detect differences between the groups, assuming an infection prevalence of 25% and an effect size of 0.25 (mean difference between groups divided by the pooled SD) for continuous birth outcomes at 5% two-sided type I error rate.

To evaluate whether any stage of caries and subsequent periapical infection development was associated with birth outcomes, we initially categorised participants into four groups according to the severity of the most advanced caries lesion (healthy, shallow caries, deep caries and periapical infection). We then compared the birth outcomes in those four groups and tested a null hypothesis of no difference with ANOVA. All groups were also tested separately against each other (with *t*-test) and also

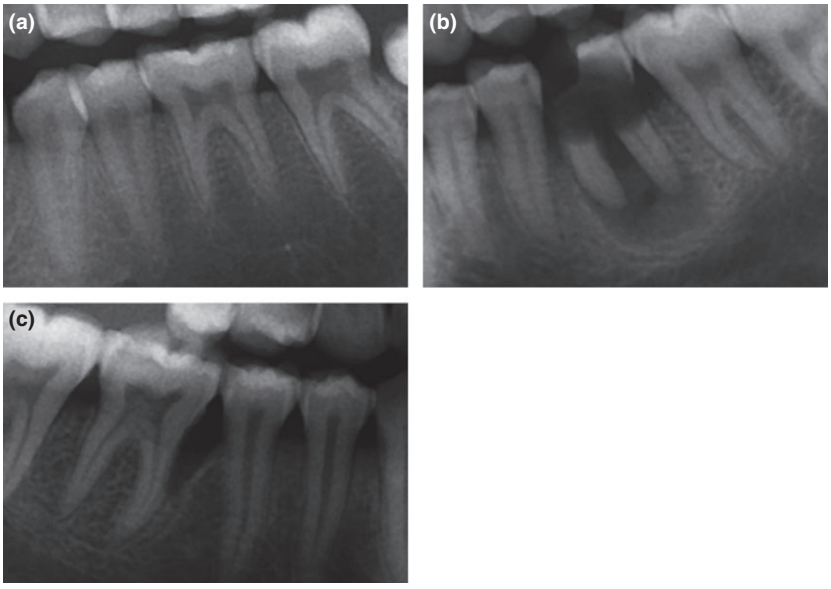


Figure 1 Examples of radiological findings in the study. (a) Healthy dentition and bone structure; (b) Periapical infection, radiolucent lesion surrounding the roots of first lower left molar with deep caries lesion; (c) Periodontitis, deep vertical bony pocket related to first lower right molar.

in combinations according to the severity (0 *vs.* 1–3, 0–1 *vs.* 2–3, 0–2 *vs.* 3). As there were no differences in mean outcomes between the groups of participants with no, shallow or deep caries without periapical infection, we collapsed those groups into one where the common denominator was the absence of periapical infection. In the final analyses, we thus compared birth outcomes between participants with or without periapical infections.

We tested the null hypothesis either with Fisher's exact test (for binary end points) or Student's t-test (for continuous end points). We calculated risk ratios (RR) for comparison of binary end points and risk differences and differences in means for comparison of continuous end points at a single time point. With a large sample size, parametric analysis of means is robust and valid regardless of the shape of outcome distribution as per the central limit theorem [12, 14]. We used multivariable log-binomial models, or log-poisson models if the algorithm failed to converge in the estimation, for binary end points and linear regression models for continuous end points to control for possible confounding and to maximise power by reducing the variance of the outcomes. All relevant and available covariates that could confound the relationship between oral infections and birth outcomes were included in the model using forced entry method. The covariates selected were maternal age, height, body mass index, socio-economic score, parity, time between delivery and the examination, and number of teeth as continuous variables, and study site, nutrition intervention [11], anaemia, HIV, malaria and periodontitis status (when analysing periapical infection) or periapical infection status (when analysing periodontitis) as categorised variables. Smoking was omitted because no participant reported that they had ever smoked. All the models were adjusted for the same set of covariates, except in the stratified analysis where the variable by which the analysis was stratified was removed from the model. We tested collinearity between the covariates using variance inflation factor analysis before conducting the multivariate analysis and found no major collinearity. We calculated adjusted population-attributable risk fraction (PAR%, the reduction in the incidence of an outcome if the whole population was unexposed) for the binary outcomes.

HIV and malaria infection were considered possible confounders because of their known association with birth outcomes and possible association with oral health. Because a covariate can be both a confounder and an effect modifier [12], and because HIV and malaria were effect modifiers in an earlier antenatal intervention trial at the same study site [11], we conducted exploratory analysis with stratification by HIV and malaria status for mean duration of pregnancy, WAZ, LAZ and head circumference-for-age *z*-score. These analyses were carried out with linear regression modelling.

Results

Of the 1391 participants who were enrolled into the iLiNS-DYAD study, 1229 (88.4%) completed the oral health examination between June 2011 and August 2013. After excluding women with twins and those who were

examined >6 weeks after delivery, 1024 participants were included in the analyses. Data on the duration of pregnancy were available for all (100%) and BW and neonatal size for 93.3% and 94.4% of the included participants, respectively (Figure 2).

The included and excluded participants had otherwise similar baseline characteristics, but the included women were on average slightly older (25 *vs.* 24 years, $P < 0.001$), less often primiparous (17.8% *vs.* 33.3%, $P < 0.001$) or malaria positive (21.4% *vs.* 28.1%, $P = 0.009$), and had higher mean score for socio-economic status (0.46 *vs.* −0.13, $P < 0.001$) and lower mean BMI (22.1 *vs.* 22.4, $P = 0.046$; Table 1).

The mean (SD) time between the delivery and the oral examination among the participants who were included in the analysis was 16.0 (8.0) days. The mean (SD) duration of pregnancy was 39.4 (2.3) gw; BW averaged 2979 (430) g and length 49.7 (2.2) cm. Mean (SD) neonatal LAZ, WAZ and head circumference-for-age *z*-scores were −0.99 (1.09), −0.54 (0.99) and −0.13 (1.07), respectively. The incidence (*n*) of PTB was 7.9% (81) and LBW occurred in 12.0% (115); prevalence (*n*) of other outcomes was 15.7% (152) for neonatal stunting, 6.9% (67) for underweight and 3.8% (37) for small head circumference.

Of the included women, 63.1% ($n = 646$) had caries, 27.8% ($n = 285$) deep caries and 23.5% ($n = 241$) periapical infection. Fifteen participants had a periapical infection without visible carious pulpal exposure. Periodontitis was diagnosed in 31.9% ($n = 327$) of the participants. More details on the dental characteristics are presented in Table S1.

The mean (SD) duration of pregnancy among the groups of participants without caries or periapical infection ($n = 370$), those with shallow caries ($n = 354$), deep caries (without periapical infection) ($n = 59$) or periapical infection ($n = 241$) was 39.5 (2.1), 39.5 (2.2), 39.3 (2.2) and 39.1 (2.7) gw, respectively, and the mean (SD) BW was 2977 (436), 3009 (428), 3029 (500) and 2926 (400) g, respectively. The mean (SD) neonatal lengths among the same participants were 49.8 (2.1), 49.8 (2.2), 49.8 (2.7) and 49.4 (2.3) cm, respectively. As there were no significant differences between the participants without caries and participants with caries in any of the assessed birth outcomes even if a pulpal exposure was present ($P = 0.758$ for duration of pregnancy, $P = 0.534$ for BW, $P = 0.975$ for length), we collapsed the first three groups into one. In the subsequent analyses, we then compared participants with no detectable periapical infection to those who had at least one periapical infection.

Table 2 shows the mean duration of pregnancy and neonatal size by maternal periapical infection status using continuous outcomes. After adjusting for possible

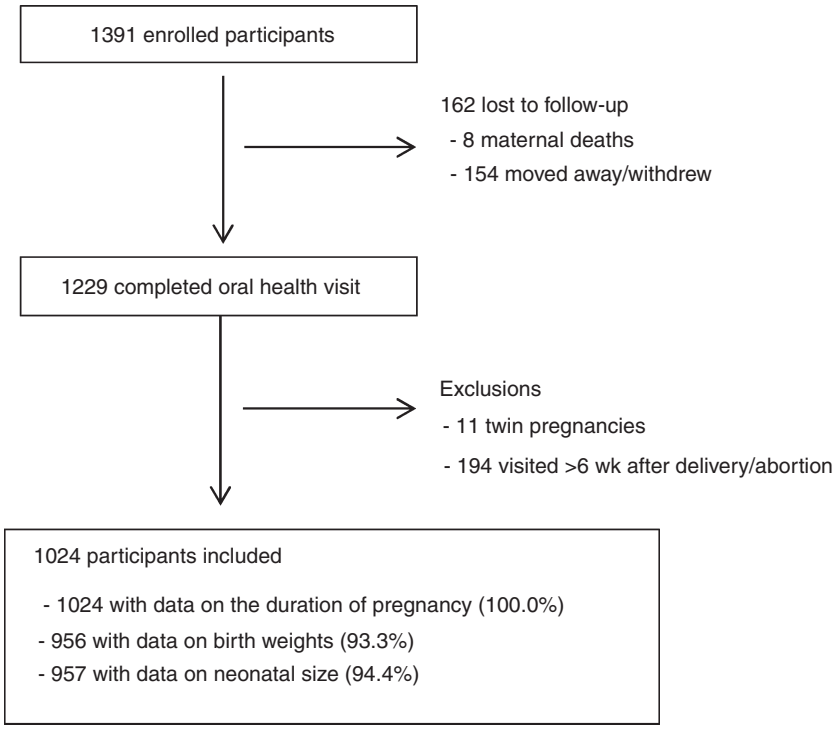


Figure 2 Participant flow.

Table 1 Baseline characteristics of the included and excluded participants

Characteristic	Included (n = 1024)	Excluded (n = 367)	P-value
Mean (SD) maternal age, years	25 (6.2)	24 (5.7)	<0.001
Mean (SD) maternal education, completed years at school	3.9 (3.4) (7 missing data)	4.3 (3.6) (58 missing data)	0.087
Mean (SD) proxy for socio-economic status*	−0.13 (1.68) (6 missing data)	0.46 (2.12) (62 missing data)	<0.001
Proportion of primiparous women (n)	17.8% (182)	33.3% (122)	<0.001
Mean (SD) BMI, kg/m²	22.1 (2.7)	22.4 (3.1)	0.046
Proportion of women with a low BMI (<18.5 kg/m²) (n)	5.4% (55)	5.2% (19)	0.891
Proportion of anaemic women (Hb < 110 g/l) (n)	19.8% (203)	23.0% (84)	0.208
Proportion of women with a positive HIV test (n)	14.1% (144) (3 missing data)	12.5% (39) (54 missing data)	0.460
Proportion of women with a positive malaria test at enrolment (RDT†) (n)	21.4% (219)	28.1% (103)	0.009
Proportion of women in iron-folic acid intervention group (n)	32.7% (335)	40.3% (83)	0.036
Proportion of women in multiple micro nutrient intervention group (n)	33.8% (346)	29.1% (60)	0.194
Proportion of women in lipid-based nutrient supplement intervention group (n)	33.5% (343)	30.6% (63)	0.417

BMI, body mass index; Hb, blood haemoglobin concentration; HIV, human immune deficiency virus.
*Created with principal components analysis by combining information on the building material of the house, main source of water and electricity, sanitary facility and main type of cooking fuel used.
†RDT, malaria rapid diagnostic test obtained from a finger prick.

confounders, women with at least one periapical infection had mean (95% CI) pregnancy duration 0.4 weeks (0.1–0.8) shorter and infants with 79 g (13–145) lower BW, 0.5 cm (0.2–0.9) shorter neonatal length, 0.27 units (0.11–0.44) lower LAZ and 0.18 units (0.01–0.35) smaller head circumference z-score compared to women without the infection.

