

## **Maternal Oral Bacterial Levels Predict Early Childhood Caries Development**

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## ABSTRACT

**Objective:** To calculate the association of maternal salivary bacterial challenge (mutans streptococci [MS] and lactobacilli [LB]) from pregnancy through 24 months' postpartum with child caries incidence ( $\geq 1$  cavitated or restored teeth) at 36 months. **Materials & Methods:** Dental, salivary bacterial, sociodemographic, and behavioral measures were collected at three- to six-month intervals from a birth cohort of low-income Hispanic mother-child dyads ( $N = 243$ ). We calculated the relative child caries incidence, adjusted for confounding, following higher maternal challenge of MS ( $>4500$  colony-forming units per milliliter of saliva [CFU/mL]) and LB ( $>50$  CFU/mL) based on multivariable models. **Results:** Salivary MS and LB levels were greater among mothers of caries-affected children versus caries-free children. Mothers with higher salivary MS challenge were more likely to have MS-positive children ( $>0$  CFU/mL), but maternal LB challenge was not a statistically significant predictor of child LB-positive status. Adjusting for sociodemographics, feeding and care practices, and maternal dental status, higher maternal salivary challenge of both MS and LB over the study period predicted nearly double the child caries incidence versus lower MS and LB (cumulative incidence ratio: 1.9; 95% confidence interval: 1.1, 3.8). **Conclusion:** Maternal salivary bacterial challenge not only is associated with oral infection among children but also predicts increased early childhood caries occurrence.

**KEY WORDS:** preschool child, mutans streptococci, lactobacillus, intergenerational health, prospective studies, life course perspective.

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## INTRODUCTION

Multiple intergenerational connections have been observed between maternal conditions and child health outcomes: from body mass index (Murrin *et al.*, 2012) to age of menarche (Deardorff *et al.*, 2012). Consequently, poor health may be transmitted from parents to children, conceivably perpetuating health disparities and providing reason to consider intergenerational determinants in disease prevention.

Correlations between parental and offspring caries status were recognized at least as early as the 1940s (Klein, 1946). Cross-sectional (Sasahara *et al.*, 1998; Weintraub *et al.*, 2010; Dye *et al.*, 2011) and longitudinal (Grytten *et al.*, 1988; Thitasomakul *et al.*, 2009; Shearer *et al.*, 2012) studies have since corroborated the association between parental dental status and offspring caries, even into adulthood (Shearer *et al.*, 2012). Multiple mechanisms likely underlie intergenerational oral health continuity, including genetic predisposition (Conry *et al.*, 1993), shared social environments (Watt, 2002), and parental oral health knowledge and attitudes (Finlayson *et al.*, 2007), as reviewed by Shearer and Thomson (2010).

Maternal-child transmission of oral bacteria is one such potential mechanism, given the essential, albeit not sufficient, role of oral infection in caries development. Maternal bacterial strains can be detected in children (Li and Caufield, 1995), and high maternal salivary mutans streptococci (MS) challenge is associated with earlier child MS acquisition (Li *et al.*, 2005). Mothers of children with caries were more likely to be positive for salivary MS than mothers of caries-free children in a cross-sectional study in New York (Smith *et al.*, 2002). Elsewhere, higher maternal salivary MS levels were associated with child caries and child salivary MS cross-sectionally but not with child caries 2 yr later (Kishi *et al.*, 2009).

The present study follows a birth cohort of low-income Hispanic children whose mothers received repeated dental and salivary bacterial assessments for both MS and lactobacilli (LB) during their children's first 3 yr as part of the Mothers and Youth Access caries prevention trial (Ramos-Gomez *et al.*, 2012). We hypothesized that higher maternal challenge of MS and LB averaged over this period would be associated with greater caries incidence among children.

## MATERIAL & METHODS

### Ethics

Institutional review boards at collaborating universities (University of California, Los Angeles; University of California, San Francisco; and San

Diego State University) approved this study. A community advisory board guided study design. Mothers granted informed consent in English or Spanish. Mothers and/or children with caries were referred for treatment.

