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By

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Abstract

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Doctor of Philosophy in Epidemiology

University of California Berkeley

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Early childhood caries (ECC) is the infectious, chronic disease responsible for tooth decay in young children. As a public health problem, ECC, and caries in general, tends to be overlooked in favor of other conditions that pose greater threats to mortality. However, as a contributor to morbidity, few conditions match dental caries in prevalence. Prevalence estimates vary, but across the globe, more than half of school-age children and nearly all adults have experienced caries. For children, tooth decay frequently remains untreated, with consequences that include pain, spreading infection, impaired eruption of the adult teeth, and difficulties in eating, sleeping, or focusing in school. Early childhood caries follows a social gradient, with least advantaged populations shouldering the greatest disease burden, however, ECC is not exclusively a disease of poverty or of resource-poor nations. Sharp rises in caries prevalence have been reported recently among children from well-off families in the United States.

The public health burden of early childhood caries must be addressed through prevention, as financial resources are not currently available to provide treatment for such a widespread condition. Many effective preventatives are known, including topical fluoride. However, the caries process can take hold in the first year of life, soon after tooth eruption – an age at which few children contact a dentist and well before school-based programs can be effective. Safe, affordable, and effective preventive strategies that can be implemented very early in life and without reliance on traditional dental service delivery systems would benefit a tremendous number of children.

Early-life feeding practices are part of the multi-factorial etiology of ECC. Broadly speaking, these practices include breastfeeding, nursing bottle use, and the quantity and frequency with which particular foods and liquids are consumed, among other behaviors. Tooth decay is a consequence of the anaerobic fermentation of dietary carbohydrates on the part of oral bacteria, creating acidic byproducts that accelerate the demineralization tooth structure. While this process could be modulated in a number of ways: from making teeth resistant to demineralization (e.g. with fluorides) to inhibiting bacterial colonization of the teeth; improvements in feeding practices might offer additional nutritional benefits above and beyond any benefits to oral health. Additionally, feeding practices could be modified from an early age without the necessary involvement of dental care providers.

In the first chapter of this dissertation, I examine the existing literature to critically and systematically compile the evidence linking early-life feeding practices to early childhood caries. The stronger the evidence, the better positioned the field may be to develop and implement dietary-based interventions to prevent ECC. I review 235 publications meeting pre-specified inclusion criteria, and synthesize the evidence connecting certain feeding practices to ECC. The review attempts to characterize the tremendous heterogeneity across these studies, stemming from differences in study design, data collection methods, analytic approaches, and study quality. The frequent consumption of fermentable carbohydrates, although measured in a variety of ways, consistently emerged as a caries risk factor, suggesting a strong causal signal above the variability in the characteristics of these widely different studies.

The study of feeding practices and caries presents numerous challenges, and the systematic review, in part, explores these difficulties. Feeding practices change considerably as a child ages, and untangling the proper temporally relationship between feeding and caries can be a thorny proposition, particularly from cross sectional data. Furthermore, context may dictate whether a given risk factor will increase the risk of caries. Yet, from a global perspective, context becomes difficult to evaluate in full when most studies have taken place in particular regions of the world (e.g. the United States, Western Europe), leaving other regions underrepresented (e.g. Africa, Southeast Asia).

In the second chapter, I present results from a dietary-based cluster-randomized intervention from southern Brazil. In this study, municipal health centers in the city of Porto Alegre were randomized to either an intervention consisting of training for health care workers in a set of recommendations for infant feeding or to a control of usual practices. The study hypothesized that this training would increase the likelihood that mothers attending these clinics would receive evidence-based complementary feeding recommendation from their health care provider and, in turn, improve their infant feeding practices, leading to oral and general health benefits in their children.

The results of the intention-to-treat analysis suggested a moderate reduction in the prevalence of ECC in the intervention group that was not statistically significant. However, in subgroup analyses that considered only mothers who had remained at the same health center from which they were initially recruited and only mothers who reported that the health center was an important source of feeding advice, there were statistically significant preventive effects of the intervention. In the second section of Chapter 2, I explore these findings in more detail. Specifically, I apply two approaches for interpreting trial results: 1. inverse probability censoring weights to account for losses to follow-up, and 2. process evaluation to provide a framework for thinking about the implementation of a trial as a series of intermediary steps between inputs and outcome. In the final pages of Chapter 2, I present a brief simulation exercise to demonstrate that whether or not losses to follow-up introduce a bias in the estimate effected of a randomized intervention depends partly on the parameter used to measure effectiveness (i.e. the relative risk or the risk difference).

In the third and final chapter, I use data collected during the Porto Alegre intervention trial to investigate a relatively controversial question in the epidemiology of early childhood caries: does continuing to breastfed a child to older ages increase caries risk? Numerous studies have reported a positive association between breastfeeding and ECC, while others have not, leading to uncertainty and, at times, conflicting messages within recommendations to mothers of young children. Treating the participants of the intervention trial as a prospective birth cohort, I hypothesized that children breastfed for at least two years would have a higher prevalence of severe-ECC at age three years than those breastfed for shorter periods. Using marginal structural models and inverse probability of treatment weighting estimators, I was able to take into account the temporal relationship between breastfeeding duration and other feeding habits, which could both influence each other and affect caries risk. Not only was breastfeeding for two years or more associated with a higher population average prevalence of severe-ECC than shorter breastfeeding durations, but this association also appeared to strengthen with more frequent breastfeeding throughout the day. This suggests that duration itself might not be the only dimension of breastfeeding relevant for determining caries risk: the frequency of breastfeeding might also play an important role.

Together, this dissertation aims to contribute a more nuanced understanding of how early-life feeding practices influence the development of dental caries in young children. The systematic review, as well as the exploration of breastfeeding and caries in Chapter 3, are both consistent with the hypothesis that frequent consumption of fermentable carbohydrates can cause tooth decay: a suggestion that has been widely accepted in the scientific literature for decades, yet remains an area of active study. Additionally, this work strives to define areas where the evidence base could be strengthened. For example, the relative lack of longitudinal studies and paucity of studies conducted in certain world regions both greatly impair our understanding of the relationship between feeding practice and caries. Interventions based on modifying diet alone have not been overwhelming effective at preventing tooth decay. The detailed analysis of the Porto Alegre intervention (Chapter 2) offers some suggestions toward strengthening future interventions. Finally, findings from both Chapter 1 and Chapter 3 imply that recommendations for the optimal duration to which breastfeeding should continue might benefit from considering potential caries risks alongside any general health benefits associated with breastfeeding to very long durations. It is entirely possible that, depending on the context, more of a very good thing is not always the best recommendation for maximizing a child’s overall health.

Dedication

To

The many people, who, when faced with a challenge related to data analysis, data management, statistical software, or just how to get from point A to point $E[\sum_{i=1, j=1}^{i=i, j=j} B_{ij}]$, turned to the Internet and posted their query

And, especially, to the many people who, upon hearing these cries from the dark, unselfishly shared their expertise and left a searchable thread that not only threw of lifeline to those lost initially, but prevented those of us who came later from drifting too far astray

And To

My parents, Spencer Chaffee and Juliette Wilk-Chaffee, my sister, Karla Chaffee,
And the talented, caring, and inimitable realist Dr. Joyce Y. Cheng
For
Unwavering confidence and patience

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Chapter 1. Early-life feeding practices and early childhood caries: a systematic review

Abstract

Early-life feeding practices are likely to play a role in the development of early childhood caries (ECC), and may represent modifiable risk factors for caries prevention. This systematic review aims to describe and critically evaluate the epidemiologic evidence on ECC and feeding practices published from 1990-2012, with particular attention to three putative risk factors: frequent consumption of foods and beverages, use of a nursing bottle at night or for sweet liquids, and breastfeeding duration. Electronic and manual searches were conducted in July 2012, together yielding 1783 non-duplicate citations, of which 235 publications from 189 independent study populations met inclusion criteria. More studies represented the United States, Brazil, China, and the European region than the African, Eastern Mediterranean, and South-East Asian regions. Relatively few studies (40/189, 21%) were longitudinal, and more than half the studies (119/189, 63%) failed to yield a publication that met all of three quality criteria: peer-review, accounting for confounding variables, and sample size ≥200. Studies were highly heterogeneous with regard to the age and caries experience of study participants and to methods of data collection and analysis. Despite this heterogeneity, a positive association between caries and more frequent consumption of fermentable carbohydrates (foods and beverages) consistently emerged across studies. Caries was not associated with bottle use practices or breastfeeding duration with universal consistency. However, breastfeeding duration and caries were consistently associated in studies that used thresholds exceeding 18 months of breastfeeding to define the longest duration category. Only five studies measured caries outcomes following randomized dietary-only interventions, yielding inconsistent results. Future studies that feature longitudinal designs, use validated dietary assessment instruments, present relevant non-statistically significant findings, and take place in previously underrepresented world regions would make important contributions.

Introduction

Early childhood caries (ECC) is the multi-factorial infectious disease process responsible for tooth decay in the primary dentition [1-3]. It is a source of pain, reduced quality of life [4,5], and heighted risk of decay in the permanent dentition [6,7]. The cost of restorative treatment is high [8,9], particularly in emergency departments and surgical facilities [10,11]. Among multiple contributing factors, early-life feeding practices represent an appealing intervention target for caries prevention. Such behaviors are potentially modifiable outside traditional dental care delivery systems, which remain inaccessible and/or underutilized for much of the world's population [12,13]. Furthermore, dental-healthy feeding practices may offer general health benefits, such as reduction in childhood obesity [14]. An improved understanding of the role of feeding practicing in ECC could aid in the construction of future evidence-based guidelines and recommendations, and might help identify behaviors to target in interventions.

A number of previous reviews have considered the potentially cariogenic role of early-life feeding practices, either specifically [15, 16] or as part of a broader review of caries risk factors [1, 17-22]. Few of these reviews have been systematic [16,17,19,21]. Of these systematic reviews, one focused on infants up to age 12 months [19], another primarily concerned socio-economic factors in relation to caries at various ages [21], and another specifically evaluated breastfeeding [16]. Harris and colleagues [17] listed numerous feeding practices among 106 reported ECC risk factors from 77 studies. The review aims to update and expand on those reviews by providing a systematic, comprehensive, and critical appraisal of over 20 years of published evidence related to ECC and infant and/or early-life feeding practices. Specifically, this systematic review has two principle objectives:

1. Describe the body of relevant epidemiologic evidence on ECC and feeding practices published from 1990-2012 with regard to study populations examined and research methodology used.
2. Compile and critically evaluate the evidence linking the occurrence of ECC to early-life feeding practices, with particular attention to three putative risk factors: frequent consumption of foods and beverages, use of a nursing bottle at night or for sweet liquids, and breastfeeding duration.

Methods

<u>Systematic Literature Search</u>

Electronic searches were conducted in July 2012 in the following bibliographic databases: BIOSIS, CINAHL, Cochrane Library, LILACS, MEDLINE, Web of Science, and WHOLIS. In MEDLINE, the search used Medical Subject Heading (MeSH) terminology; analogous searches were adapted individually for other databases (Supplemental Table 1-1). Search terms were in English, but without language restrictions. Searches were limited to publication dates starting from 1990.

Prior to reviewing citations, a set of inclusion and exclusion criteria was developed. Excluded from review were abstracts, dissertations, unpublished results, conference proceedings, commentaries, letters, review articles, position statements, and practice guidelines. Studies needed to include a clinical assessment of dental health status in a human pediatric population (primary dentition). Excluded were studies reliant on self-reported dental status or those using only measures of oral hygiene, oral bacterial infection, or dental service utilization. Studies were required to feature both caries-positive and caries-free individuals, rather than case-only reports. Feeding practices could relate to current or past diet or feeding habits of individual children, including measures of breastfeeding, bottle use, frequency of eating, consumption of specific foods or nutrients, food types (e.g. "sweets"), pacifier use, utensil sharing, or nocturnal feeding. Excluded were ecological measures of diet, markers of nutritional status (e.g. anthropometry, enamel defects), use of medications, biomarkers, or maternal diet. Intervention studies were eligible for inclusion if the intervention included components related to feeding or diet but not in combination with oral hygiene, fluoride administration, or dental treatment. To be included, a study was required to report an estimate of the association between at least one feeding or dietary practice and caries (e.g. an odds ratio or a difference in means). Studies that reported only the results of statistical tests (e.g. p-values) without expressing the magnitude of the association (or data permitting its calculation) were excluded. Only publications for which full-text copies could be obtained in English or Portuguese were considered.

Citations marked as potentially relevant based on titles and abstracts were reviewed as full-text copies. Additionally, a hand search was conducted by applying the same inclusion-exclusion criteria to the citation lists of reviewed full-text publications, in an attempt to capture relevant articles not captured by the electronic search. Due to a possible lag between publication and keyword indexing, an additional electronic search was conducted in MEDLINE without MeSH terminology to capture recent publications. The relevant results and characteristics of each study were abstracted to standardized forms (Supplemental Figure 1-2). In counting independent study populations, multiple publications were considered drawn from a single study if results were derived from the same or overlapping participants (e.g. the same wave of a national survey).

Data Synthesis

Independent studies and individual publications were organized by the following attributes to describe the relevant literature: year of publication, country, study design, sample size, average age and caries experience of participants, data collection methods, and analytic methods. Country refers to where participants were recruited, not necessarily the home country of the researchers. For this review, a cross sectional design refers to any study in which data on feeding practices and caries status were collected simultaneously, regardless of whether investigators measured current feeding practices or recalled past events, or if investigators applied different sampling fractions to caries-positive and caries-free controls during or after cross sectional recruitment and data collection. When possible, sample size refers to the analytic sample: those individuals who contributed data to results, not necessarily all participants initially recruited into the study. Analytic method refers to the technique(s) used to estimate a measure of association between feeding practices and caries and/or to account for putative confounding variables.

Included publications were separated according to whether they met three criteria, serving as a marker of publication quality: peer-reviewed, accounted for putative confounding variables, and analytic sample ≥200. A study was considered peer-reviewed if the publishing journal was designated as "refereed" at Ulrichsweb directory (http://ulrichsweb.serialssolutions.com/) or if the peer-review process was described at the journal's website. A study was deemed to have accounted for confounding variables if it included multi-variable statistical adjustment or stratification for socio-demographic variables or non-feeding related behaviors, or was a randomized controlled trial.

A quantitative synthesis of the evidence (e.g. meta-analysis) was not considered appropriate given extensive heterogeneity in study design, study populations, measurement techniques, analytic methods, and the presentation of findings. Rather, study results were organized according to the three types of feeding practice exposures most commonly investigated in the literature: feeding frequency (the number of times daily or weekly that foods or beverages were provided, not necessarily the amount or present/absence of particular items); the use of a feeding bottle (separate sub-categories for use at night and/or naptime and for the provision of sweet liquids); and the duration of breastfeeding (age to which any form of breastfeeding continued). A positive association was considered to be an increase in caries prevalence of ≥5% or ≥0.5 unit increase in mean decayed missing filled surface index (dmfs) with exposure to a putative risk factor, or analogous association measures of similar magnitude, whereas inverse associations represented a decrease in caries prevalence of ≥5% or ≥0.5 unit decrease in mean dmfs. Associations were considered to be positive or inverse, before consideration of statistical significance, which was also noted. Results were classified in this manner to provide evidence as to the overall direction of a given exposure-outcome relationship across the literature, allowing non-statistically significant to make a contribution, as would be the case in a meta-analysis.