The prevalences of all analysed adverse binary birth outcomes were also higher in the group of women with periapical infection than in the group without. The differences were statistically significant for the prevalence of neonatal stunting (adjusted RR = 1.68; P = 0.007) and small head circumference (adjusted RR = 2.52; P = 0.012). Covariate-adjusted risk difference varied between −0.5% (LBW) and 9.0% (stunting). The fraction of various adverse outcomes theoretically attributable to periapical infections in the target population (PAR %) varied from 1.8% (LBW) to 26.3% (small head circumference) (Table 3).

Figure 3 shows the distribution of pregnancy duration, LAZ, WAZ and head circumference among infants born to mothers with and without periapical infection. The difference in the distribution patterns varied by outcome. For neonatal length and head circumference, the difference between infants born to mothers with and without periapical infection was seen across the entire outcome distribution. For weight, in contrast, a difference was only seen at the right side of the distribution, that is among the heaviest infants.

There were no statistically significant differences between the groups of participants without or with periodontitis in any of the assessed continuous (Table S2) or binary (Table S3) birth outcomes.

In exploratory analyses, stratified by the participants' HIV or malaria status at enrolment, periapical infections were associated with lower LAZ in all strata, but the differences were largest among HIV or malaria positive participants (Table 4). Stratified analyses using the mean gestational age, WAZ or head circumference z-score as an outcome showed similar point estimates as LAZ analysis but often less statistical significance than the LAZ analysis (Tables S4–6).

Discussion

Our main findings were that mothers who had at least one periapical infectious lesion had significantly shorter mean pregnancy duration and they delivered infants with lower mean BW, neonatal length and neonatal head circumference than mothers who did not have periapical

Table 2 Continuous birth outcomes in participants without and with periapical infections

	Periapical infection			
	Mean (SD)		Adjusted group comparison*	
Outcome	Women without infection (n = 783)	Women with infection (n = 241)	Difference in means (95% CI)	P-value
Duration of pregnancy, weeks	39.5 (2.2)	39.1 (2.7)	−0.4 (−0.8 to −0.1)	0.014
Birthweight, g	2995 (437)	2926 (400)	−79 (−145 to −13)	0.019
Neonatal WAZ	−0.51 (0.99)	−0.63 (0.98)	−0.14 (−0.29 to −0.00)	0.057
Neonatal length, cm	49.8 (2.2)	49.4 (2.3)	−0.5 (−0.9 to −0.2)	0.002
Neonatal LAZ	−0.94 (1.08)	−1.15 (1.11)	−0.27 (−0.44 to −0.11)	0.001
Neonatal Head-Z	−0.09 (1.06)	−0.25 (1.11)	−0.18 (−0.35 to −0.01)	0.033

WAZ, weight-for-age z-score; LAZ, length-for-age z-score; Head-Z, head circumference-for-age z-score.
*Adjusted for maternal age, maternal height, BMI, HIV status, malaria status and anaemia at enrolment, number of previous pregnancies, study site, socio-economic score, periodontitis, number of teeth, time between delivery and examination, and intervention.

Table 3 Risk of adverse dichotomous birth outcomes in participants with periapical infection

	Periapical infection					
	Findings per group		Adjusted group comparison*			
Outcome	Women without infection† (%)	Women with infection (%)	RR (95% CI)	Risk difference‡,% (95% CI)	P-value	PAR%
Incidence of preterm birth	57/783 (7.3)	24/241 (10.0)	1.52 (0.93–2.47)	3.5 (−1.1 to 8.1)	0.092	9.7
Incidence of low birthweight	86/735 (11.7)	29/221 (13.1)	1.08 (0.67–1.72)	−0.5 (−6.1 to 5.2)	0.785	1.8
Prevalence of neonatal underweight	47/747 (6.3)	20/226 (8.9)	1.52 (0.83–2.75)	2.9 (−1.5 to 7.3)	0.166	10.5
Prevalence of neonatal stunting	105/742 (14.2)	47/225 (20.9)	1.68 (1.15–2.46)	9.0 (2.7 to 15.2)	0.007	12.8
Prevalence of small head circumference	21/742 (2.8)	16/224 (7.1)	2.52 (1.23–5.16)	4.5 (0.3 to 8.6)	0.012	26.3

PAR%, population-attributable risk fraction.
*Adjusted for maternal age, maternal height, BMI, HIV status, malaria status and anaemia at enrolment, number of previous pregnancies, study site, socio-economic score, periodontitis, number of teeth, time between delivery and examination, and intervention.
†Number of women with the defined adverse outcome/total number of women who had no periapical infections but who had data on the indicated outcome variable.
‡The differences are expressed in percentage points.

infections. The proportion of preterm deliveries and stunting that could theoretically be reduced if periapical infections were eliminated (PAR%) was 9.7% and 12.8%, respectively.

The probabilities of bias in this study were minimised by ultrasound-based assessment of the pregnancy duration, rigorous quality assurance in data collection and the combination of clinical and high-quality X-ray-based assessment that allowed exact diagnostics. The main weaknesses and potential sources of bias were the loss to follow-up, some differences in baseline characteristics between the participants who were included in the analyses and those excluded, our inability to document the oral health status at baseline and immediately after delivery, and possible under detection of periapical lesions due to obstruction by other anatomical structures. To overcome the weaknesses, we conducted several adjusted analyses that included the baseline characteristics that distinguished the excluded and included participants, and the time between the delivery and the examination. The fact that the adjusted analyses gave similar results to the unadjusted ones speaks against a major selection or follow-up bias. While we consider confounding by other, undiagnosed infections unlikely, we cannot positively rule out that possibility. Exposing asymptomatic women to diagnostic dental X-rays during first half of pregnancy

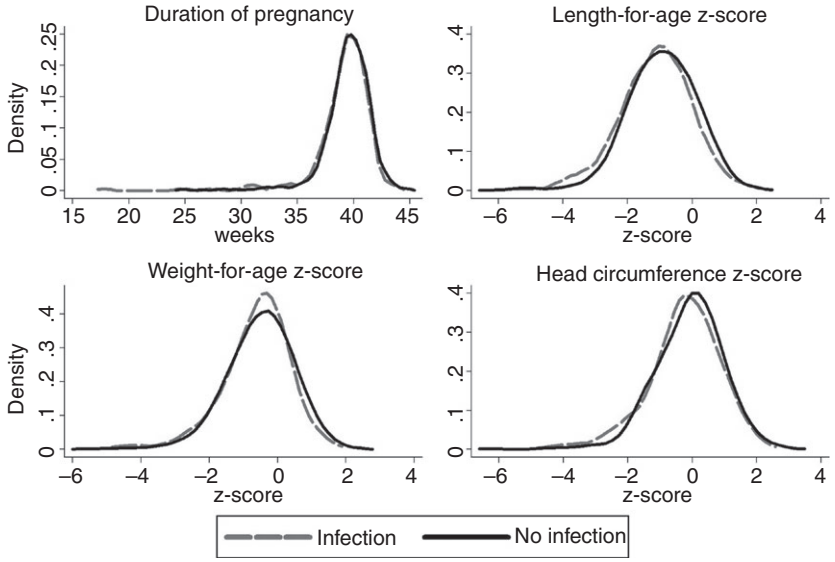


Figure 3 Distribution of pregnancy duration and *z*-scores for weight-for-age, length-for-age and head circumference-for-age in groups with and without periapical infections.

Table 4 Mean length-for-age *z*-score by infection group, stratified analyses

	Periapical infection			
Sample strata	Mean (SD) LAZ without infection*	Mean (SD) LAZ with infection*	Adjusted difference in means† (95% CI)	*P*-value†
Negative HIV test at enrolment (n = 827)	−0.92 (1.08)	−1.05 (1.07)	−0.23 (−0.41 to −0.04)	0.015
Positive HIV test at enrolment (n = 137)	−1.07 (1.09)	−1.57 (1.17)	−0.46 (−0.83 to −0.10)	0.013
Negative malaria test at enrolment (n = 762)	−0.91 (1.05)	−1.06 (1.04)	−0.23 (−0.41 to −0.05)	0.011
Positive malaria test at enrolment (n = 203)	−1.01 (1.19)	−1.60 (1.37)	−0.46 (−0.84 to −0.08)	0.016

LAZ, length-for-age *z*-score.
*Model without covariates.
†Adjusted for maternal age, maternal height, BMI and anaemia at enrolment, number of previous pregnancies, study site, socio-economic score, periodontitis, number of teeth, time between delivery and examination, intervention and maternal malaria status (when stratified by HIV) or HIV status (when stratified by malaria).

was considered unethical, especially as treatment options were limited both by the pregnancy and the trial setting. However, it is unlikely that periapical infections differed substantially after delivery from those at trial enrolment, as they were almost entirely associated with large caries lesions that normally take several years to develop [15]. We therefore believe that the sample findings were reliable and sufficiently representative of the target population to suggest that maternal periapical infections but not periodontitis were associated with shorter pregnancies and smaller infant size in the study area in general, not only in the study sample.

On the whole, there is a paucity of published data on the association between periapical infections and any systemic conditions, although an association with coronary heart diseases has been proposed [16]. Although the cross-sectional nature of our study precludes drawing definitive inferences about causality, a causal pathway from periapical infections to adverse birth outcomes is biologically plausible. Periapical infections may affect pregnancy by similar direct and indirect mechanisms that have been suggested for periodontitis [17]. The release of inflammatory mediators from the infected periapical tissues, such as interleukin 1, 2 and 6, may induce systemic inflammatory response that in turn may affect pregnancy, and periapical infections are also associated with increased serum concentrations of immunoglobulin A, G and M [18]. An alternative explanation is that oral bacteria may spread through

the blood stream causing infection and local immune response in the feto-placental unit, thereby triggering labour [19]. As an example of such bacteria, *Fusobacterium nucleatum* is the most commonly found bacteria from amniotic fluid in preterm labour [19, 20]. Consistent with the few published studies on dental caries and adverse pregnancy outcomes [21–25], we did not find significant associations between uncomplicated caries (without periapical infection) and any of the assessed outcomes. This may be related to the degree of systemic inflammation. It is plausible that only when dental infection spreads extraradicularly (outside of the dental roots) that sufficient systemic inflammation is induced to influence pregnancy outcomes.

Several earlier studies have found associations between periodontitis and adverse birth outcomes, although some studies have found conflicting results [7]. Studies that have found the association were often conducted in more affluent environments where mothers are otherwise relatively healthy. We speculate that in Malawi, other infections and inflammation are common and may override the association between low-grade periodontal infection and adverse birth outcomes while the association between more 'aggressive' periapical infection and birth outcomes may be present. Periodontal tissues have very complex local defence mechanisms that to some extent hinder the infection from spreading to other body parts [26] while the defence mechanisms in the periapical structures are less effective. Also other population characteristics, such as race and other genetic factors, may contribute to the development, manifestation and adverse effects of these oral diseases [27–29].

Conclusions

Despite persistent attempts, the currently used interventions to reduce the incidence of PTB and IUGR have not been sufficient [5]. Recently, the reduction of stunting has been recognised as an important global target because of the high prevalence of stunting and its severe adverse long-term consequences [30], but again, existing interventions have not adequately addressed the issue. On a global scale, only a small proportion of women have access to proper dental care whereby asymptomatic caries and periapical infections could be diagnosed and treated. In South Asia and sub-Saharan Africa, which account for almost two-thirds of the world's preterm babies [31], lack of preventive dental care and dentistry services is commonplace and oral diseases remain highly prevalent [10]. Our results suggest that periapical infections may reduce the duration of pregnancy and account for a substantial fraction of IUGR. If the findings are confirmed in other populations, serious attention should be paid to the prevention and treatment of this common and largely preventable risk factor for adverse pregnancy outcomes.