## Participants

The design and results of the intervention trial in which this prospective observational cohort is nested are presented elsewhere (Ramos-Gomez *et al.*, 2008; Ramos-Gomez *et al.*, 2012). Briefly, the nesting trial randomly assigned 361 mother-infant dyads either to an intervention of oral health counseling, preventive child fluoride varnish applications, and maternal chlorhexidine rinses from 4 to 7 months' postpartum or to a control of oral health counseling and "rescue" (therapeutic) fluoride varnish for children with initial carious lesions. Families were advised to obtain fluoride varnish only through the trial. At 9 months, the intervention reduced maternal salivary MS but not LB; neither was lowered significantly at any other time point (Appendix Table 1). After 36 months, there were no clinically or statistically significant differences in caries between intervention and control group children (Ramos-Gomez *et al.*, 2012).

Participants were registered patients in the second trimester of pregnancy at a health center serving a predominantly low-income community near the US-Mexico border and without water fluoridation (2003-2007). Trial eligibility criteria included maternal age of 18 to 33 yr, singleton fetus, and local residency with evidence of geographic stability (*e.g.*, proof of address). Excluded were sisters or household members of previously enrolled participants, women intending to give birth in Mexico, high-risk pregnancies, and women with existing medical conditions, pregnancy complications, or prior pregnancy-related issues that required hospitalization. Following enrollment, mothers and children returned for questionnaires, dental assessments, and saliva collection at visits corresponding to 4, 9, 12, 18, 24, 30, and 36 months' postpartum. The present cohort study includes all mother-child dyads with a 36-month child dental assessment ( $N = 243$ ).

## Caries

Trained, calibrated dental examiners followed standard diagnostic criteria (National Institute of Dental Research, 1991), as described elsewhere (Ramos-Gomez *et al.*, 2012). We calculated the number of decayed (cavitated), missing, or restored permanent teeth (excluding third molars) among mothers (*i.e.*, DMFT index) and the number of decayed (cavitated) or restored primary teeth among children ( $d_2ft$ ). The primary outcome was the cumulative incidence of  $\geq 1$  decayed or restored tooth ( $d_2ft > 0$ ) at the child's 36-month visit. Mothers were considered to have persistent untreated decay if presenting with  $\geq 1$  decayed tooth at all visits (97% completed  $\geq 6$  assessments).

## Salivary Bacteria

Bacterial assessments are detailed elsewhere (Ramos-Gomez *et al.*, 2012). Saliva samples (1.5-2.0 mL) were collected from mothers during pregnancy and with their children at the 4-, 9-, 12-, 24-, and 36-month visits. Saliva was refrigerated until laboratory transfer for serial dilution and plating within 24 hr.

Following 48- to 72-hr anaerobic incubation, colony-forming units per milliliter (CFU/mL) of saliva of MS (*Streptococcus mutans* and *Streptococcus sobrinus*) and LB were enumerated.

The primary exposures were the means of the maternal  $\log_{10}$  CFU/mL levels for MS and for LB, averaged across visits from baseline to 24 months (95% provided  $\geq 4$  samples) and dichotomized as higher bacterial challenge (yes/no). Our *a priori* cutpoint for higher MS challenge (4500 CFU/mL) was based on a previous study of maternal salivary bacteria and child caries (Kishi *et al.*, 2009). We identified no analogous study for LB; our *a priori* LB cutpoint (50 CFU/mL) placed roughly the same proportion of mothers in the higher LB group as in the higher MS group.

## Questionnaires

At each visit, trained bilingual study staff administered maternal questionnaires in English or Spanish, assessing sociodemographic and behavioral factors potentially related to caries. Data were entered into a secure web-based data management system with automated validation checks.

## Power

Recruitment and follow-up in the nesting intervention trial determined the number of participants available for this observational study. We estimated that 188 mother-child dyads would be necessary for  $>80\%$  power to detect a 2-fold difference in 36-month caries incidence (20% vs. 40%) between lower and higher bacterial challenge groups, assuming that 60% of mothers would be in the higher challenge group (2-tailed  $\alpha = 0.05$ ). With 243 eligible dyads, we deemed the statistical power sufficient for multivariable analyses.