Results

Systematic Literature Search

Figure 1-1 depicts the search process, as recommended by the Prisma Statement [23]. The electronic literature search yielded 2505 hits, of which 1749 represented non-duplicate citations. Of these, 400 citations were deemed potentially relevant and designated for full-text review. An additional 34 citations were identified through hand searching, including the non-MeSH electronic search. Three publications [24-26] were excluded after failure to obtain full-text copies, including attempts to contact the authors. Twenty-six publications were excluded due to language: Arabic [27], Chinese [28,29], Danish [30], Farsi [31], French [32-34], German [35], Italian [36], Japanese [37-42], Polish [43], Romanian [44], Russian [45], Spanish [46-51], and Turkish [52]. Altogether, 235 publications met inclusion criteria [53-287], of which hand searching identified nine [119,147,157,186,199,201,204,207,215]. Publications were drawn from 189 independent studies.

Characteristics

There were more publications in the five years from 2008-2012 (75/235, 32%) than in the 10 years from 1990-1999 (69/235, 29%). The 189 independent studies represented 47 countries, across all six World Health Organization (WHO) regions (Figure 1-2). The United States (n=31 studies), Brazil (n=26), and China (n=17) were the countries most often represented, while Africa (n=5), Eastern Mediterranean (n=9), and South-East Asia (n=10) were the WHO regions featured in the fewest studies.

About one-fifth (40/189, 21%) of the independent studies were longitudinal in design, including six intervention studies [91-93,131,132,139-141,170,230,267]. Analytic samples ranged in size from 30 [196] to >13,000 [140]; the median was 324. The age at which dental status was evaluated varied across studies, from 11-18 months [262] to a mean of 79.2 months [140] (Figure 1-3). Similarly, caries experience was heterogeneous by study. Of the studies reporting the caries prevalence (excluding non-cavitated lesions), the affected fraction ranged from 1% [272] to 92% [75] (Figure 1-4).

Most studies (135/189, 71%) reported following some form of standard or previously published protocol for dental evaluations. More than half of these (80/135, 59%) cited WHO field guides [e.g. 288]. Publications typically expressed caries experience as the prevalence of at least one affected tooth (cavitated or non-cavitated decay) or as the number of affected teeth or surfaces, with terms such as "baby bottle tooth decay," "nursing caries," or "rampant caries" rarely used to define outcomes after 2003 [85,95].

Collection of dietary information through the use of 24-hour recalls, food diaries, or food frequency questionnaires was relatively uncommon, as only 11% (21/189) of study populations were evaluated using these methods [54,87,92,101,102,114,124,132,133,138,143,150,156-163,166,171,180,185-188,195,197,198,225,248, 266,281]. The remainder of the studies used questionnaires for a limited number of specific food items and/or behaviors.

Roughly half of the study populations (95/189, 50%) provided at least one result for which confounding variables had been accounted (e.g. by randomization or multi-variable adjustment), corresponding to 57% (134/235) of the included publications. The largest number of measured variables any publication took into account was 17 [237], and the measurement, operationalization, and combination of variables used for adjustment differed greatly by publication. The most commonly used technique was multi-variable logistic regression, with some variant of this method featured in 97 publications. Forty-six publications used some type of step-wise model building algorithm.

Fewer than half the publications (97/235, 41%), representing 70 study populations, met all three minimum quality criteria: peer-review, accounting for confounding variables, and sample size ≥200. Summarized results drawn from these studies with regard to the association between dental status and feeding frequency (n=46), bottle use at night or for sweet liquids (n=28), and breastfeeding duration (n=27 studies) are compiled in Tables 1-3, respectively. Results drawn from all studies in relation to these putative risk factors, regardless of quality criteria, are depicted graphically in Figure 5.

Feeding Frequency

Study results consistently linked higher frequencies of food and beverage consumption and caries, as every study meeting the minimum quality criteria that considered feeding frequency reported a positive association for at least one frequency variable, although the associations were not always statistically significant (Table 1, Figure 5). This consistency in results was observed despite a litany of foods, beverages, and intervals used to define frequent consumption. For example, some studies required ≥6 [118,129] or ≥8 [205] between meal sweet snacks daily to reach the upper category of frequent consumption, whereas in others, thresholds as low as two times [132] or three times [117] weekly qualified children as high frequency sweets consumers.

Some notable exceptions suggested an inverse association between caries and frequent consumption of fruits and/or vegetables [86,281], staple foods [287], and dairy products, such as cheese or yogurt [133,149,188,203,251]. These potentially "protective" foods were assessed much less commonly than presumably cariogenic items, such as soft drinks, candies, and other sweet snacks.

Bottle Use

The practices of bottle use at night or during naps and bottle use for sweet liquids were inconsistently related to caries in the published literature (Table 2, Figure 5). Of studies meeting the three quality criteria, a near equal number reported a positive, statistically significant caries-night bottle association after adjustment for putative confounders [108,115,177,205,240,247,279] as reported no association or an inverse relationship [82,92,126,164,172,186,237,253,286]. Among these studies, results remained inconsistent even after considering how nocturnal bottle use was defined. Both positive and null or inverse associations were reported whether nocturnal bottle use was assessed in reference to current use [164,172,177,186,237], past use at a certain age (e.g. 12 months) [92,205,240,253,286], a history of ever use [108,115,126,279], or a combined variable of nocturnal use of the bottle for sweet liquids [82,247].

Interestingly, seven [92,155,164,172,186,229,253] of the eight [exception: 176] studies to show an inverse relationship between caries and either nighttime bottle use or sweet bottle contents was conducted in Brazil. Not all Brazilian studies found this inverse association, and one of those showing an inverse relationship with nocturnal bottle use also showed a statistically significant positive association with sweet bottle contents in a multi-variable model that included both variables [92].

Breastfeeding Duration

Study results were heterogeneous with regard to the association between breastfeeding duration and dental caries. Of the 26 independent studies meeting quality criteria, eight reported an association between greater caries experience and longer breastfeeding duration that retained statistical significance after adjustment for confounding variables [55,95,99,198,223,264,280,286] (Table 3, Figure 5). The failure of other studies to report a positive association could be related to the cut points used to categorize breastfeeding duration. Including studies that did and did not meet quality criteria, five reported an inverse association between caries and breastfeeding duration [94,169,193,217,238], and three of these [94,169,193] used thresholds ranging from ≥1 to ≥3 months to define the uppermost category of long-duration breastfeeding. Nine other studies estimated the caries experience associated with breastfeeding durations ranging from 0-2 months to 0-5 months [108,147,179,190,201,252,266,273,278], and but one [266] reported an increase in caries experience at the shortest breastfeeding durations. However, these increases were generally small in magnitude.

On the other hand, a much larger proportion studies that used thresholds exceeding 18 months of breastfeeding to define the longest duration category either found a positive association between breastfeeding duration and caries, that either remained statistically significant after adjustment for confounding variables [55,107,125,134,223,252], found a statistically significant association in an unadjusted analysis without the reporting of adjusted results [147,179,228,247,260,278,282], or found a positive association that was not statistically significant in adjusted analysis [82,166,253]. In the two studies that examined breastfeeding durations ≥18 months and did not find a positive association with caries, long duration of breastfeeding was recorded in just 36 individuals [258], or only 43 individuals had experienced carious lesions [272]. However, no study conducted in Canada, Europe, or the United States, settings where breastfeeding durations exceeding 12-24 months are uncommon, reported a positive caries-breastfeeding association that remained statistically significant in adjusted models (Figure 5).

Intervention Studies

Eleven publications [91-93,131,132,139-141,170,230,267] were drawn from six studies that featured dietary-only interventions. A randomized controlled trial in Brazil providing dietary counseling to new mothers reported a statistically significant reduction in caries occurrence at one and four years of age with the intervention [91,93,267]. However, in other trials, caries occurrence was not reduced following dietary counseling of low fat, heart healthy diets for infants in Finland [131], three years of diet-focused dental education sessions in the United Kingdom [139], or peer-led social support for healthy infant feeding in the United Kingdom [230], although the number of participants with data concerning dental caries in these trials ranged from only 85 [230] to 148 [131]. Authors reported caries reduction following a non-randomized community based intervention in the United States [170] after accounting for a rise in caries prevalence in the control community. Meanwhile, a hospital-based breastfeeding promotion program tested in a large cluster-randomized trial in Belarus did not affect dental status at age six years [141].

Other Risk Factors

A host of other early-life feeding practices were evaluated in relation to early childhood caries in the studies meeting the three minimum quality criteria, albeit a smaller number of studies assessed each practice, including factors as diverse as eating breakfast at home [231], sweet taste preference [259], percent total energy from non-milk extrinsic sugars [101], and child appetite [174]. Some studies evaluated multiple foods or beverages individually [96,161,228], while others combined dietary exposures into constructs [112,142,150,166,249].

Among variables considered in multiple studies, the use of a sweetened pacifier was associated with an increased risk of caries in several studies [58,81,108,115,165,203,247]. In some such studies, the association remained statistically significant after multi-variable adjustment [58,115], but not in all studies [81,203]. Some studies found a link between pacifier use (not specified as sweetened) and caries [215,228,264], while others did not [108,122,287]. Three studies implicated shared utensils or other measures of possible vertical transfer of oral bacterial caries risks [96,215,268], but two studies did not [81,287]. The provision of food or beverages at nighttime or before bed had a positive, statistically significant association with caries after adjustment for other variables in several studies [81,99,135,147,174,199,244,274], a positive association that was not statistically significant in adjusted models in others [85,106,167,237], a crude association without being included in adjusted models in one study [164], no association in one study [112], and an inverse association in one other [286].

Discussion

The most challenging aspect of synthesizing evidence across this body of literature is the tremendous heterogeneity of the studies that have been conducted and of the results reported. Differences in exposure and outcome definitions, baseline disease prevalence, and approaches to statistical adjustment could result in meaningful differences in the magnitude of the reported association measures, even without striking differences in the underlying biology that links risk factors and disease. A single quantitative summary, such as could be provided in a meta-analysis, overlooks this heterogeneity and was deemed inappropriate in this review.

On the other hand, despite considerable divergence in all aspects of study design, a positive association between caries experience and the frequent consumption of foods and beverages consistently emerged, adding compelling evidence that frequent consumption of fermentable carbohydrates contributes to dental decay. Decades of microbiological research [289] and experimental evidence in humans [290] and animals [291] lend additional support to this hypothesis. A previous review of caries at all ages reported that the frequency of sugar consumption was more often associated with caries than was the quantity of sugar consumed [292]. In a systematic review of studies published from 1966 to 2002, Harris and colleagues [17] found ten different measures of feeding frequency in 21 different publications to be associated with caries prevalence or incidence in children up to age six years. In this review, the relationship between feeding frequency and caries held for a wide variety of solid foods and liquids, with some notable, albeit less often studied, exceptions, such as dairy products [133,149,188,203,251] and vegetables [281].

Bottle use practices and breastfeeding duration were not associated with caries with the same consistency. Presumably, any such associations were not so universal as to persist across the multitude of methodological approaches taken in the publications reviewed. However, we should not necessarily expect uniform relationships between caries and these exposures globally. In fact, differences by context might prove informative. In the case of bottle use, a different pattern emerged among studies conducted in Brazil than in other parts of the world. It is unlikely that nighttime bottle use or the provision of sweet liquids in a bottle trigger different biological mechanisms among Brazilian children. If not a chance finding, a plausible alternative explanation is that in this setting, bottle use of this nature occurs in place of other potentially cariogenic practices.

In the case of breastfeeding duration, much of the inconsistency of findings could be attributed to the use of the widely varying cut-points used to define the upper-most category of breastfeeding duration, ranging from 40 days [193] to three years [166]. Even when cut-points do not differ, however, context could play a role. In populations where late weaning ages are normative, a single cut point of 12 months will create a long-duration breastfeeding category that includes many children breastfed to 18, 24, or ≥30 months. In populations where late weaning is uncommon, relatively few children breastfed for 12 months will continue to breastfeed to an age of 24 months. Hence, the average duration of breastfeeding among children breastfed ≥12 months may differ by setting, perhaps partly explaining the divergent findings reported from different world regions.

The possibility that some feeding practice-caries relationships are context dependent deserves further consideration. Yet, despite there being studies from all six WHO regions, the reviewed studies were predominantly from the United States, Brazil, and Europe. One contributing factor to the predominance of these regions was this review's restriction to studies published in English or Portuguese. However, even had all 26 publications excluded for language reasons contributed to this review, there would still be a relative lack of information from Africa, the Eastern Mediterranean, and South-East Asia. If we hope to develop a more nuanced understanding of caries risk factors within a global perspective, more studies of high quality will be required from these relatively under-represented world regions. The recognition that many countries in these regions shoulder a disproportionate burden of caries [293] adds urgency to this issue.

Several methodological limitations were common to multiple studies included in this review. Most studies collected feeding and dental information simultaneously, which presents the challenge of relating feeding practices that vary over time with an outcome that is strongly linked to age. Measures of "current" practices in relation to bottle use or breastfeeding, for example, conceivably represent different experiences within study populations that include children a varying ages. Reliance on caregiver recall concerning past feeding practices might introduce substantial measurement error [294]. The use of validated dietary assessment instruments in the included studies was uncommon. Significant variation persists in measures of caries experience, making it challenging to compare results across studies. Previous efforts have called for consensus definition of early childhood caries [295]. The apparent disappearance from the recent literature of etiology-based case definitions, such as "baby bottle tooth decay," represents progress toward this consensus.

Previous systematic reviews of risk factors for ECC have commented on the relative paucity of studies of high methodological quality [16,17]. In the current review, the majority of publications failed to achieve three basic criteria related to quality: peer-review, adequate sample size, and accounting for confounding. These are imperfect proxies for quality. For example, the sample size criterion placed greater focus on studies of greater statistical power and precision, although the cut-point of n=200 was chosen subjectively. Regardless, the evidence base would be strengthened if future investigations featured longitudinal data collection, used validated dietary assessment instruments, and used standard ECC case definitions.