Acknowledgements

We thank the study participants, the local communities, our research personnel at the study sites and the iLiNS Project extended research team for their positive attitude and support during the study. We particularly thank Simeon Mulewa and Davie Charlie for the clinical oral health data collection, and the iLiNS Project Steering Committee members and Project Manager Mary Arimond (http://ilins.org) for technical support. This publication is based on research funded in part by the Office of Health, Infectious Diseases, and Nutrition, Bureau for Global Health, U.S. Agency for International Development (USAID) under terms of Cooperative Agreement No. AID-OAA-A-12-00005, through the Food and Nutrition Technical Assistance III Project (FANTA), managed by FHI 360. Additional funding was provided by the Bill & Melinda Gates Foundation through a grant to the University of California, Davis. Planmega Ltd provided the X-ray machine for nominal price and 3M donated materials for dental treatment of the participants. The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of USAID, the United States Government, the Bill & Melinda Gates Foundation or the other funders. Megan Deitchler is employed by FANTA/FHI 360.

References

1. Lee AC, Katz J, Blencowe H *et al.* National and regional estimates of term and preterm babies born small for gestational age in 138 low-income and middle-income countries in 2010. *Lancet Glob Health* 2013: **1**: e26–e36.
2. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet* 2008: **371**: 261–269.
3. Katz J, Lee AC, Kozuki N *et al.* Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis. *Lancet* 2013: **382**: 417–425.
4. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet* 2008: **371**: 75–84.
5. Chang HH, Larson J, Blencowe H *et al.* Preventing preterm births: analysis of trends and potential reductions with interventions in 39 countries with very high human development index. *Lancet* 2013: **381**: 223–234.
6. Desai M, ter Kuile FO, Nosten F *et al.* Epidemiology and burden of malaria in pregnancy. *Lancet Infect Dis* 2007: **7**: 93–104.

7. Ide M, Papapanou PN. Epidemiology of association between maternal periodontal disease and adverse pregnancy outcomes – systematic review. *J Periodontol* 2013: **84**(4 Suppl): S181–S194.
8. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. *Crit Rev Oral Biol Med* 2004: **15**: 348–381.
9. Marcenes W, Kassebaum NJ, Bernabe E *et al.* Global burden of oral conditions in 1990–2010: a systematic analysis. *J Dent Res* 2013: **92**: 592–597.
10. Beaglehole J, Benzian H, Crail J & Mackay J. *The Oral-Health Atlas. Mapping a Neglected Global Health Issue*. FDI World Dental Federation: Cointrin, Swizerland, 2009.
11. Ashorn P, Alho L, Ashorn U *et al.* The impact of lipid-based nutrient supplement provision to pregnant women on newborn size in rural Malawi: a randomised controlled trial. *Am J Clin Nutr* 2015: **101**: 387–397.
12. Cheung Y. *Statistical Analysis of Human Growth and Development*. CRC Press: Boca Ration, FL, 2014.
13. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatr Suppl* 2006: **450**: 76–85.
14. Rice J. *Mathematical Statistics and Data Analysis* (2nd edn). Duxbury Press: Belmont, CA, 1995.
15. Featherstone JD. Dental caries: a dynamic disease process. *Aust Dent J* 2008: **53**: 286–291.
16. Caplan DJ, Chasen JB, Krall EA *et al.* Lesions of endodontic origin and risk of coronary heart disease. *J Dent Res* 2006: **85**: 996–1000.
17. Madianos PN, Bobetsis YA, Offenbacher S. Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms. *J Periodontol* 2013: **84**(4 Suppl): S170–S180.
18. Gomes MS, Blattner TC, Sant'Ana Filho M *et al.* Can apical periodontitis modify systemic levels of inflammatory markers? A systematic review and meta-analysis. *J Endod* 2013;**39**:1205–1217.
19. Han YW, Wang X. Mobile microbiome: oral bacteria in extra-oral infections and inflammation. *J Dent Res* 2013: **92**: 485–491.
20. Doyle RM, Alber DG, Jones HE *et al.* Term and preterm labour are associated with distinct microbial community structures in placental membranes which are independent of mode of delivery. *Placenta* 2014: **35**: 1099–1101.
21. Dasanayake AP. Poor periodontal health of the pregnant woman as a risk factor for low birth weight. *Ann Periodontol* 1998: **3**: 206–212.
22. Vergnes JN, Kaminski M, Lelong N *et al.* Maternal dental caries and pre-term birth: results from the EPIPAP study. *Acta Odontol Scand* 2011: **69**: 248–256.
23. Buduneli N, Baylas H, Buduneli E, Turkoglu O, Kose T, Dahlen G. Periodontal infections and pre-term low birth weight: a case-control study. *J Clin Periodontol* 2005: **32**: 174–181.
24. Mumghamba EG, Manji KP. Maternal oral health status and preterm low birth weight at Muhimbili National Hospital, Tanzania: a case-control study. *BMC Oral Health* 2007: **7**: 8.
25. Heimonen A, Rintamaki H, Furuholm J, Janket SJ, Kaaja R, Meurman JH. Postpartum oral health parameters in women with preterm birth. *Acta Odontol Scand* 2008: **66**: 334–341.
26. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol* 2000: **2014**;**64**: 57–80.
27. Aminoshariae A, Kulild JC. Association of functional gene polymorphism with apical periodontitis. *J Endod* 2015: **41**: 999–1007.
28. Divaris K, Monda K, North K *et al.* Exploring the genetic basis of chronic periodontitis: a genome-wide association study. *Hum Mol Genet* 2013: **22**: 2312–2324.
29. Márton IJ, Kiss C. Protective and destructive immune reactions in apical periodontitis. *Oral Microbiol Immunol* 2000: **15**: 139–150.
30. de Onis M, Dewey KG, Borghi E *et al.* The World Health Organization's global target for reducing childhood stunting by 2025: rationale and proposed actions. *Matern Child Nutr* 2013: **9**(Suppl 2): 6–26.
31. Blencowe H, Cousens S, Oestergaard MZ *et al.* National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet* 2012: **379**: 2162–2172.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Dental characteristics of iLiNS-DYAD oral health participants.

Table S2. Continuous birth outcomes in participants without and with periodontitis

Table S3. Risk of adverse dichotomous birth outcomes in participants without and with periodontitis

Table S4. Mean gestational age (GA) by infection group, stratified analyses.

Table S5. Mean weight-for-age *z*-score (WAZ) by infection group, stratified analyses.

Table S6. Mean head circumference *z*-score by infection group, stratified analyses.

Corresponding Author **Ulla Harjunmaa**, University of Tampere School of Medicine, Arvo-building, Room C219, FIN-33014 Tampere, Finland. Tel.: +358504039849; E-mail: ulla.harjunmaa@fimnet.fi

Supplementary Material

Harjunmaa et al. **The association between maternal dental periapical infections and pregnancy outcomes -** Results from a cross-sectional study in Malawi

Supplementary table 1. Dental characteristics of iLiNS-DYAD oral health participants

Characteristic	Prevalence	Mean (SD)	Median	Min	Max
Number of teeth	n/a	31.3 (1.6)	32	18	37
Filled teeth	0.5 %	0.0 (0.2)	0	0	6
Carious teeth	63.1 %	2.1 (2.8)	1	0	25
Deep caries (into the pulp)	27.8 %	0.6 (1.4)	0	0	21
Periapical infections	23.5 %	0.5 (1.2)	0	0	19
Periodontitis	31.9 %	n/a	n/a	n/a	n/a

Supplementary table 2. Continuous birth outcomes in participants without and with periodontitis

Outcome	Periodontitis			
	Mean (SD)		Adjusted group comparison[a]	
	Women without periodontitis (n=783)	Women with periodontitis (n=241)	Difference in means (95% CI)	P-value
Duration of pregnancy, weeks	39.3 (2.4)	39.5 (2.1)	0.2 (-0.1 to 0.5)	0.174
Birth weight, g	2968 (427)	3003 (436)	28 (-32 to 88)	0.360
Neonatal WAZ	-0.55 (1.00)	-0.51 (0.96)	0.01 (-0.12 to 0.15)	0.839
Neonatal length, cm	49.7 (2.2)	49.7 (2.2)	0.1 (-0.2 to 0.4)	0.525
Neonatal LAZ	-1.01 (1.09)	-0.94 (1.11)	0.06 (-0.08 to 0.21)	0.396
Neonatal Head-Z	-0.11 (1.07)	-0.18 (1.06)	-0.08 (-0.23 to 0.07)	0.310

[a]Adjusted for maternal age, maternal height, BMI, HIV status, malaria status and aenemia at enrolment, number of previous pregnancies, study site, socio-economic score, periapical infection, number of teeth, time between delivery and examination, and intervention

Abreviations: WAZ=weight-for-age z-score, LAZ=length-for-age z-score, Head-Z=head circumference-for-age z-score

Supplementary table 3. Risk of adverse dichotomous birth outcomes in participants without and with periodontitis

Outcome	Periodontitis					
	Findings per group		Adjusted group comparison[b]			
	Women without infection[a]	Women with infection	RR (95%CI)	Risk difference[c] (95% CI)	P-value	PAR%
Incidence of preterm birth	60/697 (8.6%)	21/327 (6.4%)	0.74 (0.45 to 1.22)	2.4% (-0.6 to 0.1)	0.236	-8.9%
Incidence of low birth weight	81/652 (12.4%)	34/304 (11.2%)	0.94 (0.60 to 1.46)	1.0% (-5.9 to 3.8)	0.786	-1.8%
Prevalence of neonatal underweight	46/662 (7.0%)	21/311 (6.8%)	1.02 (0.58 to 1.81)	0.1% (-3.5 to 3.3)	0.936	0.8%
Prevalence of neonatal stunting	104/660 (15.8%)	48/307 (15.6%)	1.03 (0.71 to 1.48)	0.0% (-5.2 to 5.2)	0.895	0.8%
Prevalence of small head circumference	19/657 (2.9%)	18/309 (5.8%)	1.95 (0.99 to 3.83)	2.5% (-0.4 to 5.4)	0.053	23.2%

[a]Number of women with the defined adverse outcome / total number of women who had no periodontitis but who had data on the indicated outcome variable

[b]Adjusted for maternal age, maternal height, BMI, HIV status, malaria status and aenemia at enrolment, number of previous pregnancies, study site, socio-economic score, periapical infections, number of teeth, time between delivery and examination, and intervention

[c]The differences are expressed in percentage points

PAR%= population attributable risk fraction

Supplementary table 4. Mean gestational age (GA) by infection group, stratified analyses

Sample strata	Periapical Infection			
	Mean (SD) GA without infection[a] (n=783)	**Mean (SD) GA with infection[a]** (n=241)	**Adjusted difference in means[b] (95% CI)**	**P-value[b]**
Negative HIV test (n=847)	39.5 (2.1)	39.1 (2.8)	-0.52 (-0.90 to -0.13)	0.009
Positive HIV test (n=144)	39.2 (2.7)	39.2 (2.1)	-0.10 (-0.89 to 0.68)	0.795
Negative malaria test (n=803)	39.5 (2.1)	39.2 (2.2)	-0.36 (-0.74 to 0.02)	0.065
Positive malaria test (n=219)	39.3 (2.4)	38.4 (4.5)	-0.85 (-1.65 to -0.05)	0.037

[a]Model without covariates

[b]Adjusted for for maternal age, maternal height, BMI and aenemia at enrolment, number of previous pregnancies, study site, socio-economic score, periodontitis, number of teeth, time between delivery and examination, intervention and malaria status (when stratified by HIV) or HIV (when stratified by malaria)

Supplementary table 5. Mean weight-for-age z-score (WAZ) by infection group, stratified analyses