## Statistical Analysis

Bivariable comparisons were assessed with 2-tailed hypothesis tests ( $\alpha = 0.05$ ) for dichotomous (chi-square), normally distributed continuous (Student *t* test), or nonnormally distributed continuous (Mann-Whitney *U* test) variables. General linear mixed effects models (clustering by mother-child dyads; unstructured correlation) were used to compare maternal salivary bacteria levels ( $\log_{10}$  CFU/mL) over time by child caries status, separately for MS and LB.

In the primary analysis, the parameter of interest was the cumulative incidence ratio (relative risk) of caries ( $d_2ft > 0$ ) comparing children of mothers with higher bacterial challenge to those with lower challenge. Separately for MS and LB exposures, unadjusted and adjusted log-linear regression models were estimated. Covariates were selected *a priori* considering possible causal roles in the caries process. The 3 nested adjusted models progressively added categories of variables. First, sociodemographic variables were included: maternal education ( $<$ high school or equivalent vs.  $\geq$ high school), non-English language exclusively at home (yes/no), family structure (both parents live in household, yes/no), annual household income ( $<$ \$7000, \$7000-\$35,000,  $>$ \$35,000), nationality (Mexican or Mexican American vs. other), and parity (child is first born, yes/no). Second, feeding and care behaviors were added: breastfeeding duration (0-2, 3-9, or  $\geq 10$  months), daily child tooth-brushing assistance with fluoride toothpaste at 24 months (yes/no), utensil

**Table 1.** Descriptive Characteristics and Dental Health of Study Participants: Eligible Mother-Child Dyads (*N* = 243)<sup>a</sup>

Characteristic	<i>n</i> (%)	<i>M</i> ± <i>SD</i> (Range)
Maternal and household sociodemographics		
Mother identifies as		
Hispanic and/or Latina	233 (96.7)	
Mexican and/or Mexican American	230 (95.4)	
Non-English language used exclusively at home	154 (63.9)	
Mother less than high school degree or equivalent	101 (42.3)	
Self-reported annual household income, US\$		
<7000	52 (22.2)	
7000 to 35,000	159 (67.9)	
≥35,000	23 (9.8)	
Child is mother's first	103 (42.4)	
Both parents and child live in same household	164 (67.8)	
Child feeding and care practices		
Breastfeeding duration, months		
<3	94 (38.7)	
3-9	86 (35.4)	
≥10	63 (25.9)	
Brushing with fluoride toothpaste and caregiver help at 24-month visit	107 (45.9)	
Mother ever shared utensils with child	55 (23.0)	
Total daily frequency of meals and snacks at 24 months		
<8	51 (21.9)	
8-10	151 (64.8)	
≥11	31 (13.3)	
Mother is primary caregiver	222 (92.1)	
Child ever attended daycare	71 (29.2)	
Maternal oral health status		
DMFT at baseline		12.7 ± 5.0 (1-26)
DT at baseline		7.3 ± 5.3 (0-22)
Mothers with ≥1 untreated decayed tooth at all visits	141 (58.0)	
Mean log <sub>10</sub> MS baseline to 24 months		3.9 ± 1.2 (0.3-6.6)
Mothers with mean log <sub>10</sub> MS over study period >3.65 <sup>b</sup>	147 (60.5)	
Mean log <sub>10</sub> LB baseline to 24 months		2.2 ± 1.7 (0-6.6)
Mothers with mean log <sub>10</sub> LB over study period >1.70 <sup>c</sup>	132 (54.3)	
Child oral health status at 36-month visit		
Children with <i>d</i> <sub>2ft</sub> > 0	83 (34.2)	
Children with untreated decay ( <i>d</i> <sub>2t</sub> > 0)	75 (30.9)	
<i>d</i> <sub>2ft</sub>		1.41 ± 2.54 (0-12)
Child log <sub>10</sub> MS		2.5 ± 2.5 (0-7.5)
Children MS positive	121 (55.3)	
Child log <sub>10</sub> LB		0.5 ± 1.1 (0-5.6)
Children LB positive	39 (16.4)	

Abbreviations: *d*<sub>2ft</sub>, decayed (cavitated) filled primary tooth index; *d*<sub>2t</sub>, decayed (cavitated) primary tooth index; DMFT, decayed, missing, and filled permanent tooth index; DT, decayed permanent tooth index; LB, lactobacilli; MS, mutans streptococci.