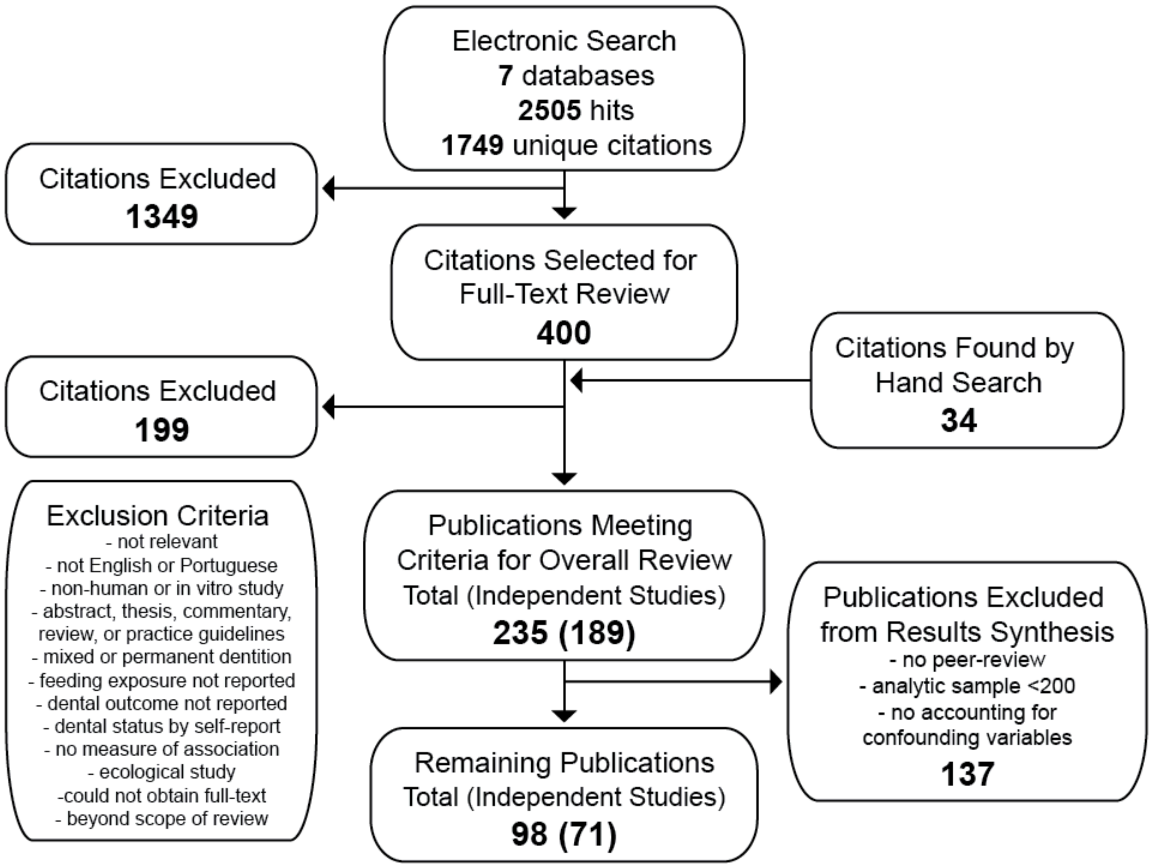
Unknown is the extent to which publication bias, often expressed as a greater tendency for statistically significant findings to reach the published literature, and hence, contribute to reviews [296], affected the findings of this review. A tendency to exclude estimated measures of association from final reports if such measures did not reach statistical significance is another form of publication bias. Step-wise model building algorithms implicitly condone this practice by designating the P-value as the arbitrator of which variables are included in adjusted models, rather than the importance of that variable with regard to the research question. Thus, merely counting up the number of studies reporting a positive result, statistically significant or not, is potentially misleading, as other, perhaps meaningful, results may have been invisible to the review process due to their removal from multi-variable models. Furthermore, this "vote counting" method does not take into account the quality of the individual studies or the magnitude of the association reported [297]. The graphical summary provided in this review attempts to sort studies findings by a proxy of quality and distinguish between the magnitude and statistical significance of associations, albeit less quantitatively than had a meta-analysis been conducted.

Together, the epidemiologic evidence supports an important role for early-life feeding practices within the multi-factorial etiology of ECC, particularly the frequent consumption of fermentable carbohydrates. Dietary interventions to reduce potentially harmful practices could be implemented even in populations of low access and utilization of traditional dental services. Yet, identification of risk factors alone will not yield substantial reductions in disease occurrence without translation of knowledge into successful prevention. Just as sweet foods have been implicated in the caries process for centuries, the necessity of parental action to reduce consumption of fermentable carbohydrates by their children has been long recognized [298]. Feeding practices are interlaced with other caries determinants, including psychosocial and behavioral factors [299], socio-economic position [21,300], and parental influence [301]. Not surprisingly then, of the few randomized trials of diet-only interventions for ECC prevention [93,132,139,230], most failed to find a significant reduction in caries occurrence [132,139,230]. Generally, multi-component interventions that begin earlier and are interactive have been most successful at preventing ECC [302].

In conclusion, despite tremendous heterogeneity in study characteristics, the epidemiologic evidence consistently implicates frequent consumption of fermentable carbohydrates in the occurrence of early childhood caries. Certain baby bottle practices and long-duration breastfeeding have also been associated with ECC, but the nature of these relationships may vary by context. Future studies that use rigorous methods and take place in previously underrepresented regions of the world would make important contributions to our understanding. Translation of this knowledge into successful prevention will require multi-faceted interventions to address the complex etiology of the caries process.

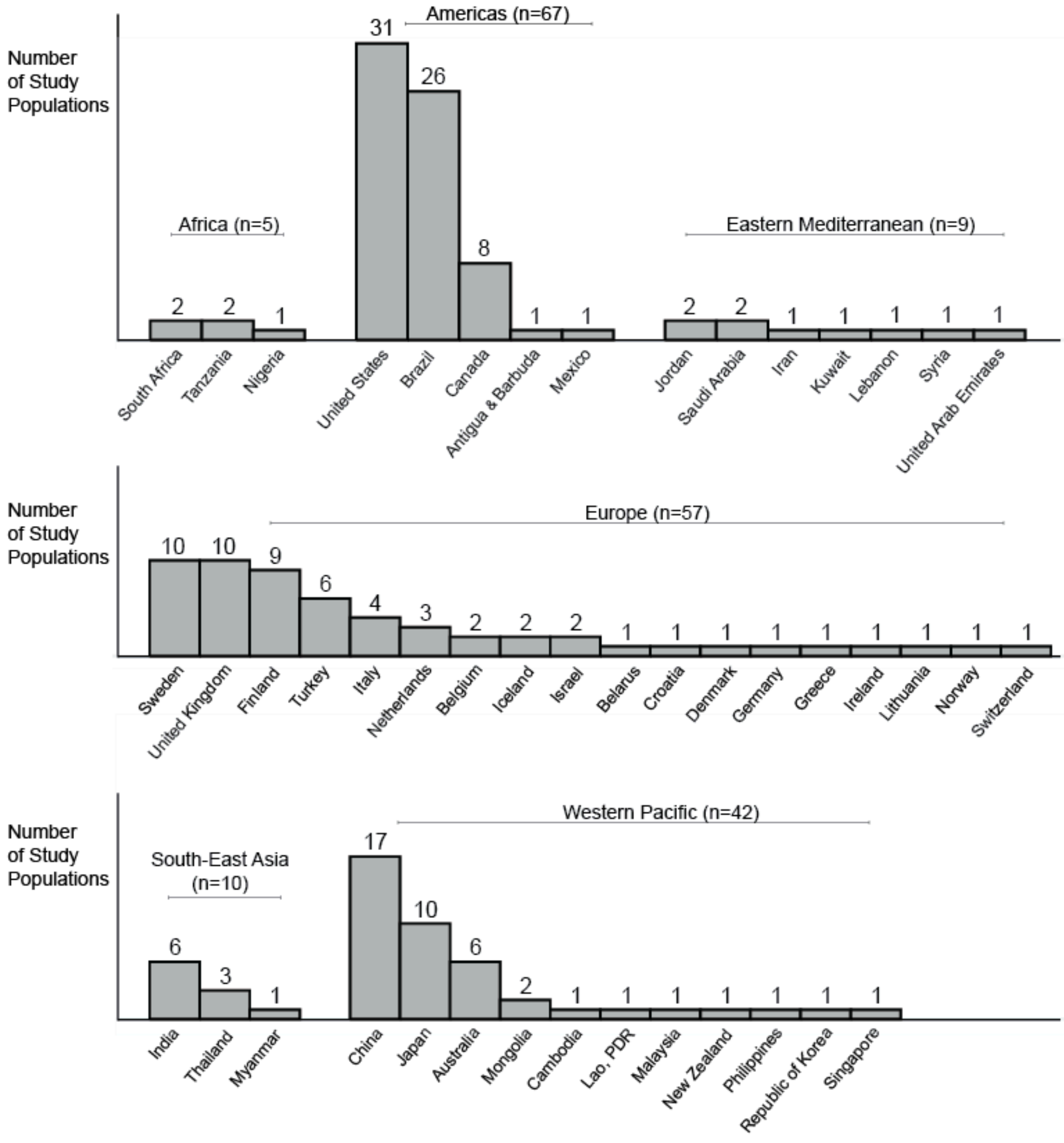
Chapter 1 Figures and Tables

Figure 1-1. Flow Diagram of Systematic Literature Search



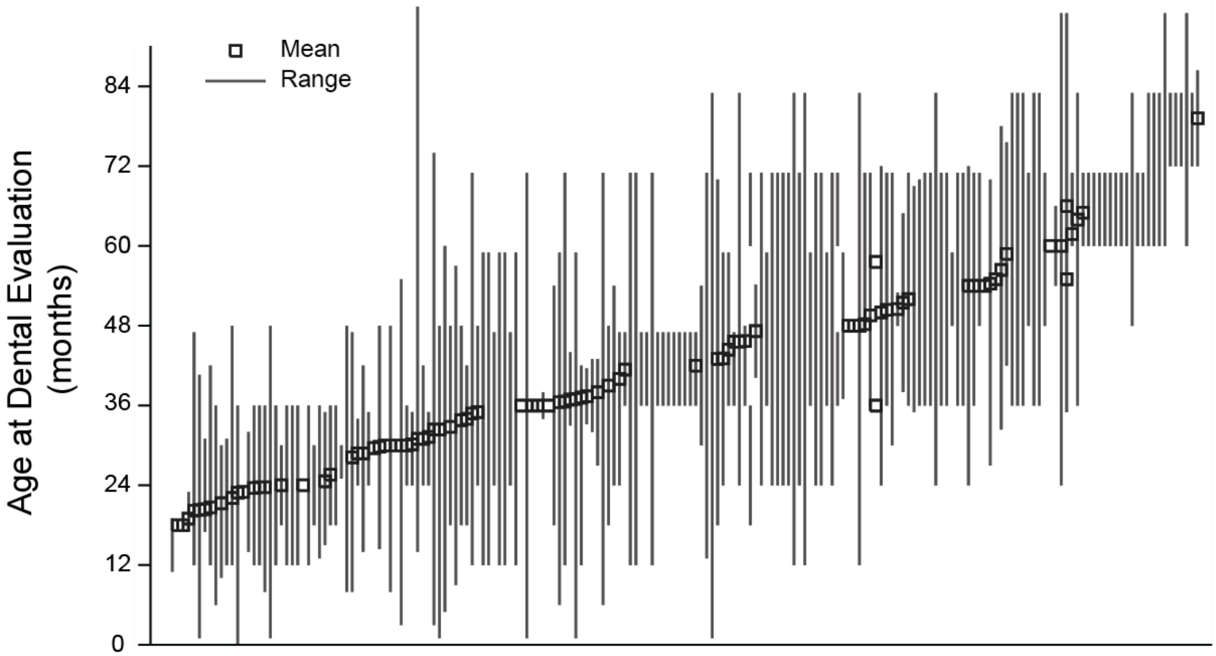
Legend: The systematic literature search is represented diagrammatically, as recommended by the Prisma Statement.

Figure 1-2. Countries and World Health Organizations Represented by Studies of Early-Life Feeding Practices and Early Childhood Caries, 1990-2012



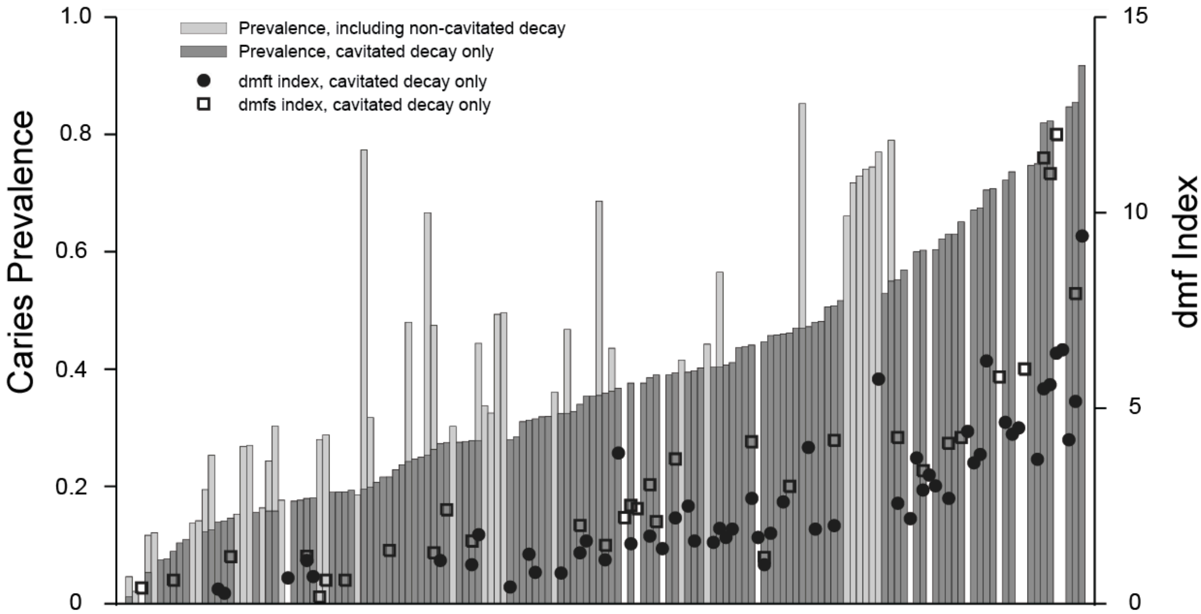
Notes: The number of studies includes independent study populations; multiple publications drawn from the same source population (e.g. a single wave of a national survey) were counted as a single study.