Sample strata	Periapical infection			
	Mean (SD) WAZ without infection[a] (n=783)	**Mean (SD) WAZ with infection[a]** (n=241)	**Adjusted difference in means[b] (95% CI)**	**P-value[b]**
Negative HIV test (n=847)	-0.50 (0.98)	-0.57 (0.94)	-0.13 (-0.29 to 0.04)	0.127
Positive HIV test (n=144)	-0.61 (1.05)	-0.88 (1.07)	-0.21 (-0.55 to 0.12)	0.204
Negative malaria test (n=803)	-0.47 (0.98)	-0.56 (0.88)	-0.12 (-0.29 to 0.04)	0.129
Positive malaria test (n=219)	-0.66 (1.03)	-1.02 (1.31)	-0.24 (-0.58 to 0.10)	0.163

[a]Model without covariates

[b]Adjusted for maternal age, maternal height, BMI and aenemia at enrolment, number of previous pregnancies, study site, socio-economic score, periodontitis, number of teeth, time between delivery and examination, intervention and malaria status (when stratified by HIV) or HIV (when stratified by malaria)

Supplementary table 6. Mean head circumference z-score by infection group, stratified analyses

Sample strata	Periapical infection			
	Mean (SD) head circumference z-score without infection[a] (n=783)	**Mean (SD) head circumference z-score with infection[a]** (n=241)	**Adjusted difference in means[b] (95% CI)**	**P-value[b]**
Negative HIV test (n=847)	-0.09 (1.03)	-0.20 (1.09)	-0.15 (-0.34 to 0.03)	0.103
Positive HIV test (n=144)	-0.14 (1.20)	-0.47 (1.17)	-0.30 (-0.68 to 0.07)	0.113
Negative malaria test (n=803)	-0.09 (1.05)	-0.14 (1.00)	-0.09 (-0.27 to 0.09)	0.337
Positive malaria test (n=219)	-0.13 (1.09)	-0.88 (1.40)	-0.65 (-1.04 to -0.27)	0.001

[a]Model without covariates

[b]Adjusted for maternal age, maternal height, BMI and anemia at enrolment, number of previous pregnancies, study site, socio-economic score, periodontitis, number of teeth, time between delivery and examination, intervention and malaria status (when stratified by HIV) or HIV (when stratified by malaria)

Received: 20 August 2017 | Revised: 26 November 2017 | Accepted: 3 December 2017

DOI: 10.1111/odi.12817

ORIGINAL ARTICLE

WILEY ORAL DISEASES

Periapical infection may affect birth outcomes via systemic inflammation

U Harjunmaa[1] | R Doyle[2,3] | J Järnstedt[4] | S Kamiza[5] | JM Jorgensen[6] | CP Stewart[6] | L Shaw[3] | L Hallamaa[1] | U Ashorn[1] | N Klein[3] | KG Dewey[6] | K Maleta[7] | P Ashorn[1]

[1]Center for Child Health Research, University of Tampere Faculty of Medicine and Life Sciences and Tampere University Hospital, Tampere, Finland

[2]Microbiology, Virology & Infection Control, Great Ormond Street Hospital NHS Foundation Trust, London, UK

[3]Institute of Child Health, University College London, London, UK

[4]Department of Radiology, Medical Imaging Center, Tampere University Hospital, Tampere, Finland

[5]Department of Pathology, University of Malawi College of Medicine, Blantyre, Malawi

[6]Program in International and Community Nutrition, Department of Nutrition, University of California, Davis, Davis, CA, USA

[7]Department of Community Health, University of Malawi College of Medicine, Blantyre, Malawi

Correspondence
Ulla Harjunmaa, University of Tampere Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland.
Email: ulla.harjunmaa@fimnet.fi

Funding information
Office of Health, Infectious Diseases, and Nutrition; Bureau for Global Health; U.S. Agency for International Development (USAID), Grant/Award Number: AID-OAA-A-12-00005; Bill & Melinda Gates Foundation through a grant to the University of California, Davis; Finnish Cultural Foundation; Finnish Dental Society Apollonia

Objectives: Maternal dental periapical infections are associated with preterm birth and intrauterine growth restriction. This study investigates whether the association is mediated through bacterial spread from periapical lesions to placenta (direct pathway) or systemic inflammatory reaction (indirect pathway).

Materials and Methods: We compared birth outcomes in Malawian mothers with and without periapical infection. As markers of a direct pathway, we identified placental bacteria using a 16S rDNA approach and assessed histological evidence of inflammation in the placenta and amniotic membranes. We measured C-reactive protein, alpha-1-acid glycoprotein, and salivary cortisol as markers of an indirect pathway. We used regression models to associate the predictor variables with duration of pregnancy and newborn size.

Results: Of 1,024 women, 23.5% had periapical infection. There was no association of periapical infection with either bacterial DNA or histological inflammation in placenta or membranes. Periapical infection was associated with C-reactive protein, alpha-1-acid glycoprotein, and cortisol concentrations in a dose-dependent manner at 36 weeks. Addition of alpha-1-acid glycoprotein or cortisol concentration into regression models attenuated the association between periapical infection and pregnancy outcomes.

Conclusion: There was no evidence of direct spread of periapical bacteria to the placenta. Periapical infections and adverse pregnancy outcomes are in part mediated through systemic inflammation.

KEYWORDS
apical infection, birth size, duration of pregnancy, placental infection, systemic inflammation

1 | INTRODUCTION

Every year, 32 million infants are born too early or too small for their gestational age (Lee et al., 2013). Of the 3.1 million annual global neonatal deaths, 35% are related to preterm birth (PTB) complications (Liu et al., 2012). The survivors are at risk of severe long-term morbidity and development delay (Saigal & Doyle, 2008). Current interventions have largely failed to substantially impact the frequency of PTB and intrauterine growth restriction (IUGR), and indeed, PTB rates are still increasing in many countries (Chang et al.,

2013). Several risk factors for PTB and IUGR have been identified of which maternal infections are among the most important. Yet the actual mechanisms by which these risk factors affect pregnancy are unclear (Goldenberg, Culhane, Iams, & Romero, 2008) which hampers the development of new, effective preventive approaches.

Our previous study results from Malawi indicated an association between maternal dental periapical infections (PAI) and shorter pregnancy duration and smaller birth size (Harjunmaa et al., 2015). PAI commonly develops when a deep caries lesion exposes the pulp of the tooth allowing oral bacteria to enter the dental root canals, from where the infection eventually spreads to the bone surrounding the root (Nair, 2004). A causal relationship between PAI and pregnancy outcomes could be through both direct and indirect pathways, which are not mutually exclusive. Firstly, a direct pathway could exist whereby oral bacteria are disseminated through the bloodstream to placental and fetal tissues, influencing birth outcomes through the generation of local inflammation, tissue damage, or changes in placental function (Han & Wang, 2013; Romero, Dey, & Fisher, 2014). Secondly, bacteria within the oral cavity could induce a systemic inflammatory response that could affect the pregnancy indirectly. Although earlier studies have shown that bacteria can translocate from mouth to placenta (Aagaard et al., 2014), and it is known that PAI may lead to systemic inflammation (Gomes et al., 2013), there is no evidence that pathways from PAI to pregnancy complications actually exist. If they do, prevention and treatment of dental caries, the most common chronic disease globally that usually precedes PAI (Marcenes et al., 2013), would be a new and feasible intervention for prevention of PTB and IUGR.

This study aimed to assess whether there is evidence of these two biologically plausible pathways between PAI and adverse pregnancy outcomes in a cohort of pregnant women from the iLiNS-DYAD study in Malawi (Harjunmaa et al., 2015). Our main hypothesis was that the associations between PAI and shortened duration of pregnancy, lower birthweight, and smaller neonatal size are mediated through spread of the periapical bacteria to the feto-placental unit (direct pathway) or systemic inflammation (indirect pathway). We examined the direct pathway by assessing the prevalence and quantity of histological inflammation and bacteria in the placenta and chorioamniotic membranes. The indirect pathway was assessed by measuring concentrations of serum C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) and salivary cortisol during pregnancy. CRP and AGP are acute phase proteins, produced in the liver in response to inflammatory stimuli, and have been associated with shortened duration of gestation and IUGR (Pitiphat et al., 2005; Tjoa et al., 2003). Cortisol is a steroid hormone released from the adrenal glands in response to emotional, physical, or immunological stress (Bellavance & Rivest, 2014), and it also regulates the maintenance of pregnancy and the timing of parturition (Giurgescu, 2009; Voltolini & Petraglia, 2014). In this study, we investigated whether the shortened duration of pregnancy, lower infant birthweight, and neonatal size in women with PAI were mediated through these pathways.

TABLE 1 Proportion of women with evidence of histological inflammation or bacterial infection in placenta, or systemic inflammation

Outcome	Proportion of women (*n*)
Direct pathway	
Chronic placental intervillositis	1.9% (19)
Acute placental intervillositis	21.2% (217)
Chorioamnionitis	19.9% (204)
Bacterial DNA detected in chorioamniotic membranes	59.2% (606)
Bacterial DNA detected in placental tissues	38.5% (394)
Indirect pathway	
Elevated CRP concentration at 14–20 weeks	39.1% (400)
Elevated CRP concentration at 36 weeks	29.7% (304)
Elevated AGP concentration at 14–20 weeks	8.2% (84)
Elevated AGP concentration at 36 weeks	4.5% (46)
Elevated cortisol concentration at 14–20 weeks	23.8% (244)
Elevated cortisol concentration at 36 weeks	23.6% (242)

PAI, periapical infection; CRP, C-reactive protein; AGP, alpha-1-acid glycoprotein; DNA, deoxyribonucleic acid.

2 | METHODS

2.1 | Study design, outcomes, and ethics statement

This study represents continued analysis of a cross-sectional oral health substudy that was nested within a randomized, controlled intervention trial, iLiNS-DYAD-M, in Malawi, sub-Saharan Africa (ClinicalTrials.gov, identifier NCT01239693). The trial was designed to examine the impact of home-fortification of pregnant women's diets with lipid-based nutrient supplements on pregnancy outcomes and maternal and child health in a rural African community. Participants were enrolled prospectively into the main trial and followed throughout pregnancy. The oral health examination to assess the association between oral infections and birth outcomes was conducted after delivery.

The outcome measures for this study were duration of pregnancy, birthweight, neonatal length, and neonatal head circumference. The potential intermediary variables were CRP, AGP, and cortisol concentrations at 14–20 weeks and at 36 gestational weeks (gw), prevalence of any or severe chorioamnionitis, prevalence of placental intervillositis, bacterial load, and prevalence of common periapical bacteria in placental tissues and fetal membranes.

Written or thumb-printed informed consent was taken from the participants at enrollment. Ethical approval was obtained from the College of Medicine Research and Ethics Committee, University of Malawi and the Ethics Committee of Pirkanmaa Hospital District, Finland (Protocol Number P.08/10/972, date of approval January 31, 2011).

TABLE 2 Prevalence of chronic and acute placenta intervillositis, any chorioamnionitis and severe chorioamnionitis by periapical infection severity

Outcome	Prevalence of placental inflammation by participants' PAI status[a]				Comparison, mild-to-mod vs no PAI		Comparison, severe vs no PAI		Comparison, severe vs mild-to-mod PAI	
	No PAI *n* = 783 (%)	Mild-to-moderate PAI *n* = 215 (%)	Severe PAI *n* = 26 (%)	*p*-value	Risk ratio (95% CI)	*p*-value	Risk ratio (95% CI)	*p*-value	Risk ratio (95% CI)	*p*-value
Chronic placental intervillositis	2.0	2.6	0.0	.883	1.28 (0.43–3.87)	.656	n/a	n/a	n/a	n/a
Acute placental intervillositis	15.8	13.9	29.9	.231	0.88 (0.54–1.44)	.513	1.90 (0.85–4.22)	.116	2.15 (0.93–4.97)	.072
Chorioamnionitis, any (>4 cells)	25.8	28.1	26.7	.867	1.09 (0.79–1.51)	.596	1.04 (0.46–2.34)	.934	0.95 (0.42–2.15)	.899
Chorioamnionitis, severe (>25 cells)	11.1	14.6	17.2	.407	1.31 (0.84–2.07)	.237	1.55 (0.58–4.14)	.382	1.18 (0.44–3.15)	.744

PAI, periapical infection.