<sup>a</sup>Sample size ranges from 234 to 243 because of missing baseline information for some variables.

<sup>b</sup>Equivalent to 4500 colony-forming units per milliliter of saliva.

<sup>c</sup>Equivalent to 50 colony-forming units per milliliter of saliva.

sharing (ever/never), combined daily meal and snack frequency at 24 months (4-7, 8-10, ≥11), mother as primary caregiver (yes/no), and daycare attendance (ever/never). Last, maternal dental factors were added: baseline decayed teeth (count) and persistent untreated decay (yes/no).

To test whether allocation status in the nesting intervention trial influenced results, models were estimated both restricted to the trial control group (*n* = 120) and pooled across trial groups (*N* = 243), the latter with terms for trial group and Trial Group

× Bacterial Challenge interaction. Restricted and pooled estimates were qualitatively similar, and no statistically significant interactions were detected (all *p* > .4). Therefore, the primary results are from pooled models that include a trial group term, even in unadjusted models. For reference, Appendix Table 2 provides restricted results. Secondly, to evaluate the association with child caries of maternal MS and LB both independently and combined, we estimated a fully adjusted model with terms for high MS, high LB, and MS × LB interaction.

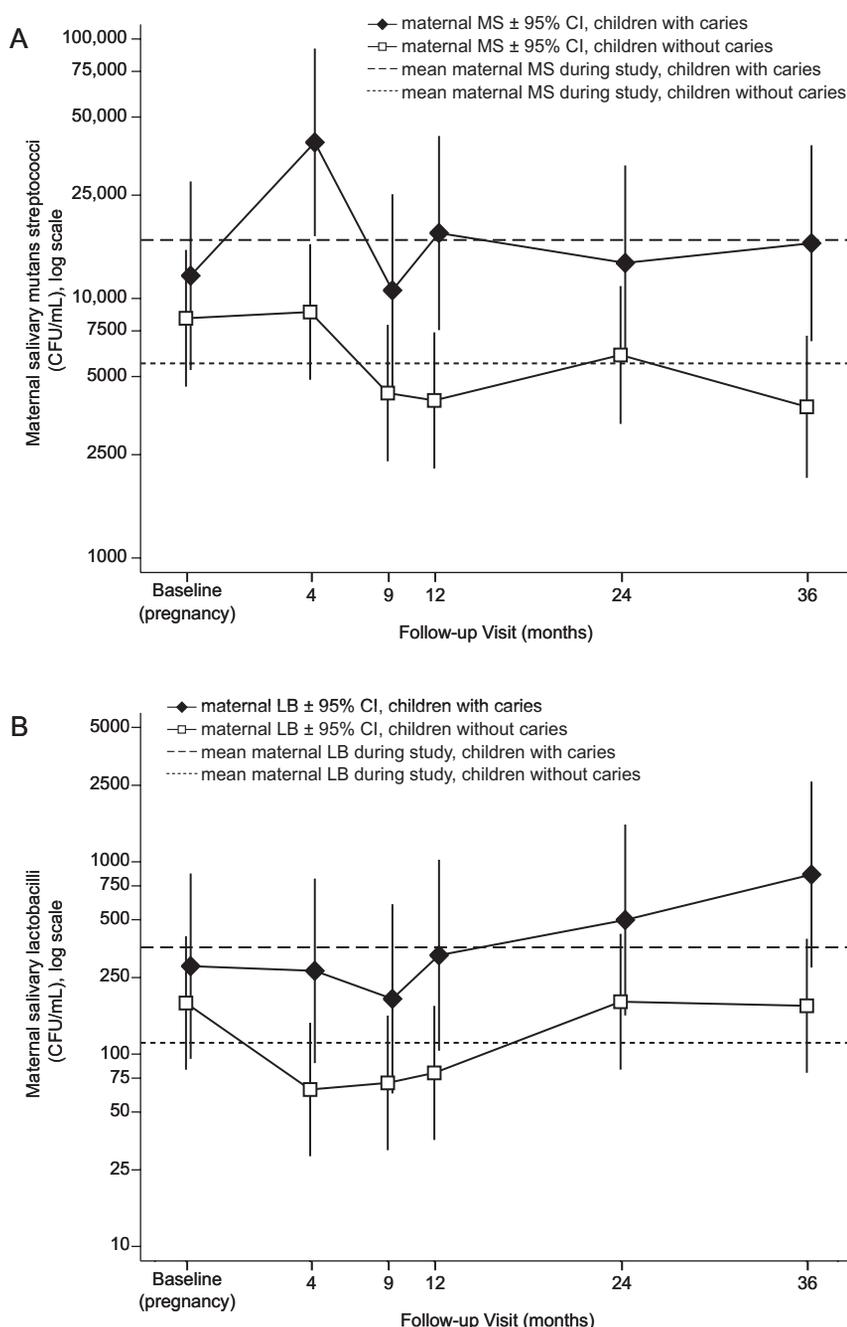
Missing covariate data (1% of all data) were addressed with multiple imputation. Point estimates were averaged over 50 imputations. We used non-parametric bootstrap resampling (5000 repetitions) to estimate 95% confidence intervals that accounted for both sampling variability and imputation. As a sensitivity check, we ran analogous zero-inflated Poisson regression models for the outcome child  $d_2ft$  (Appendix Table 3). Analyses were completed with Stata 12.1 (StataCorp LP) and R 3.0.1 (<http://r-project.org>). Study reporting followed STROBE guidelines (von Elm *et al.*, 2007).