Figure 1-3. Age at which the Dental Status of Participants was Assessed in Studies of Early-Life Feeding Practices and Early Childhood Caries, 1990-2012



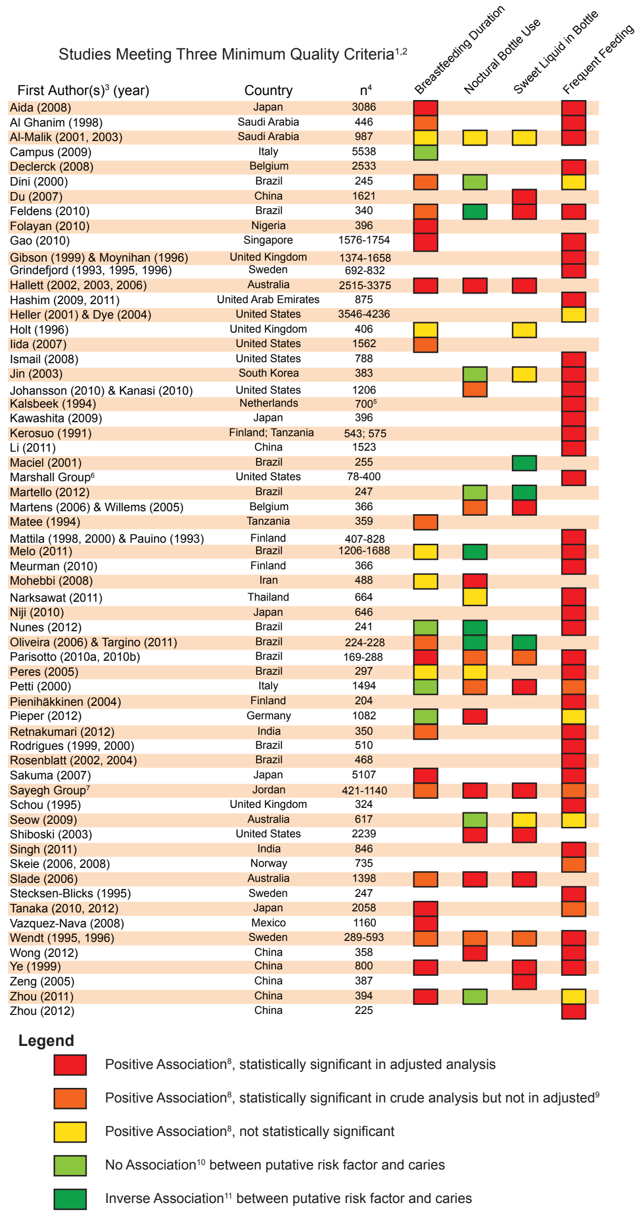
Notes: The mean age and range are shown from independent study populations that reported these data. Multiple publications drawn from the same source population (e.g. a single wave of a national survey) were counted as a single study. For longitudinal studies, age refers to age at the end of follow-up. For studies reporting age ranges in years, age in months was estimated (e.g. “3-4 years” was assumed to include ages 36-59 months). Some studies reported only the mean age or age range, not both.

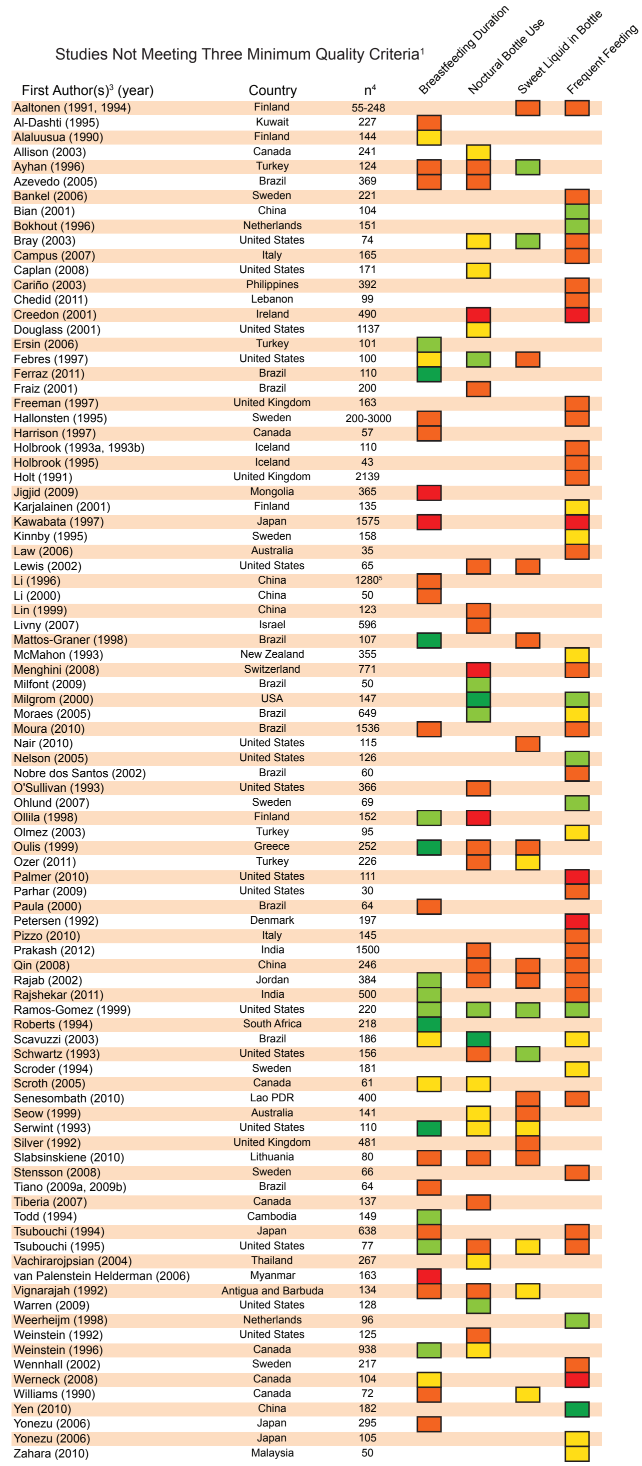
Figure 1-4. Caries Experience of Participants in Studies of Early-Life Feeding Practices and Early Childhood Caries, 1990-2012



Abbreviations: dmft = decayed missing filled teeth; dmfts = decayed missing filled surfaces.
Notes: The average caries experience, as measured by prevalence and affected teeth or surfaces from independent study populations that reported these data. Multiple publications drawn from the same source population (e.g. a single wave of a national survey) were counted as a single study. For longitudinal studies, caries experience refers to mean experience at the end of follow-up. For intervention studies, caries experience of the control (untreated) group is shown. Studies that differentially sampled caries affected cases and controls are excluded. Not all studies reported all four measures shown in the figure.

Figure 1-5. Characteristics and Graphical Depiction of Study Results in Relation to Selected Early-Life Feeding Practices and Early Childhood Caries, 1990-2012





Notes

1. At least 200 participants in analytic sample, adjustment for confounding variables, and confirmed peer-review
2. Publications that did and did not meet quality criteria were sometimes drawn from the same study population. In such instances, the results from such publications are shown together with the other publications from that study, rather than separate non-independent results. Therefore, results in this figure might differ slightly from those shown in Tables 1-3.
3. Multiple first authors listed if multiple non-independent publications met inclusion criteria
4. Range provided if multiple non-independent publications featured analytic samples of different sizes
5. Estimated (size of analytic sample not provided)
6. Includes: Chanakanka (2011), Levy (2003), Mariri (2003), & Marshall (2003, 2005, 2007a, 2007b)
7. Includes: Hillow (2004) & Sayegh (2002a, 2002b, 2005)
8. Positive association considered to be an increase in caries prevalence of ≥5% or ≥0.5 unit increase in mean decayed missing filled surface index with exposure to putative risk factor, or analogous associations of similar magnitude for other measures
9. Adjusted measure of association positve and not statistically significant or not provided
10. No association considered to be a difference in caries prevalence of <5% or <0.5 unit difference in mean decayed missing filled surface index with exposure to putative risk factor, or anlogous associations of similar magnitude for other measures
11. Inverse association considered to be a decrease in caries prevalence of ≥5% or >0.5 unit decrease in mean decayed missing filled surface index with exposure to putative risk factor, or anlogous associations of similar magnitude for other measures, regardless of statistical significance

Table 1-1. Characteristics and findings related to the frequency of food or beverage consumption from studies meeting three minimum quality criteria.

First author (year)[1]	Country	Design	n	Mean age (range), yr	Main Findings
Aida (2008)	Japan	Cross Sectional	3086	3.5 (3, 3)	Crude: higher dmft with daily sweet foods and sweet drinks Adjusted: 0.6 unit mean increase (ss) if daily sweet drinks vs. <4 weekly
Al Ghanim (1998)	Saudi Arabia	Cross Sectional	446	4.2 (3, 5)	Crude: more children frequently consumed sweets and soft drinks in high dmft group Adjusted: greater odds frequent soft drinks (OR 1.9; ss) and frequent sweets (OR 1.7; ss) in high dmft group
Al-Malik (2001, 2003)	Saudi Arabia	Cross Sectional	987	* (2, 5)	Crude: higher prevalence caries with more frequent fizzy drinks, more frequent fruit syrup in drinks, and more frequent consumption of dates Adjusted: greater odds of caries with fruit syrup ≥2 times daily (OR 2.0; ss); dates not included in m-v models; fizzy drinks not in m-v model for caries overall, only rampant caries
Declerck (2008)	Belgium	Cross Sectional	2533	3.3 (3, 3); 5.3 (5, 5)	Crude: positive caries association at ages 3 yr and 5 yr with more frequent between meal sugared drinks, but not between meal eating Adjusted: more than daily sugared drinks at 5 yr (OR 3.2, ss) and 3 yr (OR 2.0, not ss) associated with caries
Dini (2000)	Brazil	Cross Sectional	245	* (3, 4)	Crude: highest caries prevalence and dmft with sugared juices twice or more daily Adjusted: frequent juices not included in m-v model
Feldens (2010)	Brazil	Longitudinal	340	4.2 (4, 4)	Crude: greatest S-ECC prevalence with greatest number of daily meals and snacks Adjusted: greater odds S-ECC with daily meals and snacks ≥9 vs. <7 (OR 1.4, ss)
Gao (2010)	Singapore	Both Cross Sectional and Longitudinal	1576-1754	5.8[2] (4, 6)	Crude: not shown Adjusted: more frequent between meal sweets associated with greater odds of caries at baseline and 12-mo caries increment (both ss)
Gibson (1999)	United Kingdom	Cross Sectional	1374-1658	* (1, 4)	Crude: not shown Adjusted: average daily frequency of sugar confection, but not frequencies of 5 other sweet foods or drinks, maintained in m-v model and associated with great odds of caries (OR 1.3, ss)
Grindefjord (1995, 1996)	Sweden	Longitudinal	692-786	3.5 (*)	Crude: caries cases at age 3.5 yr more likely to have been frequent candy consumers and have more daily meals at age 2.5 yr and to have been more frequent candy consumers and have more frequent sugar-containing beverages at age 1 yr Adjusted: all of these variables maintain positive association (all ss)
Hashim (2009, 2011)	United Arab Emirates	Cross Sectional	875	* (5, 6)	Crude: higher prevalence caries if more frequent between meal snacks, more frequent drinks, and total combined number of snacks per day; no difference in caries prevalence with more total daily eating episodes Adjusted: of these variables, total combined snacks per day maintains a positive association (OR 1.8, ss) comparing high score threshold to low
Heller (2001) & Dye (2004)	United States	Cross Sectional	3546-4236	* (2, 5)	Crude: lower prevalence caries with ≥5 daily servings of fruits and vegetables Adjusted: caries and servings of fruits and vegetables only associated among children from households >200% of federal poverty line; mean dmft rises with increasing daily soda servings but not monotonic (not ss)
Hillow (2004) & Sayegh (2002, 2005)	Jordan	Cross Sectional	421-1140	* (4, 5)	Crude: greater proportion of children with dmft ≥8 had sweet snacks ≥5 times daily and had sweet drinks ≥5 times daily Adjusted: frequency variables not included in m-v model

Ismail (2008)	United States	Longitudinal	788	5.0^2 (2, 7)	Crude: not shown Adjusted: as increments of weekly soda consumption 2 yr earlier increase, greater odds of S-ECC (OR 1.3, ss) and ECC (OR 1.3, not ss)
Jin (2003)	South Korea	Cross Sectional	383	3.0 (<1, 4)	Crude: higher prevalence caries and S-ECC with ≥2 daily snacks Adjusted: greater odds caries (OR 2.4, ss) and S-ECC (OR 1.7, not ss) with more frequent snacks
Johansson (2010)	United States	Cross Sectional	1206	* (1, 4)	Crude: increasing prevalence caries in all age strata with increasing daily sweet snack frequency Adjusted: in partial least squares modeling, high variable importance projection placed on frequent sweet snacks
Kalsbeek (1994)	Nether-lands	Cross Sectional	700^2	* (5, 5)	Crude: not shown Adjusted: dmfs increases as daily sweet snack frequency rises from <1 to 1-5 to ≥6 times (ss)
Kawashita (2009)	Japan	Cross Sectional	396	* (3, 3)	Crude: higher prevalence caries with more frequent between meal eating and more frequent daily sports drinks Adjusted: higher odds caries at highest vs. lowest categories of frequent eating (OR 3.4, ss) and frequent sports drinks (OR 5.0, ss)
Kerosuo (1991)	Finland and Tanzania	Cross Sectional	543; 575	* (3, 7)	Crude: not shown Adjusted: higher odds of caries with more frequent sugar consumption in Finland (OR 1.3, not ss) and Tanzania (OR 1.7, ss)
Levy (2003) & Marshall (2003, 2005, 2007a,b)	United States	Longitudinal	246-400	5.2 (4, 7)	Adjusted: quantitative estimates differ by study, but group of studies together implicate greater probability of caries with more frequent consumption of snacks, soda, and juice from powder, but not milk, 100% juice, and number of daily meals
Li (2011)	China	Cross Sectional	1523	* (3, 5)	Crude: caries prevalence and dmft/s increase with increasing frequency of candy and carbonated drinks but decrease with frequency milk and dairy Adjusted: carbonated drinks and milk maintain associations (both ss); candy not included in m-v model
Mattila (1998, 2000) & Pauino (1993)	Finland	Both Cross Sectional and Longitudinal	407-828	3.0; 5.0^2	Crude: greater prevalence caries at age 3 yr if more frequent sweets at 3 yr Adjusted: sweets frequency at 3 yr associated with caries at 3 yr (OR 1.9, not ss); daily sugar at age 1.5 yr associated with caries at age 5 yr (OR 2.4, ss); increase in sweets frequency from 3 yr to 5 yr associated with increase in dmft (OR 2.3, ss)
Melo (2011)	Brazil	Cross Sectional	1688	* (1, 3)	Crude: at ages 18-36 mo and 5 yr, daily between meal sweets are more common among caries cases Adjusted: caries and daily sweet snacks associated at age 18-36 mo (OR 2.8, ss) and 5 yr (OR 3.9, ss)
Meurman (2010)	Finland	Longitudinal	366	* (4, 5)	Crude: greater probability of caries increment over 42 mo if daily sweet snacks and if drinks other than water weekly or daily at 18 mo Adjusted: greater odds caries increment with non-water drinks (OR 2.0, ss); sweet snacks not included in m-v model
Narksawat (2011)	Thailand	Cross Sectional	664	3.7 (3, 4)	Crude: higher mean dmft and odds of caries with ≥3 snacks per day Adjusted: greater odds of caries if snacking more frequent (OR 1.8, ss)
Niji (2010)	Japan	Cross Sectional	646	* (3, 3)	Crude: increasing prevalence caries with increasing daily snack frequency Adjusted: greater odds of caries if ≥4 daily snacks vs. 0-1 (OR 2.5, ss)
		Cross			Crude: increasing prevalence caries with increasing daily frequency between meal sucrose