[a]Adjusted for HIV status, malaria status, height, body mass index, age, study site, number of teeth, nutritional intervention group, anemia, periodontitis status, household assets, oral health examination visit time.

2.2 | Participants, enrollment, and data collection

Details of the main trial (Ashorn et al., 2015) and the oral health substudy (Harjunmaa et al., 2015) have been published previously. In brief, we enrolled 1,391 pregnant women attending antenatal care before 20 gestational weeks between February 2011 and August 2012 through four health facilities in Mangochi District, southern Malawi. Participants received intermittent preventive malaria treatment with sulfadoxine–pyrimethamine at their first antenatal visit and between 28 and 34 gw as part of standard Malawian antenatal care. Research personnel assessed the duration of pregnancy with ultrasonography, performed health and antenatal examinations and recorded participants' medical and obstetric history and socioeconomic background. Participants, who were enrolled in the main trial, had singleton pregnancies, and completed the oral health examination, were eligible for the oral health substudy. Two dental therapists conducted full-mouth oral health examinations of the mothers and took digital panoramic radiographs of their jaws as soon as possible after delivery. A radiologist and an experienced dentist analyzed the radiographs jointly. Research personnel recorded delivery events within 48 hr after delivery and measured infant's birthweight (BW). At 1–6 weeks after delivery, anthropometrists measured infant's length, head circumference, and neonatal weight.

2.2.1 | Saliva and blood collection and processing

Research nurses collected saliva and venous blood samples at the study clinic at baseline (14–20 gw) and 36 gw. Saliva was collected after 30 min fast with a polymer swab placed under the tongue. Laboratory technicians measured salivary cortisol concentrations using an ELISA method and plasma CRP and AGP by immunoturbidimetry. Details of the saliva collection and processing are published elsewhere (Stewart et al., 2015).

2.2.2 | Placental tissue and fetal membrane collection and processing

Two pieces of the chorionic and amniotic membranes were cut from the edge of the rupture site and two full thick pieces of placental tissue from near the umbilical cord insertion. One placental and one membrane sample were fixed in 10% formalin, embedded in paraffin wax, cut, and stained with hematoxylin and eosin on glass slides for histological analysis. The remaining two samples were used for bacterial DNA analysis as published earlier (Doyle et al., 2017). In brief, all DNA extracted from the samples was screened for bacterial DNA using a qPCR targeting the V5-7 regions of the 16S rRNA gene (785F: 5′-GGATTAGATACCCBRGTAGTC-3′, 1175R: 5′-ACGTCRTCCCCDCCTTCCTC-3′). Samples with detectable amounts of bacterial DNA were multiplexed, pooled, cleaned, and sequenced. Paired-end sequenced reads were demultiplexed and assigned OTUs at 97% similarity against a small custom database of full-length 16S rDNA sequences. Any sequences that failed to match at 97% were assigned against the full Greengenes database.

TABLE 3 Comparison of bacterial loads from selected genera that are commonly found in periapical infectious lesions, in the placenta and fetal membranes, presented by periapical infection status groups

Bacterial genera in placental tissues	Mean log 10 16S rRNA copies (*SD*) in placental tissues			Mean log 10 16S rRNA copies (*SD*) in fetal membranes		
	No PAI *n* = 641	PAI *n* = 188	*p*-value	No PAI *n* = 687	PAI *n* = 200	*p*-value
Actinomyces	0.10 (0.53)	0.03 (0.27)	.070	0.02 (0.26)	0 (0)	.242
Bacteroides	0.08 (0.53)	0.04 (0.37)	.351	0.12 (0.72)	0.06 (0.40)	.206
Dialister	0.02 (0.22)	0.02 (0.21)	.938	0.08 (0.52)	0.07 (0.54)	.893
Enterococcus	0.21 (0.92)	0.16 (0.81)	.559	0.35 (1.20)	0.23 (0.98)	.173
Fusobacterium	0.17 (0.76)	0.08 (1.01)	.120	0.14 (0.76)	0.11 (0.72)	.605
Parvimonas	0.01 (0.09)	0.09 (0.50)	<.001	0.12 (0.67)	0.17 (0.80)	.394
Peptostreptococcus	0.16 (0.69)	0.16 (0.69)	.952	0.32 (1.11)	0.47 (1.36)	.131
Porphyromonas	0.13 (0.63)	0.09 (0.49)	.418	0.04 (0.36)	0.01 (0.18)	.253
Prevotella	0.72 (1.42)	0.82 (1.56)	.398	1.18 (1.86)	1.20 (1.89)	.869
Propionibacterium	0.25 (0.03)	0.35 (0.07)	.197	0.13 (0.59)	0.07 (0.45)	.135
Streptococcus	0.52 (1.23)	0.59 (1.34)	.481	1.02 (1.83)	1.08 (1.94)	.680
Treponema	0.06 (0.41)	0.06 (0.42)	.959	0.15 (0.73)	0.15 (0.73)	.922
Veillonella	0.25 (0.89)	0.18 (0.76)	.311	0.48 (1.33)	0.39 (1.12)	.347

PAI, Periapical infection.

2.3 | Definitions

We diagnosed PAI if an unambiguous osteolytic finding (>1 mm) related to the dental root apex was seen in the radiographs. We graded PAI as not present or present. We considered that a participant had PAI if at least one finding was recorded. We determined mild-to-moderate PAI as one to three lesions and severe PAI as four lesions or more. We calculated infants' sex–age-standardized length-for-age (LAZ), weight-for-age (WAZ), and head circumference-for-age z-scores using the WHO Child Growth Standards.

We considered high CRP concentration as CRP > 5 mg/L, high AGP concentration as AGP>1 g/L, and high cortisol concentration as >75th centile of all cortisol concentrations at that time point. We defined histological chorioamnionitis as ≥5 and severe chorioamnionitis as ≥25 neutrophil granulocytes on average per 10 high-power fields present in either the chorionic plate or the amniotic membrane. We defined acute intervillositis as ≥5 neutrophils and chronic intervillositis as ≥5 lymphocytes or monocytes on average per 10 high-power fields in the placental intervillous space. We assessed the presence of bacteria in the placental or fetal membranes by 16S rDNA broad-range SYBR green quantitative PCR assay. A result was positive for bacterial DNA if their Ct value was lower than 28 ± 3 cycles (equivalent to 40 CFU/μl) depending on variation between runs and determined using calibration curves generated from serial dilution of a known amount of *Escherichia coli* culture.

2.4 | Statistical analysis

We performed statistical analysis with Stata 12.1 (StataCorp, College Station, USA). We included in the analyses participants who completed the oral health visit within the 6-week puerperal period. The sample size was originally calculated for the main objective of the iLiNS-DYAD-M trial. In the current analyses, we used the same sample of 1,024 women as in our previous analyses on oral infections and birth outcomes that offered approximately 93% power to detect differences between the groups, assuming an infection prevalence of 25% and an effect size of 0.25 for continuous birth outcomes at 5% two-sided type I error rate (Harjunmaa et al., 2015).

We used multivariate linear regression to estimate adjusted means for continuous outcomes, and modified Poisson regression models (Zou, 2004) to estimate adjusted proportions for binary outcomes, and to estimate the associations between PAI and inflammation or infection variables (except bacterial prevalence and mean loads) and cortisol. The same covariates that were considered potential confounders of the relationship between oral infections and birth outcomes in our previous study (Harjunmaa et al., 2015) were used in this study for all outcomes to make the estimates comparable. We considered that these covariates were also biologically plausible confounders for the inflammation outcomes. The covariates were included in the models using forced entry method. The included covariates were maternal age, height, body mass index, socioeconomic score (household assets), parity, time between delivery and the oral health examination, and number of teeth as continuous variables, and study site, nutrition intervention group, anemia, HIV, malaria, and periodontitis status as categorical variables. In addition, time between wake-up and saliva collection, and time between last meal and saliva collection were used in cortisol analysis models as continuous variables. We added square terms for maternal height, BMI, age, and socioeconomic score into the models to test the assumption of linear relationships between continuous outcomes and covariates. Because adding nonlinear covariates

TABLE 4 Mean CRP, AGP, and cortisol concentrations at 14–20 weeks and at 36 weeks of pregnancy by maternal periapical infection severity

Outcome	Mean (SD) concentration by participants' PAI status[a]				Comparison, mild-to-mod vs no PAI		Comparison, severe vs no PAI		Comparison, severe vs mild-to-mod PAI	
	No PAI *n* = 783	Mild-to-moderate PAI *n* = 215	Severe PAI *n* = 26	*p*-value	Difference in means (95% CI)	*p*-value	Difference in means (95% CI)	*p*-value	Difference in means (95% CI)	*p*-value
CRP mg/L, 14–20 weeks	8.89 (19.13)	8.82 (16.00)	11.85 (18.93)	.740	−0.07 (−3.06 to 2.93)	.966	2.96 (−4.71 to 10.63)	.449	3.03 (−4.75 to 10.81)	.445
CRP mg/L, 36 weeks	6.33 (14.81)	8.68 (17.06)	14.41 (19.00)	.048	2.35 (−0.48 to 5.18)	.104	8.07 (0.40–15.75)	.039	5.73 (−2.06 to 13.51)	.149
AGP g/L, 14–20 weeks	0.72 (0.24)	0.71 (0.21)	0.70 (0.20)	.926	0.00 (−0.04 to 0.03)	.838	−0.02 (−0.11 to 0.08)	.718	−0.01 (−0.11 to 0.08)	.781
AGP g/L, 36 weeks	0.57 (0.22)	0.60 (0.29)	0.69 (0.26)	.040	0.03 (−0.01 to 0.07)	.140	0.12 (0.02–0.23)	.022	0.09 (−0.02 to 0.20)	.093
Cortisol nmol/L, 14–20 weeks	5.62 (3.79)	5.60 (3.06)	5.41 (2.65)	.963	0.03 (−0.56 to 0.63)	.910	−0.18 (−1.70 to 1.34)	.781	−0.21 (−1.71 to 1.29)	.818
Cortisol nmol/L, 36 weeks	7.97 (2.91)	8.85 (4.62)	9.87 (3.24)	.006	0.84 (0.19–1.50)	.012	1.90 (0.25–3.36)	.024	1.06 (−0.63 to 2.75)	.218

CRP, C-reactive protein; AGP, alpha-1-acid glycoprotein; PAI, periapical infection.
Mild-to-moderate PAI = 1–3 lesions, severe PAI > 3 lesions.
[a]Adjusted for HIV status, malaria status, height, body mass index, age, study site, number of teeth, nutritional intervention group, anemia, periodontitis status, household assets, oral health examination visit time. Cortisol adjusted also for saliva collection time and time from last meal.

did not markedly improve the adjusted *R*-square, we excluded the nonlinear terms from the final models. To assess whether an inflammation variable was on a pathway between PAI and birth outcomes, we added those variables that were associated with PAI severity one by one in the models. We used White's general test for heteroscedasticity to test for unequal variances using non-imputed data. The null hypothesis of homoscedasticity was not rejected.

We used multiple imputed data (50 imputations) based on chained equation methods (van Buuren, Boshuizen, & Knook, 1999) for all multivariable model analyses. We imputed missing data for all variables that were included in the analyses, except for the 16S rRNA data. Cortisol concentration at 28 weeks was used in the imputation but not in the analyses themselves. The number of originally missing values that were substituted with values obtained by multiple imputation ranged from 0 to 371 (0.0% to 36.2%) per variable (Table S1).

We assessed the prevalence of any bacteria (bacterial 16S rRNA copies) in the placental and fetal membrane tissues by the PAI severity categories (no, mild-to-moderate, and severe infection). Comparison between the groups was made with Fisher's exact test.