### RESULTS

Eligible study participants ( $N = 243$  mother-child dyads) were primarily from low-income Mexican American families (Table 1). Among mothers, caries experience was universal (100% baseline DMFT > 0), and persistent active caries was prevalent (58% with untreated decay at all visits). There was no statistically significant association of any Table 1 variable with treatment allocation in the nesting trial or with postenrollment loss to follow-up. At 36 months, 34% of children were caries positive ( $d_2ft > 0$ ), including 31% with untreated decay (Table 1), while 53% and 16% of children were positive for MS and LB, respectively.

For both MS and LB, maternal salivary bacterial levels—at each visit and averaged over the study period—were higher among mothers of children with caries ( $d_2ft > 0$ ) at 36 months *versus* those caries-free (Figure 1). Compared with children of mothers with lower levels, children of mothers with higher maternal salivary MS averaged from baseline to 24 months were more likely to be MS positive at 36 months (63% *vs.* 43%,  $p < .01$ ), although mean  $\log_{10}$  MS counts among MS-positive children were similar with or without higher maternal MS challenge (4.6 *vs.* 4.4, respectively,  $p = .42$ ). Higher average maternal LB was not statistically significantly associated with child LB positivity (19% *vs.* 13%,  $p = .18$ ) or with mean  $\log_{10}$  LB counts among LB-positive children (2.8 *vs.* 2.8,  $p = .94$ ).

Higher average maternal salivary bacterial challenge was associated with greater child caries incidence for MS, but this relationship was weaker and not statistically significant for LB



**Figure 1.** Maternal salivary bacterial counts by child dental status. Maternal salivary bacterial counts of (A) MS and (B) LB by 36-month child caries status ( $\geq 1$  cavitated or restored teeth *vs.* caries-free). Maternal bacterial levels of MS ( $p < .01$ ) and LB ( $p = .03$ ) over the study period were greater among mothers of children with caries (Wald test), based on 1369 (MS) and 1405 (LB) salivary measurements clustered in 243 mother-child dyads. Abbreviations: CFU, colony-forming units; CI, confidence interval; LB, lactobacilli; MS, mutans streptococci.