Parisotto (2010)	Brazil	Cross Sectional	288	* (3, 4)	Crude: larger proportion of caries cases had frequent solid sugars and liquid sugars Adjusted: greater odds caries with frequent solid sugars ≥3 daily episodes vs. <3 (OR 4.5, ss)
Peres (2005)	Brazil	Both Cross Sectional and Longitudinal	297	* (6, 6)	Crude: higher prevalence caries and proportion of children dmft ≥4 with at least daily current sweet consumption Adjusted: greater odds dmft ≥4 with frequent sweet consumption (OR 2.4, ss)
Petti (2000)	Italy	Cross Sectional	1494	* (3, 5)	Crude: among caries cases, greater proportion had daily sweets at least twice Adjusted: sweet frequency not included in m-v model
Pienihäkkinen (2004)	Finland	Longitudinal	204	* (5, 5)	Crude: greater probability of positive caries increment by 5 yr with more frequent weekly candy consumption at 2 yr Adjusted: candies at least once weekly associated with caries increment (OR 3.6, ss)
Pieper (2012)	Germany	Cross Sectional	1082	* (5, 7)	Crude: more frequent daily sugar-sweetened food (≥ 3 times), sugar drinks (≥3 times) and between meal sweet snacks (≥ 2 times) associated with greater mean dmft and prevalence caries Adjusted: no frequency variables included in m-v model
Retnakumari (2012)	India	Cross Sectional	350		Crude: not shown Adjusted: greater odds of caries if ≥10 daily food intakes (OR 1.7, ss)
Rodrigues (1999, 2000)	Brazil	Longitudinal	510	4.5est	Crude: probability of "high" 1-yr caries increment greater with more frequent sugar intake at home, at the nursery, and combined Adjusted: greater odds caries increment if daily sugar intakes ≥5 vs. 1-2.9 (OR 4.3, ss)
Rosenblatt (2002)	Brazil	Cross Sectional	468	* (1, 3)	Crude: grater prevalence caries if ≥6 daily sugary meals Adjusted: greater odds of caries with more frequent meals, but magnitude not shown (ss)
Sakuma (2007)	Japan	Longitudinal	5107	* (3, 3)	Crude: not shown Adjusted: greater odds of caries increment by 3 yr with more frequent daily sweets and daily sugar-containing beverages at 1.5 yr (OR ranges 1.2-1.6 by site, ss in all municipalities)
Schou (1995)	United Kingdom	Cross Sectional	324	* (5, 5)	Crude: prevalence caries increases as daily sweet intake rises from less than daily to once to ≥2 Adjusted: stratified by parental occupation, highest caries prevalence with most frequent sweet intake in all three strata
Seow (2009)	Australia	Cross Sectional	617	3.1 (<1, 4)	Crude: total eating frequency roughly similar among ECC children recruited from various sites and controls; sugar in fluids more common among ECC children at one site Adjusted: sweet drinks more strongly associated at one site (OR 4.0, ss) than other (OR 1.2, not ss); total eating frequency not included in m-v model
Singh (2011)	India	Cross Sectional	846	* (5, 5)	Crude: not shown Adjusted: increasing odds of caries with each increase in episodes of between meal sugar from 1 to 2 to 3 to ≥4 times daily (OR 1.2, ss)
Skeie (2006)	Norway	Cross Sectional	735	3.0 & 4.8	Crude: higher mean dmfs with "high degree" of frequent sugar Adjusted: frequent sugar not included in m-v models
Stecksen-Blicks (1995)	Sweden	Cross Sectional	247	* (4, 4)	Crude: greater mean dmft with higher scores on index of frequent consumption of several sweet snacks Adjusted: stratified by tooth brushing, dmfs is 1.1 to 2.7 units greater when scoring higher on dichotomized index (both ss)
Tanaka (2010)	Japan	Cross Sectional	2058	* (3, 3)	Crude: lower prevalence caries with more frequent consumption of yogurt, cheese, and milk; higher prevalence with more frequent butter Adjusted: direction of associations persist, but only ss for yogurt (≥4 times weekly vs. less than once)

Wendt (1996)	Sweden	Longitudinal	289	* (3, 3)	Crude: not shown Adjusted: greater odds of <2 weekly soft drinks at 2 yr if caries-free at 3 yr (OR 2.4, ss)
Wong (2012)	China	Longitudinal	358	* (5, 6)	Crude: greater 2-yr caries increment and greater probability of positive caries increment if more frequent daily snacks Adjusted: compared to <1 daily snack, 1-2 daily snacks and ≥3 daily snacks associated with 2.2-fold and 2.9-fold greater number of new lesions, respectively (both ss)
Ye (1999)	China	Cross Sectional	800	* (2, 5)	Crude: daily snack frequency greater among rampant caries cases Adjusted: snacking frequency included in all models, but unclear how defined (ss)
Zhou (2011)	China	Cross Sectional	394	2.5 (2, 3)	Crude: prevalence caries increases with increasing frequencies of sweet and soft drink consumption Adjusted: both frequency variables not included in m-v models
					Crude: greater odds of caries with at least once daily sweets

* Mean age of participants not provided
1. Multiple first authors listed if multiple non-independent publications met inclusion criteria
2. Estimated ECC
Abbreviations: ECC = early childhood caries; dmft/s = decayed missing (extracted) filled tooth/surface index; mo = month; m-v = multi-variable; OR = odds ratio; PR = prevalence ratio; ss = statistically significant; S-ECC = severe early childhood caries; yr = year

Table 1-2. Characteristics and findings related to the use of a bottle baby at night or for sweet liquids from studies meeting three minimum quality criteria.

First author (year)[1]	Country	Design	n	Mean age [range], yr	Main Findings
Al-Malik (2001, 2003)	Saudi Arabia	Cross Sectional	987	* (2, 5)	Crude: higher prevalence caries with history of bedtime fruit juice in bottle Adjusted: bottle content not included in m-v model
Dini (2000)	Brazil	Cross Sectional	245	* (3, 4)	Crude: no clear association between milk in bottle at bedtime and caries Adjusted: nighttime bottle not included in m-v model
Du (2007)	China	Cross Sectional	1621	* (3, 5)	Crude: slightly higher caries prevalence with history of fruit juice in bottle; no difference with history of tea in bottle Adjusted: caries and fruit juice in bottle associated (OR 1.6; ss); tea in bottle not included in m-v model
Feldens (2010)	Brazil	Longitudinal	340	4.2 (4, 4)	Crude: lower prevalence severe-ECC with nighttime bottle use; but higher prevalence with bottle use for non-milk drinks Adjusted: nighttime bottle not included in m-v model; greater odds of severe-ECC with juice or soda in bottle (OR 1.4; ss)
Hallett (2003, 2006)	Australia	Cross Sectional	2515-3375	* (4, 6)	Crude: greater prevalence caries and mean dmft if sleeping with bottle and with sweet bottle contents Adjusted: greater odds caries if sleep with bottle (OR 1.6; ss) and with sweet bottle contents (OR 3.8; ss)
Hillow (2004) & Sayegh (2002, 2005)	Jordan	Cross Sectional	421-1140	* (4, 5)	Crude: greater proportion children with dmft≥8 had sweetened milk from bottle and had bottle at nap or nighttime Adjusted: greater odds of sweet liquid in comforter (OR 2.6 ss) and of bottle feeding at nap or night (OR 3.2; ss) if dmft≥8 vs caries free
Holt (1996)	United Kingdom	Cross Sectional	406	* (1, 4)	Crude: greater prevalence caries if given sweet drinks in a bottle Adjusted: bottle content not included in m-v model
Jin (2003)	South Korea	Cross Sectional	383	3.0 (<1, 4)	Crude: greater prevalence caries with sweetened solution in bottle, but no difference with bedtime bottle Adjusted: higher odds caries (OR 1.4; not ss), but not S-ECC, with sweetened contents; no association with bedtime bottle (caries or S-ECC)
Johansson (2010)	United States	Cross Sectional	1206	* (1, 4)	Crude: greater prevalence caries with bottle use in bed at night or nap time Adjusted: in partial least squares modeling, low variable importance projection placed on bottle use
Maciel (2001)	Brazil	Cross Sectional	255	* (4, 5)	Crude: lower dmfs with sweetened milk in bottle Adjusted: dmfs 0.9 units lower (not ss) with sweetened milk in bottle
Martello (2012)	Brazil	Cross Sectional	247	* (2, 3)	Crude: lower prevalence caries with bottle use at night; same caries prevalence with added sugar in bottle Adjusted: inverse association with bottle at night (OR 0.5; not ss); bottle content not included in m-v model
Martens (2006) & Willems (2005)	Belgium	Cross Sectional	366	2.5 (2, 2)	Crude: greater prevalence caries and higher mean dmfs if sleep with bottle and if sweet drinks in bottle Adjusted: higher odds of caries going to sleep with bottle (OR 1.3; not ss) and sweet drinks in bottle (OR 3.1; ss)
Melo (2011)	Brazil	Cross Sectional	1206	* (1, 3)	Crude: at age 18-36 mo, nighttime bottle use less common in caries cases Adjusted: inverse association (OR 0.6; ss)
Mohebbi (2008)	Iran	Cross Sectional	488	* (1, 3)	Crude: current milk-bottle at night not associated with number of decayed teeth Adjusted: greater odds of caries with nighttime milk-bottle (OR 5.5; ss)
Narksawat (2011)	Thailand	Cross Sectional	664	3.7 (3, 4)	Crude: higher mean dmft and odds of caries if ever slept with bottle of milk or milk late at night Adjusted: odds of caries greater with this practice (OR 1.3; not ss)

Nunes (2012)	Brazil	Cross Sectional	241	2.8 (1, 3)	Crude: lower prevalence caries if nocturnal bottle with infant formula Adjusted: adjusted result not shown
Oliveira (2006) & Targino (2011)	Brazil	Longitudinal	224-228	3.0 & 4.5[2]	Crude: lower prevalence caries at 54 mo with nighttime bottle at 18 mo and with sugar in the bottle at each of 18, 24, 30, and 36 mo Adjusted: inverse association with nighttime bottle feeding (OR 0.5; ss); bottle content not included in m-v model at some time points; for others, inverse association persists (not ss)
Parisotto (2010)	Brazil	Cross Sectional	288	* (3, 4)	Crude: greater proportion caries cases had sugar in bottle ≥3 times daily, and greater proportion went to bed with bottle of sweet liquid Adjusted: neither bottle variable included in m-v model
Peres (2005)	Brazil	Both Cross Sectional & Longitudinal	297	* (6, 6)	Crude: higher prevalence caries and dmft ≥4 if ever used bottle at night Adjusted: nighttime bottle not included in m-v model
Petti (2000)	Italy	Cross Sectional	1494	* (3, 5)	Crude: greater proportion caries cases ever used nocturnal bottle, and greater proportion ever used bottle with sweet beverage Adjusted: nocturnal bottle associated with rampant caries (not ss), but not non-rampant caries; sweet contents associated with rampant (OR 4.7; ss) and non-rampant (OR 2.2; not ss)
Pieper (2012)	Germany	Cross Sectional	1082	* (5, 7)	Crude: greater prevalence caries and dmft if used nighttime bottle ≥8 mo Adjusted: greater odds caries with nighttime bottle ≥8 mo (OR 2.1; ss)
Seow (2009)	Australia	Cross Sectional	617	3.1 (<1, 4)	Crude: ECC cases from some sites, but not all, more likely to have added sweetener to bottle than ECC-free children; sleeping with bottle more common among ECC children at one site only Adjusted: sleeping with bottle inversely associated at one site (OR 0.2; ss) and positively at another (OR 1.3; not ss); bottle contents not included in m-v model
Shiboski (2003)	United States	Cross Sectional	2239	* (2, 6)	Crude: not shown Adjusted: caries associated with falling asleep with sweet substance in bottle at 12 mo (OR 4.0; ss) and at time of survey (OR 1.7; not ss)
Slade (2006)	Australia	Cross Sectional	1398	* (5, 5)	Crude: higher prevalence caries if ever slept with sweet drink in bottle Adjusted: positive association (PR 1.4; ss)
Wong (2012)	China	Longitudinal	358	* (5, 6)	Crude: greater 2-yr caries increment and greater probability of any caries increment if bottle use during sleep Adjusted: nighttime bottle associated with 1.6-fold greater number of new lesions (ss)
Ye (1999)	China	Cross Sectional	800	* (2, 5)	Crude: greater odds of bottle containing sweetened cow's milk and of bottle with sweet liquids among rampant caries cases Adjusted: sweetened cow's milk in bottle associated (OR 1.7; ss)
Zeng (2005)	China	Cross Sectional	387	* (3, 5)	Crude: not shown Adjusted: greater mean dmft (0.9 units; ss) and greater odds of caries (OR 2.1; ss) with history of fruit juice in bottle
Zhou (2011)	China	Cross Sectional	394	2.5 (2, 3)	Crude: caries prevalence similar with never vs. sometimes or always bottle at night Adjusted: nighttime bottle not in m-v models

* Mean age of participants not provided; 1. Multiple first authors listed if multiple non-independent publications met inclusion criteria; 2. Estimated ECC
Abbreviations: ECC = early childhood caries; dmft/s = decayed missing (extracted) filled tooth/surface index; mo = month; m-v = multi-variable; OR = odds ratio; PR = prevalence ratio; ss = statistically significant; S-ECC = severe early childhood caries; yr = year

Table 1-3. Characteristics and findings related to the duration of breastfeeding from studies meeting three minimum quality criteria.