We selected twenty-one periapical pathogen genera that are commonly mentioned in the literature (Fujii et al., 2009; Siqueira & Rocas, 2009; Somma, Castagnola, Bollino, & Marigo, 2011) and compared their mean loads (mean number of 16S rRNA copies) and prevalence in the placental and fetal membrane tissues between participants who did not have and who had PAI. The selected bacteria genera were *Actinomyces*, *Anaeroglobus*, *Atopobium*, *Bacteroides*, *Dialister*, *Enterococcus*, *Eubacterium*, *Filifactor*, *Fusobacterium*, *Mogibacterium*, *Olsenella*, *Parvimonas*, *Peptostreptococcus*, *Porphyromonas*, *Prevotella*, *Propionibacterium*, *Pseudoramibacter*, *Streptococcus*, *Tannerella*, *Treponema*, and *Veillonella*. Bacterial OTUs were first filtered at 0.01% mean relative abundance across samples and then clustered by genus. Tables of the particular genera were produced for women with both sample and the dental assessment data. In the group comparisons, the Mann–Whitney *U* test was used for the means and Fisher's exact test for the prevalence.

We assessed also the mean bacterial loads (mean number of 16S rRNA gene copies) of each bacteria genera found in the placentas or membranes of the mothers without or with PAI. Comparison of the mean values between the infection groups was made with Mann–Whitney *U* test. The analysis was adjusted by Benjamini–Hochberg equation to control the false discovery rate due to the number of comparisons.

3 | RESULTS

A total of 1,391 participants were enrolled in the iLiNS-DYAD study. Of those, 1229 (88.4%) completed the oral health examination between May 2011 and August 2013. After excluding mothers with twins and those who completed the oral examination later than 6 weeks after delivery, 1024 (79.6%) participants were included in the analyses (Figure S1). The baseline characteristics of the included and excluded participants were similar except that the included women were on

TABLE 5 Continuous birth outcomes by periapical infection status, adjusted for AGP, CRP, cortisol, or both AGP and cortisol at 36 gw, or chorioamnionitis

	Primary analysis[a]				Adjusted for AGP	
Outcome	Women without PAI (*n* = 783)	Women with PAI (*n* = 241)	Difference in means (95% CI)	*p*-value	Diff. in means (95% CI)	*p*-value
Pregnancy duration (*SD*), gw	39.5 (2.05)	39.1 (2.60)	−0.4 (−0.8 to −0.1)	.015	−0.3 (−0.7 to −0.0)	.049
Birthweight (*SD*), g	2,982 (426)	2,891 (415)	−90 (−157 to −22)	.009	−75 (−142 to −8)	.028
WAZ (*SD*)	−0.56 (1.03)	−0.73 (1.07)	−0.19 (−0.35 to −0.02)	.026	−0.14 (−0.31 to 0.02)	.077
Neonatal length, cm (*SD*)	49.7 (2.19)	49.2 (2.28)	−0.56 (−0.92 to −0.21)	.002	−0.48 (−0.83 to −0.13)	.007
LAZ (*SD*)	−0.98 (1.10)	−1.27 (1.12)	−0.29 (−0.47 to −0.11)	.001	−0.25 (−0.42 to −0.07)	.005
Head Z (*SD*)	−0.15 (1.15)	−0.38 (1.24)	−0.22 (−0.41 to −0.04)	.017	−0.18 (−0.37 to 0.00)	.051

PAI, Periapical infection; AGP, alpha-1-acid glycoprotein; CRP, C-reactive protein; WAZ, weight-for-age z-score; LAZ, length-for-age z-score; Head Z, head circumference-for-age z-score.

[a]Adjusted for HIV status, malaria status, height, body mass index, age, study site, number of teeth, nutritional intervention group, anemia, periodontitis status, household assets, oral health examination visit time.

average slightly older (25 vs 24 years), less often primiparous (17.8% vs 33.3%) or malaria positive (21.4% vs 28.1%), and had a higher mean score for socioeconomic status (0.46 vs −0.13) and lower mean BMI (22.1 vs 22.4) (Table S2). The participant flow and the baseline characteristics have been published earlier (Harjunmaa et al., 2015).

Among the included participants, the mean (*SD*) duration of pregnancy was 39.4 (2.3) gw, birthweight was 2979 (430) g, and neonatal length was 49.7 (2.2) cm. The prevalence of PAI was 23.5% (*n* = 241). Mean (*SD*) time between delivery and oral examination was 16.0 (8.0) days. The mean values for the laboratory measurements, histology findings, and bacterial DNA of the participants are presented in Table 1.

3.1 | Markers of a direct pathway

There were no statistically significant differences between the PAI categories in any of the placental or fetal membrane histology-based inflammation markers. However, although statistically non-significant, the relative risk was usually higher when comparing the severe PAI group to the mild-to-moderate or no PAI groups, or the mild-to-moderate group to the no PAI group (Table 2). Similarly, no statistically significant differences were detected between the PAI categories in the prevalence of any bacteria in the placental (healthy 66.7%, mild-to-moderate 74.0%, and severe 58.5% $p = .242$) or membrane (healthy 44.8%, mild-to-moderate 53.7%, and severe 38.6% $p = .470$) samples.

Of the selected 21 bacteria genera that are commonly involved in PAI, 13 were found in both the fetal membranes and the placental tissues. However, there were no statistically significant differences between the groups of women with and without PAI in the mean bacterial loads (mean number of 16S rRNA copies) (Table 3) or in the prevalence of any of those bacteria genera (Table S3) that were found in at least 10 participants in the placental tissues. Similarly, no statistically significant differences between the PAI groups were found in the mean bacterial loads even when any bacteria genera that were found from the placental or membrane tissues were analyzed (data not shown).

3.2 | Markers of an indirect pathway

There was a positive, dose-dependent association between PAI severity and the mean plasma concentrations of CRP and AGP, and salivary concentration of cortisol at 36 gw. Compared to the no PAI group, women in the severe PAI group had 8.07 (0.40–15.75) mg/L higher mean CRP and 1.90 nmol/L higher mean cortisol concentration, and women in the mild-to-moderate PAI group had 0.84 nmol/L higher mean cortisol concentration at 36 weeks. No association was found with the 14- to 20-week samples (Table 4). No association was found with the 14- to 20-week samples (Table 4).

The patterns in the prevalence of elevated CRP, AGP, and cortisol by PAI categories were similar to those for the continuous variables, except that the differences were statistically significant only for high cortisol at 36 gw (healthy 21.3%, mild-to-moderate 28.7%, severe 45.8%, $p = .033$) (data not shown).

3.3 | Mediation of PAI–birth outcome association by systemic inflammation markers and cortisol

In the primary analyses, women with PAI had 0.4-week shorter mean pregnancy duration ($p = .015$) and infants with 90 g lower mean BW ($p = .009$) and 0.56 cm shorter mean neonatal length ($p = .002$) compared to those who did not have PAI. Adjustment of the regression model for maternal AGP concentration at 36 weeks reduced the difference in means by 0.1 weeks in the duration of pregnancy, 15 g in BW, and 0.08 cm in neonatal length, and increased the *p*-values for each of the models. Adjustment for salivary cortisol concentration at 36 weeks reduced the difference in pregnancy duration by 0.1 weeks, BW by 10 g, and neonatal length by 0.02 cm. When adjusting for both AGP and cortisol at the same time, the results did not differ

Adjusted for CRP		Adjusted for cortisol		Adjusted for AGP and cortisol		Adjusted for severe chorioamnionitis	
Diff. in means (95% CI)	*p*-value	Diff. in means (95% CI)	*p*-value	Diff. in means (95% CI)	*p*-value	Diff. in means (95% CI)	*p*-value
−0.4 (−0.8 to −0.1)	.021	−0.3 (−0.7 to 0.0)	.056	−0.3 (−0.7 to 0.1)	.097	−0.4 (−0.7 to −0.1)	.022
−85 (−153 to −17)	.014	−80 (−149 to −10)	.024	−70 (−139 to −2)	.043	−86 (−153 to 18)	.013
−0.17 (−0.34 to −0.01)	.040	−0.17 (−0.34 to −0.00)	.047	−0.14 (−0.31 to −0.02)	.086	−0.18 (−0.34 to −0.01)	.035
−0.54 (−0.90 to −0.18)	.003	−0.54 (−0.90 to −0.17)	.004	−0.49 (−0.84 to −0.13)	.008	−0.54 (−0.89 to −0.19)	.003
−0.27 (−0.46 to −0.10)	.002	−0.28 (−0.47 to −0.10)	.002	−0.26 (−0.43 to −0.08)	.005	−0.28 (−0.46 to −0.10)	.002
−0.21 (−0.41 to −0.03)	.024	−0.22 (−0.41 to −0.03)	.026	−0.19 (−0.41 to −0.03)	.049	−0.21 (−0.5 to −0.03)	.022

substantially from the AGP only adjustment; that is, the effects were not additive. Adjustment for plasma CRP concentration or maternal severe chorioamnionitis did not markedly affect the strength of the association with any of the outcomes (Table 5).

4 | DISCUSSION

We aimed to investigate whether infection in placenta or within the chorioamniotic membranes (direct pathway) or systemic inflammation (indirect pathway) mediates the association between maternal PAI and shortened pregnancy duration, lower birthweight, or smaller neonatal size. We did not find any evidence of a direct pathway linking periapical infection and pregnancy outcomes. However, PAI was associated with CRP, AGP, and cortisol at 36 weeks of gestation in a dose-dependent manner. The associations between PAI and the adverse pregnancy outcomes were partially mediated by AGP and cortisol.

4.1 | Strengths and weaknesses

Strengths of this study include a large sample size, rigorous quality control, multiple biological samples collected at various time points during pregnancy and after delivery, and clinical and X-ray-based oral health assessment that allowed high-quality diagnostics. The main weaknesses were some missing biological sample data, some differences in baseline characteristics between the participants who were included in analyses and those lost to follow-up, for example, primiparity, and our inability to document the oral health status at baseline and immediately after delivery, that is, at the same time as the biological sample collection, due to ethical restrictions (e.g., exposure to X-rays) and complexity of the study logistics in this low-resource context. In addition, the placental tissue of mothers who delivered very preterm was mostly not available, and naturally they did not undergo the 36 gw sample collection. To reduce bias due to the lost to follow-up, we conducted adjusted analyses that included the baseline characteristics that distinguished the excluded and included participants, and imputed the missing data with multiple imputations (excluding bacterial DNA). PAI was almost entirely related to large caries lesions that take several years to develop and are rarely treated in this low-resource setting; thus, it is unlikely that their prevalence after delivery differed substantially from that at enrollment or 36 gw. Finally, as the research team did not have the responsibility of providing the intermittent malaria treatment to the mothers, it was not possible to confirm whether some participants received less than the standard amount of three doses.

4.2 | Placental infection

In previous studies, bacteria, unequivocally demonstrated to have originated from infected root canals, have been isolated from blood (Debelian, Olsen, & Tronstad, 1996), (Debelian et al., 1996) and, in many studies, found in the feto-placental unit, especially in preterm births (Han & Wang, 2013). While we did detect bacteria in placental tissue, previously reported to have originated from the oral cavity, we did not see any differences between those who did or did not have PAI. Furthermore, no differences were found in placental inflammation. This may be explained by the lack of samples from the pregnancies that ended in miscarriage and very early deliveries that took place at home where the tissues were discharged.