(Table 2). In the interaction model, high maternal challenge of MS was more strongly associated with greater child caries incidence if maternal LB challenge was low (Table 3). The relative increase in caries prevalence associated with high maternal challenge from MS and LB together (cumulative incidence ratio, 1.9; 95% confidence interval, 1.1, 3.8) was not markedly

**Table 2.** Child Caries Incidence ( $d_{2ft} > 0$ ) at 36 Months by Higher versus Lower Mean Maternal Salivary Bacterial Challenge ( $N = 243$ )

Model	Exposure <sup>a</sup> : Maternal Mean MS >4500 CFU/mL		Exposure <sup>a</sup> : Maternal Mean LB >50 CFU/mL	
	Cumulative Incidence Ratio	95% Confidence Interval	Cumulative Incidence Ratio	95% Confidence Interval
Unadjusted <sup>b</sup>	1.7	1.1, 2.7	1.2	0.8, 1.8
Adjusted model 1 <sup>c</sup>	1.7	1.1, 2.6	1.2	0.8, 1.8
Adjusted model 2 <sup>d</sup>	1.8	1.2, 2.9	1.3	0.9, 1.9
Adjusted model 3 <sup>e</sup>	1.7	1.1, 2.8	1.2	0.8, 1.8

Abbreviations: CFU/mL, colony-forming units per milliliter saliva;  $d_{2ft}$ , decayed (cavitated) filled primary tooth index; LB, lactobacilli; MS, mutans streptococci.

<sup>a</sup>Mean maternal bacterial levels from baseline (in pregnancy) to 24 months' postpartum.

<sup>b</sup>Includes allocation status from nesting intervention study only.

<sup>c</sup>Includes allocation status from nesting intervention study and maternal education, language spoken at home, family structure, household income, maternal nationality, and parity.

<sup>d</sup>Includes all adjusted model 1 variables and breastfeeding duration, child tooth brushing, utensil sharing, feeding frequency, mother as primary caregiver, and daycare attendance.

<sup>e</sup>Includes all adjusted model 2 variables and maternal baseline decayed tooth index and persistent untreated maternal decay.

**Table 3.** Interaction of Maternal Salivary Mutans Streptococci and Lactobacilli Challenge in Association with Child Caries Incidence ( $d_{2ft} > 0$ ) at 36 Months

	Exposure	Reference	Cumulative Incidence Ratio	95% Confidence Interval
MS	High <sup>a</sup>	Low	2.4	1.3, 4.9
LB	Low <sup>b</sup>	Low		
<i>n</i>	41	70		
MS	High	Low	1.2	0.6, 2.7
LB	High	High		
<i>n</i>	106	26		
MS	Low	Low	1.6	0.7, 3.7
LB	High	Low		
<i>n</i>	26	70		
MS	High	High	0.8	0.5, 1.3
LB	High	Low		
<i>n</i>	106	41		
MS	High	Low	1.9	1.1, 3.7
LB	High	Low		
<i>n</i>	106	70		

Abbreviations:  $d_{2ft}$ , decayed (cavitated) filled primary tooth index; LB, lactobacilli; MS, mutans streptococci; *n*, mothers with this combination of MS/LB challenge.

<sup>a</sup>Cutpoint for higher versus lower maternal mean salivary MS = 4500 colony-forming units per milliliter of saliva, averaged from baseline (in pregnancy) to 24 months' postpartum.

<sup>b</sup>Cutpoint for higher versus lower maternal mean salivary LB = 50 colony-forming units per milliliter of saliva, averaged from baseline (in pregnancy) to 24 months' postpartum.

Test for statistical MS × LB interaction:  $p = .14$ .

Adjusted for allocation status from nesting intervention study and maternal education, language spoken at home, family structure, household income, maternal nationality, parity, breastfeeding duration, child tooth brushing, utensil sharing, feeding frequency, mother as primary caregiver, daycare attendance, maternal baseline decayed tooth index, and persistent untreated maternal decay.

stronger than that associated with high challenge from either MS alone, suggesting no synergistic relationship.

## DISCUSSION

Poor maternal dental health might enhance caries development in subsequent generations through multiple mechanisms—genetic, behavioral, infectious, and social (Shearer and Thomson, 2010). Here, we demonstrated that, when adjusted for numerous factors, higher maternal salivary bacterial challenge was prospectively, longitudinally associated with child caries in a low-income Hispanic cohort. Multiple studies have documented maternal-to-child transfer of caries-causing oral bacteria (Li and Caufield, 1995; Klein *et al.*, 2004). To our knowledge, this study is the largest prospective longitudinal investigation showing that maternal MS and LB predicted not only oral infection among children but also a meaningful increase in caries incidence. Maternal bacterial challenge continues to merit consideration among the multiple individual, familial, and community risk factors for early childhood caries.