First author (year)[1]	Country	Design	n	Mean age [range], yr	Main Findings
Aida (2008)	Japan	Cross Sectional	3086	3.5 (3, 3)	Crude: higher mean dmft if breastfed ≥18 mo Adjusted: 0.9 unit mean increase in dmft (ss)
Al Ghanim (1998)	Saudi Arabia	Cross Sectional	446	4.2 (3, 5)	Crude: more children in high dmft group had breastfed ≥13 mo Adjusted: breastfeeding duration not included in m-v model
Al-Malik (2001, 2003)	Saudi Arabia	Cross Sectional	987	* (2, 5)	Crude: if ever breastfed, higher caries prevalence if breastfed ≥13 mo Adjusted: breastfeeding duration not included in m-v model
Campus (2009)	Italy	Cross Sectional	5538	3.9 (3, 4)	Crude: small increase in caries prevalence if breastfed ≥12 mo Adjusted: positive association (OR 1.2; not ss) in logit portion but not negative binomial portion of ZINB model
Dini (2000)	Brazil	Cross Sectional	245	* (3, 4)	Crude: highest caries prevalence and dmft in those breastfed ≥25 mo Adjusted: caries associated with combined category of never breastfed or breastfed ≥25 mo (OR 2.3; not ss)
Feldens (2010)	Brazil	Longitudinal	340	4.2 (4, 4)	Crude: higher prevalence S-ECC if breastfed ≥12 mo Adjusted: breastfeeding duration not included in m-v model
Folayan (2010)	Nigeria	Cross Sectional	396	* (<1, 5)	Crude: highest mean dmft if breastfed ≥19 mo Adjusted: caries prevalence (not ss) and dmft (ss) rise with each increasing mo of breastfeeding
Gao (2010)	Singapore	Both Cross Sectional and Longitudinal	1576-1754	5.8^2 (4, 6)	Crude: not shown Adjusted: increased odds of caries at baseline and 12-mo caries increment with each increasing mo of breastfeeding (both ss)
Hallett (2003, 2006)	Australia	Cross Sectional	2515-3375	* (4, 6)	Crude: higher caries prevalence and dmft if breastfed ≥13 mo vs. 3-12 mo but not vs. <3 mo Adjusted: increased odds of caries if breastfed ≥13 vs. no breastfeeding (OR 1.5; not ss)
Hillow (2004), Sayegh (2002, 2005)	Jordan	Cross Sectional	421-1140	* (4, 5)	Crude: higher prevalences caries and dmft ≥4 if breastfed ≥18 mo Adjusted: breastfeeding duration not included in m-v model
Holt (1996)	United Kingdom	Cross Sectional	406	* (1, 4)	Crude: higher prevalence caries if breastfed <13 weeks or ≥37 weeks Adjusted: breastfeeding duration not included in m-v model
Iida (2007)	United States	Cross Sectional	1562	* (2, 5)	Crude: higher prevalence caries if breastfed ≥12 mo Adjusted: greater odds of caries (OR 1.4; not ss) if breastfed ≥12 mo vs. no breastfeeding
Matee (1994)	Tanzania	Cross Sectional	359	1.9^2 (1, 4)	Crude: rampant caries cases breastfed longer than controls Adjusted: greater odds of breastfeeding ≥36 mo (OR 2.4; not ss)
Melo (2011)	Brazil	Cross Sectional	1211	* (1, 3)	Crude: at ages 18-36 mo, current breastfeeding slightly more common among those with caries Adjusted: breastfeeding duration not included in m-v model
Mohebbi (2008)	Iran	Cross Sectional	488	* (1, 3)	Crude: higher mean number decayed teeth if breastfed ≥7 mo for children ages 24-36 mo but not ages 12-23 mo Adjusted: breastfeeding duration not associated (OR 1.0; not ss)
Nunes (2012)	Brazil	Cross Sectional	241	2.8 (1, 3)	Crude: lower prevalence caries among if currently breastfeeding vs. having stopped before12 mo Adjusted: higher prevalence if currently breastfed (PR 1.2; not ss)
Oliveira (2006), Targino (2011)	Brazil	Longitudinal	224-228	3.0 & 4.5^2	Crude: higher prevalence caries at 54 mo if breastfed at night at 24 mo Adjusted: greater odds of caries if breastfed at night (OR 1.8; not ss)

Parisotto (2010)	Brazil	Cross Sectional	288	* (3, 4)	Crude: greater proportion of caries cases had breastfed ≥12 mo Adjusted: greater odds breastfeeding ≥12 mo if caries case (OR 2.0; ss)
Peres (2005)	Brazil	Both Cross Sectional and Longitudinal	297	* (6, 6)	Crude: higher prevalences caries and dmft ≥4 if breastfed ≥9 mo Adjusted: breastfeeding duration not included in m-v model
Petti (2000)	Italy	Cross Sectional	1494	* (3, 5)	Crude: mean breastfeeding duration shortest among rampant caries cases but longest among non-rampant caries cases Adjusted: breastfeeding duration not included in m-v model
Pieper (2012)	Germany	Cross Sectional	1082	* (5, 7)	Crude: no change in mean dmft and caries prevalence if breastfed ≥8 mo Adjusted: breastfeeding duration not included in m-v model
Retnakumari (2012)	India	Cross Sectional	350	* (1, 3)	Crude: children in greater dmfs categories more often in longer breastfeeding duration categories Adjusted: breastfeeding duration not included in m-v model
Sakuma (2007)	Japan	Longitudinal	5107	* (3, 3)	Crude: not shown Adjusted: if breastfeeding at 1.5 yr, greater odds of caries increment by age 3 yr (OR differs by municipality, 1.9-2.2; ss in all but one)
Slade (2006)	Australia	Cross Sectional	1398	* (5, 5)	Crude: higher prevalence caries if breastfed ≥18 mo Adjusted: breastfeeding duration not included in m-v model
Vazquez-Nava (2008)	Mexico	Cross Sectional	1160	4.5 (4, 5)	Crude: higher prevalence caries if breastfed at night ≥12 mo Adjusted: greater odds of caries if breastfed ≥12 mo (OR 3.6; ss)
Ye (1999)	China	Cross Sectional	800	* (2, 5)	Crude: longer mean breastfeeding duration if rampant caries case Adjusted: breastfeeding duration (not defined; ss) associated with caries if ever breastfed
Zhou (2011)	China	Cross Sectional	394	2.5 (2, 3)	Crude: highest prevalence caries if breastfed ≥12 mo Adjusted: greater odds caries if breastfed ≥12 mo (OR 2.6; ss)

* Mean age of participants not provided
1. Multiple first authors listed if multiple non-independent publications met inclusion criteria; 2. Estimated
Abbreviations: dmft/s = decayed missing (extracted) filled tooth/surface index; mo = month; m-v = multi-variable; OR = odds ratio; PR = prevalence ratio; ss = statistically significant; S-ECC = severe early childhood caries; yr = year; ZINB = zero inflated negative binomial

Supplemental Table 1-1. Search terms used in MEDLINE

("dental caries"[MeSH Terms] OR ("dental"[All Fields] AND "caries"[All Fields]) OR "dental caries"[All Fields]) AND (("diet"[MeSH Terms] OR "diet"[All Fields]) OR ("feeding behaviour"[All Fields] OR "feeding behavior"[MeSH Terms] OR ("feeding"[All Fields] AND "behavior"[All Fields]) OR "feeding behavior"[All Fields]) AND ("1990/01/01"[PDAT] : "2012/12/31"[PDAT])) AND (("child"[MeSH Terms] OR "child"[All Fields]) OR ("infant"[MeSH Terms] OR "infant"[All Fields]) OR ("child"[MeSH Terms] OR "child"[All Fields] OR "children"[All Fields]) OR preschool[All Fields] OR ("pediatrics"[MeSH Terms] OR "pediatrics"[All Fields] OR "pediatric"[All Fields]))

Notes:
Search completed July 23, 2012
Analogous searches were individually adapted for other databases: BIOSIS, CINAHL, Cochrane Library, LILACS, Web of Science, and WHOLIS.

Supplemental Figure 1-2. Data Abstraction Form
Information from studies under consideration for review was entered into a spreadsheet with the following column headings:

					Publication Characteristics: Search						
author, first	year	journal	peer review (1=yes)	title	full text available (1=yes)	meets inclusion criteria (1=yes)	meets 3 quality criteria (1=yes)	reason to reject	found by electronic=1 hand=2	publication part of group of studies (1=yes)	group number
				Publication Characteristics: Design, Sample, Age Range							
country	WHO region	study design	design (notes)	n (analytic sample)	follow-up % (if applicable)	analytic sample ≥200 (1=yes)	mean age at baseline (for longitudinal), mo	mean age at dental exam, mo	age SD	age range	community fluoride
					Dental Caries Assessment						
dental exam method	outcome defintion: dmf	outcome defintion: binary: caries vs. caries free	outcome defintion: rampant, nursing, or BBTD	other definitions	other notes on caries definitions	prevalence d2+ (cavitated or above)	prevalence d1+ (white spot or above)	mean d1mfs	mean d1mft	mean d2mfs	mean d2mft
					Bottle Use Information						
		Timing of feeding data collection wrt outcome	Bottle Use Itself	Bottle Contents	Night or Bedtime Bottle	Age of bottle weaning	Misc. Bottle Related Exposures	Notes on bottle use	Pacifier Use	Bacterial Transfer via feeding	
				Food and Beverages: Frequencies, Amounts, and Others							
		beverages	multiple beverages/ notes:	candy sugar sweets	multiple candy sweets / notes	frequency of snacking or eating	multiple: freq of eating / notes	age of food introduction	night time feeding	others	
		Breastfeeding Information					Interventions				
	ever BF vs never	other definitions of BF itself	duration /termination of BF or exclusive	nocturnal BF	frequency of BF or Other		intervention (describe)				
				Statistical Adjustment & Results							
	Analytic set-up (e.g. prevalence by exposure groups)	Adjustment method	Provides crude results (1=yes)	Provides adjusted results (1=yes)	fits minimum criterium for adjustment? (1=yes)	socio-demographic confounders (list)	feeding-practices in model (list)	Notes on potential sources of bias	Main findings, crude and adjusted	Other notes	

Chapter 2. Healthcare worker training in infant nutrition for dental caries prevention in young children: a cluster-randomized field trial in southern Brazil

Section 1.
Primary and Subgroup Analyses for Dental Caries Outcomes[1,2]

1. An earlier version of this chapter section has been accepted for publication (in press):
Chaffee BW, Feldens CA, and Vítolo MR. 2013. Cluster-randomized trial of infant nutrition training for caries prevention. *J Dent Res.* DOI: 10.1177/0022034513484331

2. A preliminary report was presented at the International Association for Dental Research General Session and Exhibition, Foz do Iguaçu, Brazil:
Chaffee BW, Feldens CA, and Vítolo MR. 2012. Caries prevention through healthcare worker training: a randomized controlled trial. *J Dent Res.* 91(Spec Iss B): 2988

Abstract
OBJECTIVE: Examine the impact on caries of providing training in infant feeding guidelines to workers at urban public primary care clinics in Brazil.
METHODS: In a cluster-randomized controlled trial involving 20 health centers, patient care staff was trained in either: 1. a Brazilian guide for infant nutrition, which stressed healthful complementary feeding, or 2. usual practices (control). Eligible pregnant women attending participating clinics were invited to enroll to track health outcomes in their children. Assessments occurred at approximately 6, 12, and 36 months; the last evaluation measured early childhood caries (ECC).
RESULTS: Dental data were available for 458 children at age 2-3 years. The impact of the intervention on ECC (relative risk 0.92; 95% confidence interval 0.75, 1.12) and severe-ECC (0.87; 0.64, 1.19) was not statistically significant. In subgroup analyses, there was a protective effect of the intervention among mothers who exclusively remained at the same health center (0.68; 0.47, 0.99) and among those who listed the health center as their most important source of infant feeding advice (0.53; 0.29, 0.97).
CONCLUSIONS: Healthcare worker training had a small overall impact on dental caries, with a greater reduction seen when mothers were more connected to their health center.
TRIAL REGISTRATION: ClinicalTrials.gov (NCT00635453)

Introduction

In childhood, dental caries is a frequent, often untreated disease, particularly in low-resource populations (Mouradian, 2000; Selwitz, 2007; AAP, 2011), and it has important negative implications for quality of life (Abanto *et al*., 2011; Leal, 2012). Diet-based programs delivered through the medical care system might help address this problem, as infant feeding behaviors contribute to caries development (Selwitz, 2007; Aida, 2008; Mobley, 2009; Thitasomakul *et al*., 2009; Feldens, 2010a), and medical providers can play a key preventive role (Keels, 2008; Kressin *et al*., 2009; Pahel, 2011). Though complementary feeding guidelines generally contain no specific oral health messages, recommendations to limit added sugar, reduce bottle use, and serve defined meals or snacks could have a positive oral health impact. This common risk-factor approach to disease prevention is highlighted in World Health Organization (WHO) priorities (WHO, 2009).

In a previous trial in São Leopoldo, Brazil, new mothers were randomized to an intervention of ten in-home infant feeding counseling sessions spanning one year (Vítolo, 2005) or a control condition of limited contact. Guidance for the intervention was based on the "Ten Steps of a Healthy Diet for Brazilian Children Under Two Years of Age (Brazilian Ministry of Health, 2002; Coitinho, 2002)," a collection of complementary feeding recommendations based on WHO guidelines, which stresses healthful infant and complementary feeding, such as longer durations of exclusive breastfeeding and, later, the gradual introduction of fruits, vegetables, and animal proteins. Trial targets included improving feeding practices (e.g. increased duration of exclusive breastfeeding) and clinical outcomes (e.g. hospitalizations, diarrhea occurrence, anemia status) of the children born to participating mothers. Results showed that, in addition to general health benefits (Vítolo, 2005), the occurrence of ECC was significantly reduced at ages one and four years (Feldens, 2007; Feldens, 2010b).

While these results are promising, it is unknown whether a less intensive approach whereby the intervention is delivered through participants' medical facilities will have a similar impact. Such a program could offer general and oral health benefits at lower costs and be more easily and rapidly scaled to a regional or national level. Thus, a cluster-randomized intervention was implemented in municipal primary healthcare centers in the neighboring city of Porto Alegre, aiming to reproduce these health benefits.

The Porto Alegre trial initially aimed to improve feeding practices and the nutritional status of children born to clinic attendees. After first observing a significant positive effect of this intervention on the mean duration of exclusive breastfeeding (Bernardi, 2011), this study expands the suite of evaluated outcomes to assess dental status. We hypothesized that fewer children from intervention group health centers would have dental caries at age three years.

Methods

Objective:

We assessed whether healthcare worker training in the content and delivery of infant feeding guidelines reduced the caries experience of children born to mothers attending clinics in the Porto Alegre trial. We hypothesized that fewer of the children from the intervention group health centers would have dental caries at age three years.

Setting and Design:

Porto Alegre is a southern Brazilian city of 1.4 million residents with a fluoridated water supply at 0.7 ppm (Municipality of Porto Alegre). Of its 52 municipal health centers, 31 met eligibility criteria for this cluster-randomized controlled trial (Figure 2.2.1). Reasons for exclusion were having fewer than 100 infant patient visits in 2006, staff sharing with other clinics, and participation in a contemporaneous community-based dietary program. Of the eligible health centers, 16 were initially selected via a witnessed drawing of labeled markers from an opaque container by the principal investigator (MRV) under the stipulation that only the first two drawn from each of the city's eight geo-administrative districts would be retained. These health centers were block-randomized by district, with the first health center drawn allocated to an intervention of staff training and the other to a control of usual practices. To increase statistical power, four additional health centers, not paired by district, were drawn and assigned at random. These 20 health centers were invited to participate without being informed of allocation status, and all consented, resulting in 9 intervention and 11 control group health centers.

Patient Participants:

Following staff training at intervention sites, all pregnant women with scheduled visits to the 20 participating clinics were invited to enroll for outcome tracking. These women were contacted by fieldworkers not involved in health center recruitment or the training session and masked to health center allocation status. Recruitment of individuals took place from April-December 2008, with births occurring from May 2008 to February 2009. Women reporting a positive HIV test were not eligible due to concerns of HIV transmission through breastfeeding. Of 736 eligible women, 715 (97.1%) agreed to enroll. Informed consent was reached with mothers on behalf of their children at each stage of data collection.