4.3 | Systemic inflammation

Our study supports the earlier findings that PAI may induce systemic inflammatory responses (Gomes et al., 2013; Marton & Kiss, 2000; Somma et al., 2011), with elevated concentrations of CRP (Gomes et al., 2013) and AGP (Glurich et al., 2002; de Soet et al., 2003). We could not find any earlier studies linking PAI and cortisol concentrations, but cortisol concentrations have previously been associated with increased growth and virulence of oral pathogens (Jentsch, Marz,

& Kruger, 2013). In our study, the associations between PAI and the inflammatory markers and cortisol were stronger at 36 weeks than earlier in gestation. We believe that this finding relates to the alteration in the immune system during pregnancy. In early gestation, the host immune responses are able to control local, low-grade infections, but pregnancy-related attenuation in the immune response increases the susceptibility to and consequences of infections in later gestation (Kourtis, Read, & Jamieson, 2014). The underlying mechanisms include limited T-cell activity (Taylor, Sullivan, Eblen, & Gercel-Taylor, 2002), decreased neutrophil functions and antibody production (Zachariasen, 1993), and hormonal changes which contribute to increased vascular permeability and bone resorption (Komm et al., 1988).

Systemic inflammation, measured by elevated CRP concentration (Pitiphat et al., 2005; Tjoa et al., 2003), and salivary cortisol concentrations (Giurgescu, 2009) during pregnancy have previously been associated with shortened duration of gestation and IUGR. In line with those results, inflammation was associated with pregnancy outcomes also in our study sample. We also found that the association between PAI and adverse pregnancy outcomes was attenuated modestly when AGP or cortisol concentration was added in regression models, suggesting partial mediation of the association by the inflammatory pathway. The modest association may be related to the missed "before delivery" samples from those who delivered very early and who may have had more marked inflammation. Another important consideration is that our analyses were limited to the available markers, which may not have fully reflected systemic inflammation. Some oral diseases-related salivary interleukins, immunoglobulins, or metalloproteinases, released from infected periapical tissues (Gomes et al., 2013; Javaid, Ahmed, Durand, & Tran, 2016) and known to be associated with adverse pregnancy outcomes (Romero et al., 2014), might have been more sensitive markers.

5 | CONCLUSIONS

The study findings suggest that PAI causes adverse pregnancy outcomes partially via an indirect pathway of systemic inflammation but not through a direct pathway of local placental infection. Further studies are needed to investigate whether the associations between PAI, elevated inflammatory markers, and adverse birth outcomes are more pronounced when very early preterm deliveries are included. It is also important to study other inflammatory pathways and the impact of PAI elimination on pregnancy outcomes in future studies.

ACKNOWLEDGEMENTS

This publication is based on research funded in part by the Office of Health, Infectious Diseases, and Nutrition, Bureau for Global Health, U.S. Agency for International Development (USAID) under terms of Cooperative Agreement No. AID-OAA-A-12-00005, through the Food and Nutrition Technical Assistance III Project (FANTA), managed by FHI 360. Additional funding was provided by the Bill & Melinda Gates Foundation through a grant to the University of California, Davis. UH received personal working grant also from the Finnish Cultural Foundation and Finnish Dental Society Apollonia. Planmega Ltd. provided the X-ray machine for nominal price and 3M donated materials for dental treatment of the participants. The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of USAID, the United States Government, the Bill & Melinda Gates Foundation, or the other funders. We thank the study participants, the local communities, our research personnel at the study sites and the iLiNS Project extended research team for their positive attitude and support during the study. We particularly thank Simeon Mulewa and Davie Charlie for the clinical oral health data collection, and the iLiNS Project Steering Committee members and Project Manager Mary Arimond (http://ilins.org) for technical support.

CONFLICT OF INTERESTS

None to declare.

AUTHOR CONTRIBUTIONS

The authors' responsibilities were as follows: UH, UA, NJK, KGD, KM, and PA designed research; UH, RD, JJ, SK, CPS, JMJ, LS, and PA conducted research; UH, LH, RD, and LS analyzed data; UH and PA wrote the manuscript with critical input and comments from all other authors. All authors read and approved the final manuscript.

ORCID

U Harjunmaa http://orcid.org/0000-0003-0598-3918

REFERENCES

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. (2014). The placenta harbors a unique microbiome. *Science Translational Medicine*, *6*(237), 237ra65. https://doi.org/10.1126/scitranslmed.3008599

Ashorn, P., Alho, L., Ashorn, U., Cheung, Y., Dewey, K., Harjunmaa, U., … Maleta, K. (2015). The impact of lipid-based nutrient supplement provision to pregnant women on newborn size in rural Malawi: A randomised controlled trial. *American Journal of Clinical Nutrition*, *101*, 387–397. https://doi.org/10.3945/ajcn.114.088617

Bellavance, M. A., & Rivest, S. (2014). The HPA – Immune axis and the immunomodulatory actions of glucocorticoids in the brain. *Frontiers in Immunology*, *5*, 136. https://doi.org/10.3389/fimmu.2014.00136

van Buuren, S., Boshuizen, H. C., & Knook, D. L. (1999). Multiple imputation of missing blood pressure covariates in survival analysis. *Statistics in Medicine*, *18*(6), 681–694. https://doi.org/10.1002/(ISSN)1097-0258

Chang, H. H., Larson, J., Blencowe, H., Spong, C. Y., Howson, C. P., Cairns-Smith, S., … Born Too Soon Preterm Prevention Analysis Group (2013). Preventing preterm births: Analysis of trends and potential reductions with interventions in 39 countries with very high human development index. *Lancet*, *381*(9862), 223–234. https://doi.org/10.1016/S0140-6736(12)61856-X

Debelian, G. J., Olsen, I., & Tronstad, L. (1996). Electrophoresis of whole-cell soluble proteins of microorganisms isolated from bacteremias in endodontic therapy. *European Journal of Oral Sciences*, *104*(5–6), 540–546. https://doi.org/10.1111/j.1600-0722.1996.tb00139.x

Doyle, R. M., Harris, K., Kamiza, S., Harjunmaa, U., Ashorn, U., Nkhoma, M., … Klein, N. (2017). Bacterial communities found in placental tissues are associated with severe chorioamnionitis and adverse birth outcomes. *PLoS ONE*, *12*(7), e0180167. https://doi.org/10.1371/journal.pone.0180167

Fujii, R., Saito, Y., Tokura, Y., Nakagawa, K. I., Okuda, K., & Ishihara, K. (2009). Characterization of bacterial flora in persistent apical periodontitis lesions. *Oral Microbiology and Immunology*, *24*(6), 502–505. https://doi.org/10.1111/j.1399-302X.2009.00534.x

Giurgescu, C. (2009). Are maternal cortisol levels related to preterm birth? *Journal of Obstetric, Gynecologic, and Neonatal Nursing*, *38*(4), 377–390. https://doi.org/10.1111/j.1552-6909.2009.01034.x

Glurich, I., Grossi, S., Albini, B., Ho, A., Shah, R., Zeid, M., … De Nardin, E. (2002). Systemic inflammation in cardiovascular and periodontal disease: Comparative study. *Clinical and Diagnostic Laboratory Immunology*, *9*(2), 425–432.

Goldenberg, R. L., Culhane, J. F., Iams, J. D., & Romero, R. (2008). Epidemiology and causes of preterm birth. *Lancet*, *371*(9606), 75–84. https://doi.org/10.1016/S0140-6736(08)60074-4

Gomes, M. S., Blattner, T. C., Sant'Ana Filho, M., Grecca, F. S., Hugo, F. N., Fouad, A. F., & Reynolds, M. A. (2013). Can apical periodontitis modify systemic levels of inflammatory markers? A systematic review and meta-analysis. *Journal of Endodontics*, *39*(10), 1205–1217. https://doi.org/10.1016/j.joen.2013.06.014

Han, Y. W., & Wang, X. (2013). Mobile microbiome: Oral bacteria in extra-oral infections and inflammation. *Journal of Dental Research*, *92*(6), 485–491. https://doi.org/10.1177/0022034513487559

Harjunmaa, U., Jarnstedt, J., Alho, L., Dewey, K. G., Cheung, Y. B., Deitchler, M., … Ashorn, P. (2015). Association between maternal dental periapical infections and pregnancy outcomes: Results from a cross-sectional study in Malawi. *Tropical Medicine & International Health*, https://doi.org/10.1111/tmi.12579

Javaid, M. A., Ahmed, A. S., Durand, R., & Tran, S. D. (2016). Saliva as a diagnostic tool for oral and systemic diseases. *Journal of Oral Biology and Craniofacial Research*, *6*(1), 66–75. https://doi.org/10.1016/j.jobcr.2015.08.006

Jentsch, H. F., Marz, D., & Kruger, M. (2013). The effects of stress hormones on growth of selected periodontitis related bacteria. *Anaerobe*, *24*, 49–54. https://doi.org/10.1016/j.anaerobe.2013.09.001

Komm, B. S., Terpening, C. M., Benz, D. J., Graeme, K. A., Gallegos, A., Korc, M., … Haussler, M. R. (1988). Estrogen binding, receptor mRNA, and biologic response in osteoblast-like osteosarcoma cells. *Science (New York, NY)*, *241*(4861), 81–84. https://doi.org/10.1126/science.3164526

Kourtis, A. P., Read, J. S., & Jamieson, D. J. (2014). Pregnancy and infection. *The New England Journal of Medicine*, *370*(23), 2211–2218. https://doi.org/10.1056/NEJMra1213566

Lee, A. C., Katz, J., Blencowe, H., Cousens, S., Kozuki, N., Vogel, J. P., … CHERG SGA-Preterm Birth Working Group (2013). National and regional estimates of term and preterm babies born small for gestational age in 138 low-income and middle-income countries in 2010. *The Lancet Global Health*, *1*(1), e26–e36. https://doi.org/10.1016/S2214-109X(13)70006-8

Liu, L., Johnson, H. L., Cousens, S., Perin, J., Scott, S., Lawn, J. E., … Child Health Epidemiology Reference Group of WHO and UNICEF (2012). Global, regional, and national causes of child mortality: An updated systematic analysis for 2010 with time trends since 2000. *Lancet (London, England)*, *379*(9832), 2151–2161. https://doi.org/10.1016/S0140-6736(12)60560-1

Marcenes, W., Kassebaum, N. J., Bernabe, E., Flaxman, A., Naghavi, M., Lopez, A., & Murray, C. J. (2013). Global burden of oral conditions in 1990–2010: A systematic analysis. *Journal of Dental Research*, *92*(7), 592–597. https://doi.org/10.1177/0022034513490168; 10.1177/0022034513490168

Marton, I. J., & Kiss, C. (2000). Protective and destructive immune reactions in apical periodontitis. *Oral Microbiology and Immunology*, *15*(3), 139–150. https://doi.org/10.1034/j.1399-302x.2000.150301.x

Nair, P. N. (2004). Pathogenesis of apical periodontitis and the causes of endodontic failures. *Critical Reviews in Oral Biology and Medicine*, *15*(6), 348–381. https://doi.org/10.1177/154411130401500604

Pitiphat, W., Gillman, M. W., Joshipura, K. J., Williams, P. L., Douglass, C. W., & Rich-Edwards, J. W. (2005). Plasma C-reactive protein in early pregnancy and preterm delivery. *American Journal of Epidemiology*, *162*(11), 1108–1113. https://doi.org/10.1093/aje/kwi323

Romero, R., Dey, S. K., & Fisher, S. J. (2014). Preterm labor: One syndrome, many causes. *Science (New York, NY)*, *345*(6198), 760–765. https://doi.org/10.1126/science.1251816

Saigal, S., & Doyle, L. W. (2008). An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet*, *371*(9608), 261–269. https://doi.org/10.1016/S0140-6736(08)60136-1

Siqueira, J. F. Jr, & Rocas, I. N. (2009). Diversity of endodontic microbiota revisited. *Journal of Dental Research*, *88*(11), 969–981. https://doi.org/10.1177/0022034509346549

de Soet, J. J., Schriks, M. C., Kratz, E., Poland, D. C., van Dijk, W., & van Amerongen, W. E. (2003). Dental caries related to plasma IgG and alpha1-acid glycoprotein. *Caries Research*, *37*(2), 79–84. https://doi.org/69014

Somma, F., Castagnola, R., Bollino, D., & Marigo, L. (2011). Oral inflammatory process and general health. Part 2: How does the periapical inflammatory process compromise general health? *European Review for Medical and Pharmacological Sciences*, *15*(1), 35–51.