Association estimates changed little after adjustment for various sociodemographic, feeding/care, and maternal dental measures, suggesting limited confounding by these variables. We hypothesize, however, that the association between maternal cariogenic salivary bacteria and child caries might be strongest under contexts entailing close maternal contacts (*e.g.*, breastfeeding, utensil sharing) and weaker under contexts that imply broader potential sources of infection (*e.g.*, daycare attendance, having siblings). For example, greater fidelity of maternal and child MS genotypes was observed among MS-positive children who were breastfed (Li *et al.*, 2000). If observed, such effect measure modification would strengthen the mechanistic evidence inculcating maternal bacterial transfer as a caries determinant among children, representing an area for future study.

While maternal salivary MS and LB levels over the study period were associated with child caries, the nesting intervention trial—which lowered maternal bacterial levels only temporarily and only among a subset of mothers—had no caries effect among children (Ramos-Gomez *et al.*, 2012). Thus, sustained reductions in maternal oral bacterial levels might be more critical than short-term declines for preventing child caries. As a

methodological consideration, averaging maternal bacterial levels over repeated measurements likely permitted more valid classification of mothers into higher challenge groups in light of intrasubject variability.

This study builds on previous work by considering maternal salivary MS and LB, rather than MS alone, in relation to child caries. However, we did not observe synergistic interaction between MS and LB. Instead, we found that higher maternal challenge from MS or LB alone predicted child caries, albeit not statistically significant for LB, and that each exposure added little information if the other was already present. This contrasts a recent finding in adults, which reported the greatest number of decayed surfaces associated with high levels of MS and LB together (Featherstone *et al.*, 2012). However, because of correlation between MS and LB, fewer mothers were discordant with regard to their salivary challenge from each bacterial group, making the evaluation of statistical interaction more challenging. Thus, our findings await confirmation in larger samples. Several bacteria have been associated with early childhood caries (Tanner *et al.*, 2011). Future studies are needed measuring multiple groups of maternal oral bacteria, individually and in relation to one another.

As a limitation, we did not isolate bacterial strains for genetic analysis and therefore could not directly confirm maternal-to-child transfer. Studies have shown that oral bacteria in children has both maternal and nonmaternal origins (Mitchell *et al.*, 2009; Zhan *et al.*, 2012). As children age, additional sources of oral bacteria (*e.g.*, other caregivers, siblings, peers) are likely to contribute to caries susceptibility. Thus, despite adjustment for numerous factors, unmeasured correlations in genetics, diets, and environments, rather than infection alone, could partially account for interrelationships between maternal and child bacteria. Also, this study population featured a heightened maternal caries burden; whether the results apply more generally requires further investigation. To prevent unnecessary exposure, radiographic examinations were not conducted, potentially lowering sensitivity to detect some carious lesions.

Our study highlights the contribution of maternal bacterial challenge in early childhood caries, supporting ongoing interruption of vertical transmission as a potential preventive strategy. A multifaceted intervention to reduce oral bacterial challenge in mothers was associated with both short- and long-term reductions in MS colonization (Köhler *et al.*, 1984; Köhler and Andréen, 2012) and caries prevention in primary and permanent teeth (Köhler and Andréen, 1994, 2012). Interventions that provided mothers with xylitol-containing chewing gum successfully prevented child MS colonization and reduced child caries (Thorild *et al.*, 2006; Söderling, 2009), although longer duration and higher dose regimens yielded greater effects (Söderling, 2009). Thus, early initiated and sustained interventions, perhaps integrating life course and socioenvironmental determinants perspectives (Kenney *et al.*, 2012), might be best positioned for favorable outcomes. Broadly implemented maternal-child health initiatives might contribute meaningful reductions in oral health disparities by helping to break an intergenerational chain of oral health disadvantage.

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