Title
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Journal
Maternal and Child Nutrition, 12(1)

ISSN
1740-8695

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Publication Date
2016

DOI
10.1111/mcn.12204

Peer reviewed

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

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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.

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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

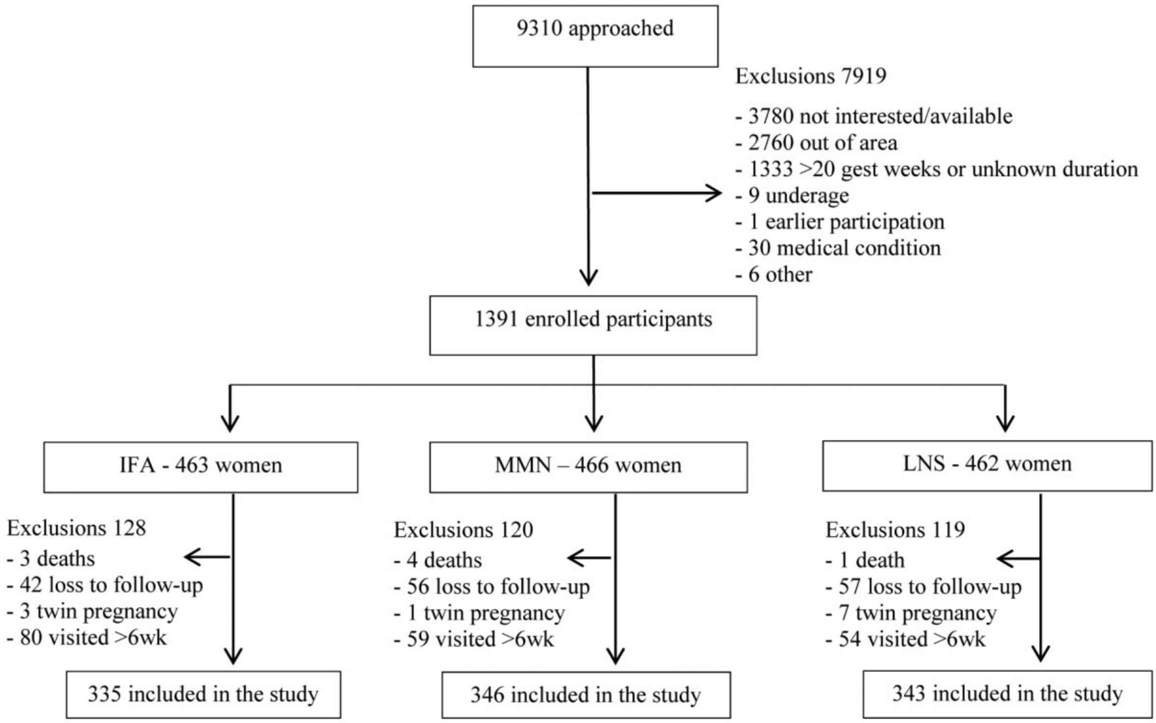

Fig. 1. Participant flow in CONSORT-recommended form.

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.

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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.