Intervention:

In early 2008, an experienced nutritionist (MRV) delivered a standardized, one-hour training session for pediatricians, other physicians, nurses, and administrative staff that outlined the Ten Steps recommendations and strategies for their incorporation into maternal consultations. Intervention health centers were given posters to display in areas where patients circulate and pamphlets to distribute to pregnant and lactating women. The intervention was designed as a low-cost program that could ultimately result in large-scale implementation and dissemination.

In brief, the Ten Steps recommendations are: 1.) exclusive breastfeeding to six months; 2.) continued breastfeeding to two years, with the gradual introduction of complementary foods; 3.) at six months, start complementary feeding (grains, cereals, meat, vegetables, fruits) three times a day while continuing to breastfeed; 4.) mealtimes should be at regular intervals, adjusted to the child’s internal hunger cues; 5.) new foods should gradually get thicker until the child is able to eat a family meal, but never liquefied; 6.) provide a variety of healthy foods everyday; 7.) daily intake of different fruits and vegetables; 8.) avoid sugar, candies, sweets, soft drinks, salty snacks, processed and fried foods; 9.) good hygiene practices in food preparation and handling; and10.) adequate, responsive feeding during illness.

Statistical Power:

The sample size was based on a desired power of 90% to detect a 60% relative increase in the frequency of exclusive breastfeeding at age four months (Bernardi, 2011). For dental outcomes, we estimated a 25% relative reduction in the prevalence of caries at 2-3 years, a 55% caries frequency in the control group, and 30% loss to follow-up, based on interpolation from the São Leopoldo study (Feldens, 2010b), which measured caries at different ages. At an alpha of 5% for two-sided tests and a design effect of 1.5, the estimated power of the study, given the number of mother-child pairs actually enrolled, was 71%.

Measurement:

Trained field workers, masked to allocation status and not part of healthcare worker training, contacted participating families at baseline and as enrolled children reached approximately 6, 12, and 36 months of age. Demographic information and maternal perceptions were collected by questionnaire (detailed in: Bernardi, 2011).

At the final visit, visual oral health assessments were completed following WHO protocol (WHO, 1997), with the additional recording of non-cavitated (white spot) lesions. Assessments took place in participants' homes under ambient light with the aid of a lighted intraoral mirror. All teeth were brushed before drying with gauze. Tooth surfaces were recorded as sound, decayed non-cavitated (white spot), cavitated (frank lesion), missing due to caries, or restored. Following case definitions from the National Institutes of Health (Drury, 1999), ECC was classified as one or more decayed, missing, or filled tooth surface (dmfs ≥1). Severe early childhood caries (S-ECC) was defined by the presence of one or more affected maxillary anterior tooth or a total dmfs ≥4. For children <36 months, one or more affected smooth surface also qualified as S-ECC.

One dentist-examiner (BWC) completed 94.7% (434/458) of the assessments, with the remainder performed by a second calibrated dentist following the identical protocol. To estimate reliability, each examiner independently evaluated 24 children aged 3-5 years on each of two occasions, one week apart. Inter-rater reliability was based on the identification of sound, cavitated, missing, or filled teeth (unweighted kappa 0.75), as was intra-rater reliability (unweighted kappa 0.83 for both examiners).

<u>Analysis</u>:

Primary analysis was by intention-to-treat, with proportions (ECC, S-ECC) and means (dmfs) compared for children whose mothers were initially recruited from either intervention or control group health centers, regardless of whether attendance at those clinics continued. For statistical inference, binary events were compared using log-linear regression and dmfs counts using negative binomial regression. Variance estimates were derived from the clustered sandwich estimator (command: vce) in Stata 12.0 (StataCorp, College Station, USA) to account for non-independence within health centers. In secondary analyses, the intervention effect was estimated across six *a priori*-determined demographic subgroups: maternal age, maternal education, household income, family structure (nuclear vs. other), parity (first-time mother vs. other), and social class to determine whether certain characteristics might identify families with greater or lesser benefits of the intervention taking place in their health center. Similarly, we estimated the intervention effect across three *a priori*-determined behavioral subgroups: primary caregiver of the child (mother vs. other), exclusive use of the same health center (mother continuing to attend the same health center from which she was recruited through the age 11-15 month visits vs. change of health center), and health center as main source of feeding guidance (mother reporting that the health center or health center staff is the most important influence in making feeding decisions for her child vs. other). The final two of these behavioral categories were intended to identify, respectively, mothers most likely to have continually received health center-based feeding advice consistent with either an intervention or control facility experience and those mothers most likely to be receptive to any guidance received.

<u>Ethical Review</u>:

The Ethics Committee in Human Research at the Federal University of Health Sciences of Porto Alegre and the Committee for the Protection of Human Subjects at the University of California Berkeley approved this study. Children with caries or suspected anemia, under-nutrition, or overweight status were referred for care at their local health center.

Results

Dental assessments took place from August 2011 to June 2012. At the time of assessment, child ages ranged from 31-46 months, with 92.6% (424/458) from 35-42 months. Previous contact with a dentist was uncommon (117/440, 26.6%). Of the initial 715 mother-child pairs, dental outcome data were available for 458 (64.1%, Figure 2.1). Baseline characteristics were similar by allocation status (Table 2.1), as was the case at baseline (data not shown). Principle reasons for missing outcomes were withdrawal from the study and inability to locate. Losses were similar by allocation status (p=0.41). Children available for analysis differed from those lacking dental information to a statistically significant extent for three measured variables: they were, on average, born to older mothers, had fathers with fewer years of education, and were from higher social class households. There were no other statistically significant differences between losses and the analytic sample, including for maternal education and self-identified race (data not shown).

Overall, 78.2% (495/633) and 70.7% (383/542) of the enrolled mothers were exclusively attending the same health center from which they were recruited at the 5-9 month assessment and at the 11-15 month assessment, respectively (Figure 2.2). Only in the intervention group did a substantial number of mothers report seeing the Ten Steps posters or receiving pamphlets that were distributed to intervention group health centers (Figure 2.2) suggesting that there was no contamination of the comparison group in that regard.

A lower proportion of intervention group children experienced ECC (52.3% vs. 57.0%), any cavitated decay (37.1% vs. 42.1%), or S-ECC (32.1% vs. 36.7%), although these differences were not statistically significant (Table 2.2). The mean number of affected tooth surfaces was lower in the intervention group.

In subgroup analyses (Figure 2.3) there was no indication of a difference in the impact of the intervention on S-ECC by family or demographic characteristics. However, among those mothers who at the 11-15 month assessment were still exclusively attending the same health center from which they were recruited, and among those who listed the health center as their principle source of infant feeding guidance, there was a statistically significant reduction in the occurrence of S-ECC (Figure 2.3). This same pattern was observed for ECC and cavitated decay (data not shown).

Discussion

Rarely has caries been measured following randomized infant feeding interventions lacking specific oral health components. Caries reduction was not found following peer-led “social support” for recommended feeding practices in economically disadvantaged areas of London (Scheiwe, 2010). In a large hospital-based cluster-randomized trial of breastfeeding promotion, no caries effect was reported at age six years (Kramer *et al*., 2007). Here, healthcare worker training in pre-existing infant feeding guidelines resulted in lower caries experience among children born to mothers attending intervention group health centers, although the difference was not statistically significant.

Results from São Leopoldo showed a stronger effect when dietary counseling based on the same guidelines was provided directly to mothers in their homes (Feldens, 2010b). Although the mean duration of exclusive breastfeeding was extended with the Porto Alegre intervention (Bernardi, 2011), this effect, too, was weaker than that observed in São Leopoldo (Vítolo, 2005). The single training session of the Porto Alegre study might have lacked the intensity needed for sustainable behavior change among physicians, such as repeated sessions, program individualization, or health center-level changes to encourage and allow for more meaningful physician-patient interactions. The consistency and accuracy with which messages were relayed to mothers are unknown, but ≥40% of mothers in the intervention group did not report receiving the Ten Steps pamphlets that we provided to intervention group health centers for patient distribution. Additionally, many participants were receiving care at different facilities within one year. Others did not list health professionals as their most valued source of infant feeding advice. These factors likely contributed to the inability to observe a statistically significant effect in the overall population. More intensive future interventions might seek to incorporate multi-level and/or multi-stage elements to the healthcare worker training to reinforce knowledge and behavior change, as well as assessments of knowledge transfer from trainer to healthcare workers and from healthcare workers to mothers.

Among those mothers who remained at the same health center or those who did name the health center as a primary source of guidance, there were statistically significant protective effects. This suggests that the intervention might have been more effective had it been more widely or more intensively implemented, helping ensure that the Ten Steps messages reached mothers. Had the intervention been available at more healthcare sites, allowing mothers to hear consistent messages despite attending different clinics, its impact might have been greater. S-ECC was, in fact, less common among children of intervention group mothers who received a Ten Steps pamphlet than those who did not (see: Chapter 2.2.A). These findings must be interpreted cautiously, as departures from intention-to-treat analysis should be considered exploratory (Lee, 1991).

The lack of a caries preventive effect among children of mothers who changed health center or did not value health center advice highly indicates that greater overall effectiveness might have been achieved had continuity of care and patient-provider trust been stronger in municipal clinics. Care delivery patterns and other attributes of the quality of care delivered were not measured in this study population. However, frequent changes in the source of medical care suggest a breakdown in the establishment of a pediatric medical home – a continuous, comprehensive, central resource for the patient's ongoing care (McLeod 2012; Trivedi 2010). Under a medical home model, caries prevention through medical facilities might have been more effective for pediatric patients.

It should be noted, that while non-dental health professionals under a well-functioning pediatric medical home are important in oral health promotion (Keels, 2008; Kressin *et al.*, 2009; Pahel, 2011), infants and toddlers at high risk for caries should ultimately be referred a qualified dentist for the establishment of a dental home, as well (Kighara 2009). Thus, medical care based interventions are only part of a long-term solution for caries prevention. Coordinated improvements in the access and utilization of dental services are needed for children at the greatest risk for caries.

This study offers strengths and limitations. Strengths include the randomized design and the ability to compare results to those of a related intervention that differed by implementation scheme. In the main analysis was by intention-to-treat, a favored approach for most trials, however, this conservative approach may have hampered the ability to detect any intervention effect in this population in which many mothers changed the health centers they attended. In the subgroup analysis, subgroups were defined *a priori*, though there was no adjustment for multiple hypothesis tests. For the main analysis, statistical power was estimated with a stronger expected intervention effect than was observed. The proportion of participants lost to follow-up was typical of a long-term trial completed in low resource settings. Losses were similar by allocation status, making a meaningful bias due to censored outcomes unlikely.

Despite its modest impact, this is one of the first studies to demonstrate the feasibility of adding oral health assessments to the evaluation spectrum of dietary interventions in early childhood. This approach not only helps better define oral health benefits owing to improved feeding behaviors, but also increases important cross-disciplinary collaboration in a common risk-factor approach to disease prevention, particular in low-income population with medical care access.

Summary:

Previous research indicates that one-on-one maternal counseling in infant dietary practices can reduce ECC in a low-income Brazilian population. A less expensive and more easily implemented training program for medical providers featuring the same infant feeding guidelines was tested in this cluster-randomized trial. This intervention yielded a small caries reduction that did not achieve statistical significance in the full sample, but significantly reduced S-ECC by one-third to one-half in subgroup analyses based on health center attendance and attitudes regarding health professionals' advice, respectively. These results suggest that oral health benefits can be achieved via the medical care system for a population of low dental service access and utilization. However, interventions of greater intensity should be tested to maximize oral health improvements.

Funding

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Chapter 2. Section 2.
Secondary and Exploratory Analyses Related to the Porto Alegre Ten Steps Trial

Part A.
Dental Caries Impact in Relation to "Process" and "Validation" Outcomes

The overall impact of the Porto Alegre "Ten Steps" healthcare worker training intervention on the occurrence of dental caries did not reach statistical significance. However, children of mothers who remained at the same health clinic, perhaps indicating more continuous access to consistent dietary counseling (and subsequently leading to better feeding practices) as would be associated with a medical home model (McLeod 2012; Trivedi 2010), appeared to fair better. The non-statistical significant effect for the group overall, however, indicates that, for a study of this size, the magnitude of the observed reduction in dental caries occurrence was comparable to a difference that plausibly could have emerged due to sampling variability alone if there were no causal impact of the intervention. As with all results lacking statistical significance, it cannot be known whether a true underlying effect exists, despite the inability to reject the traditional null hypothesis. It is plausible in studies of modest size that chance sampling could yield a study population from which the findings are "null" even if a true effect exists. Further, incomplete, inefficient, or otherwise flawed transmission of a truly efficacious intervention to the people it is intended to benefit could lead to null results (e.g. a trial of a truly beneficial pharmaceutical fails to shows an overall effect owing to few participants completing a full treatment regimen of multiple doses). The secondary analyses that follow explore similar possibilities in the Porto Alegre trial.

The most conservative interpretation of the Porto Alegre trial would be that the intervention offers no true dental caries benefit. Even this "null" conclusion provides value, however, as it highlights the considerable challenges in taking a promising intervention to scale. Our experience should inform future interventions in the search for sustainable, affordable practice-based interventions to improve pediatric general and oral health. Mainly, our findings suggest that for all the promise that the Ten Steps guidelines demonstrated as a caries preventive strategy in the São Leopoldo trial, going from a more intensive intervention (in a sense, "efficacy") to an implementation scheme suitable for a large-scale program (analogously, "effectiveness") rarely delivers similar impact.

To assess the probability that the null hypothesis was rejected falsely if the impact of the intervention was in fact weaker than originally anticipated, we can revise our power estimates *post hoc*. Prior to obtaining results, we expected the probability of a type II error to be 29% (Chapter 2.1). However, this was based on a number of assumptions: notably, that the intervention would lead to a 25% relative reduction in the proportion of children with early childhood caries (ECC). If, instead, we base the power calculation on the observed parameters of the study: 57% caries frequency in the control group, 458 observations, a design effect of 1.5 (intra-cluster correlation coefficient = 0.0144 for 20 clusters), and a relative risk of 0.90; then, at an alpha of 5%, the power of study would be only 14% (86% probability of a type II error). The power of the study would also be 14% for the outcome severe-ECC

One might question whether it was reasonable to expect a 25% relative reduction in ECC after the more intensive São Leopoldo study had reduced ECC at age 4 by 22% and S-ECC by 32% (Feldens, 2010b). Had a less ambitious target been chosen *a priori*, the estimated power would have appeared inadequate. At the time, there was no reasonable way to increase study power (the sample size was fixed, based on the number of health centers recruited in 2008). An ethical argument could be made against the pursuit of underpowered trials, as erroneously negative results could discourage the future implementation of otherwise effective interventions. That said, given that the much more common criticism of public health interventions is the tendency to push forward with ineffective interventions due to a lack of proper evaluation, there was an opportunity to demonstrate the feasibility of assessing oral health in a primarily nutrition-focused intervention trial. Ideally, the greater contribution of this study will be to motivate more rigorous evaluations in future studies, rather than suggest that infant feeding be abandoned in caries prevention, as evidence still supports the merits of this approach.