Stewart, C. P., Oaks, B. M., Laugero, K. D., Ashorn, U., Harjunmaa, U., Kumwenda, C., … Dewey, K. G. (2015). Maternal cortisol and stress are associated with birth outcomes, but are not affected by lipid-based nutrient supplements during pregnancy: An analysis of data from a randomized controlled trial in rural Malawi. *BMC Pregnancy and Childbirth*, *15*, 346-015-0793-8. https://doi.org/10.1186/s12884-015-0793-8

Taylor, D. D., Sullivan, S. A., Eblen, A. C., & Gercel-Taylor, C. (2002). Modulation of T-cell CD3-zeta chain expression during normal pregnancy. *Journal of Reproductive Immunology*, *54*(1–2), 15–31. https://doi.org/10.1016/S0165-0378(01)00067-5

Tjoa, M. L., van Vugt, J. M., Go, A. T., Blankenstein, M. A., Oudejans, C. B., & van Wijk, I. J. (2003). Elevated C-reactive protein levels during first trimester of pregnancy are indicative of preeclampsia and intrauterine growth restriction. *Journal of Reproductive Immunology*, *59*(1), 29–37. https://doi.org/10.1016/S0165-0378(02)00085-2

Voltolini, C., & Petraglia, F. (2014). Neuroendocrinology of pregnancy and parturition. *Handbook of Clinical Neurology*, *124*, 17–36. https://doi.org/10.1016/B978-0-444-59602-4.00002-2

Zachariasen, R. D. (1993). The effect of elevated ovarian hormones on periodontal health: Oral contraceptives and pregnancy. *Women and Health*, *20*(2), 21–30. https://doi.org/10.1300/J013v20n02_02

Zou, G. (2004). A modified Poisson regression approach to prospective studies with binary data. *American Journal of Epidemiology*, *159*(7), 702–706. https://doi.org/10.1093/aje/kwh090

SUPPORTING INFORMATION

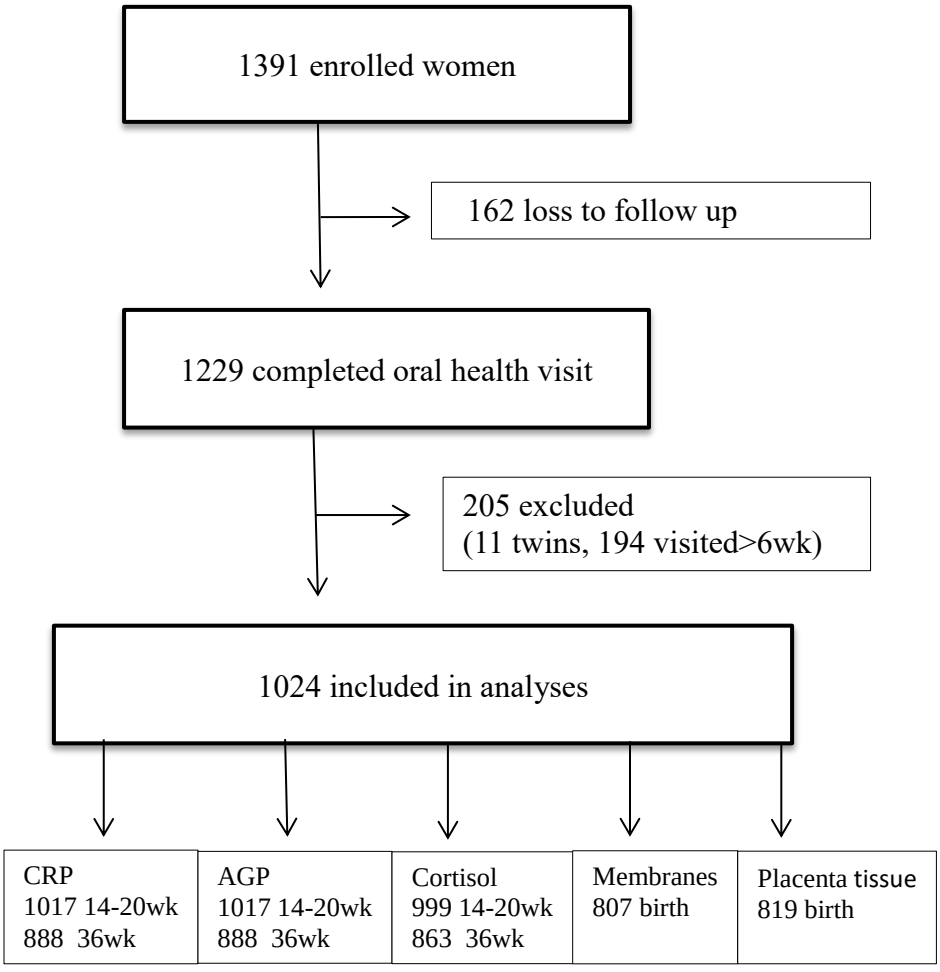
Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Harjunmaa U, Doyle R, Järnstedt J, et al. Periapical infection may affect birth outcomes via systemic inflammation. *Oral Dis*. 2018;24:847–855. https://doi.org/10.1111/odi.12817

Periapical infection may affect birth outcomes via systemic inflammation

Supplementary figures and tables

Supplementary figure 1. Participant flow in the iLiNS-DYAD Oral Health study.



CRP= C-reactive protein, AGP= alpha-1-acid glycoprotein

Supplementary table 1. Variables used in the analyses of the 1024 included participants

Variable	**Variable type**	**# imputed**	**% imputed**
Acute intervillositis	dichotomous	205	20.0%
Anemia	dichotomous	1	0.0%
AGP 14-20 weeks	continuous	7	0.7%
AGP at 36 weeks	continuous	137	13.4%
Periapical infections	categorical	0	0.0%
Birth weight	continuous	68	6.6%
Chorioamnionitis	dichotomous	217	21.2%
Chronic intervillositis	dichotomous	205	20.0%
Cortisol 14-20 weeks	continuous	25	2.4%
Cortisol at 28 weeks	continuous	286	27.9%
Cortisol at 36 weeks	continuous	161	15.7%
CRP 14-20 weeks	continuous	7	0.7%
CRP at 36 weeks	continuous	137	13.4%
Gestational age at delivery	continuous	0	0.0%
Head circumference	continuous	58	5.7%
HIV status	dichotomous	3	0.3%
Intervention group	categorical	0	0.0%
Length of the infant	continuous	57	5.6%
Length-for-age z-score	continuous	57	5.6%
Maternal age	continuous	0	0.0%
Maternal bmi	continuous	6	0.6%

Maternal height	continuous	4	0.4%
Maternal malaria	dichotomous	2	0.2%
Number of 16S rRNA copies in membranes	continuous	137	13.4%
Number of 16S rRNA copies in placentas	continuous	195	19.0%
Number of previous pregnancies	continuous	2	0.2%
Number of teeth	continuous	1	0.0%
Periodontitis status	dichotomous	0	0.0%
Research site	categorical	0	0.0%
Severe chorioamnionitis	dichotomous	217	19.0%
Socioeconomic score	continuous	6	0.6%
Time between delivery and oral health visit	continuous	0	0.0%
Time between meal and saliva collection at 28 weeks	continuous	371	36.2%
Time between meal and saliva collection at 36 weeks	continuous	263	25.7%
Time between meal and saliva collection 14-20 weeks	continuous	117	11.4%
Time between waking up and saliva collection at 28 weeks	continuous	371	36.2%
Time between waking up and saliva collection at 36 weeks	continuous	262	25.6%
Time between waking up and saliva collection 14-20 weeks	continuous	116	11.3%
Weight-for-age z-score	continuous	51	5.0%
Years of education completed	continuous	7	0.7%

Supplementary table 2. Baseline characteristics of the included and excluded participants

Characteristic	Included[b] (n=1024)	Excluded (n=367)	P-value
Mean (SD) maternal age, years	25 (6.2)	24 (5.7)	<0.001
Mean (SD) maternal education, completed years at school	3.9 (3.4) (7 missing data)	4.3 (3.6) (58 missing data)	0.087
Mean (SD) proxy for socioeconomic status[a]	-0.13 (1.68) (6 missing data)	0.46 (2.12) (62 missing data)	<0.001
Proportion of primiparous women, (n)	17.8% (182)	33.3% (122)	<0.001
Mean (SD) BMI, kg/m²	22.1 (2.7)	22.4 (3.1)	0.046
Proportion of women with a low BMI (< 18.5 kg/m²), (n)	5.4% (55)	5.2% (19)	0.891
Proportion of anemic women (Hb < 110 g/l) (n)	19.8 % (203)	23.0% (84)	0.208
Proportion of women with a positive HIV test, (n)	14.1 % (144) (3 missing data)	12.5 % (39) (54 missing data)	0.460
Proportion of women with a positive malaria test at enrollment (RDT), (n)	21.4% (219)	28.1% (103)	0.009

Abbreviations: BMI=body-mass index, Hb=blood hemoglobin concentration, HIV=human immune deficiency virus, RDT=malaria rapid diagnostic test obtained from a finger-prick

[a]Created with principal components analysis by combining information on the building material of the house, main source of water and electricity, sanitary facility and main type of cooking fuel used

[b]Included = visited<6wk after delivery, singleton pregnancy, data on at least one presented outcome

Published earlier on: Harjunmaa U et al.

Supplementary table 3. Comparison of prevalence of selected bacteria that are commonly found in PAI lesions, in the placenta and fetal membranes, presented by periapical infection status groups

Bacterial genera in placental tissues	Prevalence (n) in placental tissues			Bacterial genera in fetal membranes	Prevalence (n) in fetal membranes		
	No PAI n=641	PAI n= 188	P-value (chi-squared)		No PAI n=687	PAI n= 200	P-value (chi-squared)
Actinomyces	3.6% (23)	1.1% (2)	0.090	*Actinomyces*	0.7% (5)	0.0% (0)	0.593
Bacteroides	2.2% (14)	1.1% (2)	0.546	*Bacteroides*	3.2% (22)	2.0% (4)	0.480
Dialister	0.6% (4)	0.5% (1)	1.000	*Dialister*	2.3% (16)	2.0% (2)	1.000
Enterococcus	5.3% (34)	4.3% (8)	0.706	*Enterococcus*	8.4% (58)	5.5% (11)	0.229
Fusobacterium	5.3% (34)	2.7% (5)	0.170	*Fusobacterium*	3.5% (24)	2.5% (5)	0.652
Parvimonas	0.2% (1)	3.2% (6)	0.001	*Parvimonas*	3.2% (22)	4.5% (9)	0.384
Peptostreptococcus	5.0% (32)	5.3% (10)	0.851	*Peptostreptococcus*	8.6% (59)	11.5% (23)	0.213
Porphyromonas	4.1% (27)	3.2% (6)	0.745	*Porphyromonas*	1.5% (10)	0.5% (1)	0.472
Prevotella	21.2% (136)	22.9% (43)	0.616	*Prevotella*	30.3% (208)	30.0% (60)	1.000
Propionibacterium	8.7% (56)	11.7% (22)	0.255	*Propionibacterium*	5.2% (36)	2.5% (5)	0.126
Streptococcus	15.8% (101)	17.0% (32)	0.653	*Streptococcus*	25.5% (175)	25.5% (51)	1.000
Treponema	2.0% (13)	2.1% (4)	1.000	*Treponema*	4.4% (30)	4.0% (8)	1.000
Veillonella	7.5% (49)	5.3% (10)	0.334	*Veillonella*	12.4% (85)	10.5% (21)	0.537

PAI=Periapical infection