Although it was not possible to increase the number of participants in the trial, options do remain to attempt to pull "signal from the noise" among the trial results. Two general approaches would be subgroup analysis (Ask: Were there certain groups for whom the intervention was either more or less effective?) and a treatment-on-the-treated approach (Ask: Was the intervention more effective for the individuals who had greater exposure to the components of the intervention?). While all intervention group *clusters* did receive the intervention of healthcare worker training, we can explore effects across *individuals* whose experiences with the intervention differed. Whether and when such analyses are appropriate is point of contention (Lee, 1991). Thus, any findings of such analyses must be considered hypothesis or theory generating and await confirmation in other studies.

In Chapter 2.1, we concluded that the overall non-statistically significant effect of the intervention could be due, at least in part, to inadequate transmission of the infant feeding information from the trained health care providers to mothers. This could have been due to a number of factors, including mothers opting to seek care at other sites; mothers not valuing recommendations from the health center; or the providers not correctly or forcefully incorporating the content of the Ten Steps training into patient consultations. Notably, statistically significant intervention effects were seen among mothers who did not change health centers and those who placed a high value on feeding advice from the health center (Figure 2.3).

An alternative explanation for the statistically significant subgroup findings is that they arose by chance alone; something that is increasingly more likely for any one subgroup as the number of subgroups increases. This concern, in part, drove the *a priori* decision to examine a limited number of subgroups, all chosen based on prior knowledge that these factors could plausibly modify the effect of the intervention, rather than examine an unlimited number of factors non-systematically. Additionally, there is a realistic possibility of of important outcome predictors ending up unevenly distributed across the intervention and control groups (empirical confounding) in one or more of the subgroups. For example, among mothers who changed health centers, intervention group mothers were less likely to have >8 years of formal education (56% versus 72%, p=0.01). However, an adjusted model (log-linear regression) suggests that any such confounding had minimal impact on the results (Table 2.3).

<u>Validation Outcomes</u>:

Positive and negative validation tests can be helpful in addressing findings potentially due to chance. In the context of this study, a positive validation outcome would be one with a strong theoretical basis for expecting a causal intervention effect: for example, breastfeeding behavior, which is specifically stressed in the Ten Steps guidelines. A negative validation outcome would be some measureable aspect of health not targeted by the intervention and with little theoretical basis for expecting an intervention effect. If the secondary analysis results (i.e.

subgroup and treatment-on-the-treated) observed for dental caries are mirrored by the positive validation outcomes (but not by the negative validation outcomes), we have evidence that the dental caries outcomes operate through a causal mechanism related to the intervention. If all outcomes follow the same pattern, general trends in health or disease resistance unrelated to the intervention might be responsible for the findings. If no pattern is observed, chance is a likely explanation.

In the analyses that follow, negative validation outcomes are represented by skin rash observed at 5-9 months and birth weight <3000g. Three thousand grams was chosen as a cut-point for birth weight due to a low number of births under 2500g. Neither outcome represents an ideal option for negative validation. "Low" birth weights in the range of 2500-3000g might not carry significant health relevance. It is arguable, too, whether skin rash is an adequate "negative" validation test for a nutrition intervention, as some studies (e.g. Kull 2002), although not all (e.g. Fergusson, 1981; Bergman *et al.,* 2002), suggest a protective effect of breastfeeding feeding and atopic dermatitis. Nonetheless, these outcomes were considered the least likely to show an intervention effect of all the outcomes measured. Neither of these outcomes differed across the intervention and control groups to a statistically significant extent (Table 2.4), although the magnitude of the difference was comparable to the overall effects observed for dental caries outcomes (Table 2.2).

Three nutrition / infant feeding outcomes were selected *a priori* as positive validation outcomes: exposure to soft drinks before age six months, exclusive breastfeeding to age four months or more, and having family food (sharing the same meal consumed by older household members) every day of the week at age 11-15 months. All of these behaviors were directly targeted in the Ten Steps guidelines. As expected, the proportion of children introduced to soda before six months was reduced by the intervention at a statistically significant level, however, the proportion exclusively breastfeed to at least four months, although increased, and the proportion consuming family meals at 11-15 months did not differ by allocation status to a statistically significant extent (Table 2.4).

Based on the subgroup findings for dental caries, we had proposed that any beneficial effect of the intervention would be magnified among those mothers with stronger ties to their health center. The two subgroups we considered presumably represent those mothers who had more opportunities to hear Ten Steps messages from their medical providers (subgroup: "exclusive use of same health center") and those were most receptive to the advice their were presumably given (subgroup: "health center main source of feeding guidance"). If being more strongly connected to the health center in these ways is an actual mechanism through which the intervention affects health outcomes, we would expect that each of the positive validation outcomes would mirror, at least directionally, the subgroup pattern observed for the dental caries outcomes, while the negative validation outcomes would not.

In fact, the subgroup results for the outcomes soda before six months and birth weight <3000g both display a pattern, at least directionally, that supports the hypothesis that maternal connections to the health center (i.e. continual attendance and high valuation of advice) enhance intervention effects. The positive validation outcome early soda introduction was reduced with the intervention to a greater extent among those mothers with stronger health center ties, but the impact on the negative validation outcome birth weight <3000g followed the opposite pattern (Figure 2.4). However, in the case of each of the three other validation outcomes, there was at least one violation of the expected pattern. For example, the negative validation outcome skin rash was reduced with the intervention to a greater extent among mothers exclusively seeking care that the same health center, while the positive validation outcome exclusive breastfeeding to at least four months displayed the strongest beneficial intervention effect among mothers who did not list the health center as their main source of feeding guidance.

Taken in total, the subgroup findings for the validation outcomes do not provide convincing evidence that the intervention operated in a stronger, causal way among the mothers with the strongest health center connections, as proposed. This hypothesis was based on the subgroup findings for dental caries outcomes, and the inability to consistently replicate the dental caries subgroup pattern for the positive validation outcomes (and, conversely, the ability to replicate it for at least one negative validation outcome) suggest that the caries findings could be artifact. On the other hand, given only a small number of imperfect validation outcomes to examine, along with the limited ability of these subgroups, as defined and measured, to discriminate between the mothers most receptive to the intervention, this validation exercise does not entirely preclude the still reasonable hypothesis that advice on infant feeding could prevent caries in the children of mothers who actually receive and internalize such advice. However, in light of the validation findings, it appears even less likely that the Porto Alegre Ten Steps intervention, if replicated in the same manner as it was implemented, would offer any more than a very modest oral health benefit.

<u>Process Outcomes</u>:

Rather than focus solely on the health outcomes targeted by an intervention, a process evaluation considers the intermediate steps and components along a path through which the health outcomes are realized (CDC, 2008). Health outcomes can often be distal to the intervention itself, either in time or in the number of mechanical, behavioral, or biological steps necessary to cause changes in health status. By assessing the intermediary steps, which can be simple (e.g. "How many informational pamphlets were delivered to participating health centers?") or complex (e.g. "How were physicians' attitudes and behaviors changed?"), an evaluator can develop a more complete view of an intervention as a process rather than focusing only on end points. For example, a "null" intervention effect in terms of health outcomes might result from a breakdown of important component related to the implementation of an intervention rather than a lack of a true behavioral or biological action of the intervention itself. Without monitoring of the intermediary components, it can be difficult to determine which aspects of an intervention were most critical to its success or failure.

The process components of an intervention are often conceptualized as acting along a linear path from intervention inputs (the content or attributes of the intervention itself), through the mediating components (activities and outputs), and arriving at short and long-term health outcomes (Figure 2.5). Measuring and reporting the entire process might benefit various stakeholders by helping to build more effective program models, leading to better program monitoring, informing future improvements, and adding accountability (CDC, 2008). Table 2.5 provides examples of how these components can be conceptualized in relation to the Porto Alegre Ten Steps trial.

The Porto Alegre Ten Steps Intervention was not designed with a plan to conduct a formal process evaluation. As a result, data are not available for many of the components that would be assessed in such an evaluation (Table 2.5). However, from Figure 2.2 there is little doubt that suboptimal execution of program of activities played a credible role in reducing the overall effectiveness of the intervention. For example, only 59% of mothers in the intervention group reported receiving the Ten Steps pamphlet.

<u>Treatment-on-the-Treated</u>:

If we compare the dental caries experience of children born to mothers who reported exposure to some of the activities (e.g. seeing the Ten Steps poster; receiving the Ten Steps pamphlet; having the pamphlet on-hand) and outputs (e.g. following the guideline/recommendations they were given) to those children born to mothers who did not, there is a trend of decreasing caries with increasing exposure to these process components (Figure 2.6).

Important caveats apply. Assignment to these categories was not random, and thus confounding factors might contribute to the observed trend. The control group does not contribute to the treatment-on-the-treated analysis, because one cannot identify which control group mothers would have viewed posters or received pamphlets had their clinic been allocated to the intervention (i.e. a reasonable approximation of the counterfactual for the intervention group is not identifiable). Finally, the number of children in some categories is small (e.g. n=34 for having the pamphlet on hand and following guidelines), leading to imprecise estimates of caries occurrence.

Figures 2.7 and 2.8 compare the treatment-on-the-treated trend seen for dental caries outcomes with the negative and positive validation outcomes defined earlier, respectively. There is some suggestion that the trend seen for caries outcomes is not mirrored by the negative validation outcomes, supporting a caries-specific intervention effect. However, the positive validation outcomes also show no obvious pattern as exposure to process components increases, consistent with the caries trend being artifact.

Summary:

The subgroup findings presented in Chapter 2.1 suggested that the Porto Alegre Ten Steps trial was effective among mothers who shared certain characteristics, but further exploration reveals the challenges in differentiating a true effect from a chance finding. Validation outcomes, process evaluation, and treatment-on-the-treated analyses can be valuable in augmenting an impact evaluation, but rely on access to additional measured variables and assumptions about the causal pathways connecting the intervention to health outcomes. A major take-away message of Chapter 2.1 was that the intervention might have had a greater overall impact if it were implemented more intensively. This is both logical and consistent with outside knowledge, though does not identify specific ways in which the intervention might have been enhanced. Secondary analyses might have been able to elucidate specific pathways to target in program improvement had the appropriate qualitative and quantitative data been available. The general finding that the relatively low strength of this intervention was the primary reason for the modest-to-null dental caries impact appears valid.

Chapter 2. Section 2.
Secondary and Exploratory Analyses Related to the Porto Alegre Ten Steps Trial

Part B.
Estimating the Intervention Impact Under Different Missing Data Assumptions

In the Porto Alegre trial, dental outcomes were not recorded for 257 of the 715 children initially enrolled (35.9%), principally due to loss to follow-up. The study population underwent frequent changes of address and phone numbers, complicating retention efforts. The 458 participants with observed outcomes (the "complete cases") might not be representative of the initial 715. In this section, I attempt to make inference to the entire cohort without relying on the main assumption of a complete case analysis: that data are missing completely at random.

In brief, the complete case analysis assumes that unrecorded values are missing completely at random (MCAR). In other words, the process that led to missingness was not influenced by any factors (measured, unmeasured, known, or unknown) aside from chance. Therefore, the complete observations represent a random sample of the initial cohort and will give an unbiased effect estimate with respect to the initial cohort (albeit less precise, due to having fewer observations). The MCAR assumption is highly unlikely to be true, although it cannot be tested. Donders (2006) provides an approachable overview of missing data assumptions.

Figure 2.9 shows a more plausible scenario. Here, we allow that some variable or set of variables, W (e.g. socio-economic status), may be a shared parent both of the outcome, Y, and of having that outcome measured, D. We also allow that treatment, A, could be a cause of missingness (e.g. drug side-effects led to more drop-outs in the intervention group). The complete case estimate of the effect of A on Y may be biased in this situation, as this approach is conditional on the outcome being measured (D=1), opening a non-causal path from A to Y via D (a collider) and W.

When data are missing not at random (MNAR), missingness is predicted by unknown or unmeasured variables (e.g. study participants are less likely to provide responses to sensitive or personal questionnaire items, dependent on what their response would be). Figure 2.9 still applies when W is not measured. In such situations, no estimation strategy can completely remove the bias.

If we assume that the data are missing at random (MAR), missingness is not predicted by any unknown or unmeasured factors within strata of the known and measured variables. This assumption, which is similar to "no unmeasured confounding," allows one to use information collected on co-variables (e.g. those in W) in identification of the causal effect of A on Y. One estimation approach commonly used when outcome data are missing is inverse proportion censoring weighting (IPCW), which is described in more detail in Chapter 3.

In the Porto Alegre trial, follow-up data were collected at approximately 6, 12, and 36 months, with loss to follow-up occurring at frequencies of 11.5%, 23.8%, and 33.7%[1], respectively. Because the vast majority of losses at the six-month assessment were due to withdrawal of consent, the investigative team opted to discontinue following any participants lost

[1] The frequency of missing dental outcomes was higher (35.9%), as not all observed participants completed a dental evaluation.

at the six-month assessment. Therefore, in the causal diagram depicting the Porto Alegre trial (Figure 2.10), the complete case analysis is conditional on being present at both the six and 36-month assessments. Having a measured dental outcome was not contingent on being present at the 12-month assessment (20.6% of participants with missing 12-month data were later recovered). However, being absent at 12 months was a strong predictor of lacking dental data at 2-3 years (62% for those present versus 22% for those absent, among participants followed to at least six months).

In Figure 2.10, we allow that the intervention, A, could be a predictor of missingness. There is no strong theoretical argument to support that the intervention (providing training to health care workers) would structurally lead to a greater or lesser frequency of missing outcomes among clinic attendees enrolled in the trial. However, in a cluster-randomized trial of 20 independent clinics, it is plausible that an imbalance in follow-up proportions could arise empirically. In Chapter 2.1, we stated that, "losses were similar by allocation status, making a meaningful bias due to censored outcomes unlikely." The proportion of missing outcome data in the intervention group was 34.2%, compared to 37.8% in the control group. Thus, the results of the IPCW and the complete case analysis should be similar.

That said, the possibility of an empirical imbalance increases over multiple subgroups. For example, among those who named the health center as their main source of feeding guidance, the proportion of missing outcome data in the intervention group was 25.4%, compared to 16.7% in the control group.

One of the main oral health findings of the Porto Alegre trial was an apparent protective effect of the intervention among those mothers who reported strong connections to their health center. To account more fully for missing data, we must also consider individuals who did have dental examinations but were missing information on the variables that defined the subgroups: change of health center (39 observations) and main source of feeding guidance (42 observations). Therefore, multiple imputation (MI) was utilized to assign values to these individuals based on their observed baseline characteristics.

The predicted probabilities of being assigned to either subgroup were estimated from baseline covariates using Super Learner (van der Laan, 2007; Polley, 2011). The probability of having observed dental information was a two-part probability, also estimated using Super Learner: the probability of being observed at the six-month assessment, given baseline characteristics, multiplied by the probability of having observed dental data given baseline characteristics and presence at 12-month assessment, conditional on being observed at the six-month assessment. Point estimates were averaged over 100 imputations. Bootstrap re-sampling was performed 1000 times to estimate non-parametric 95% confidence intervals. This represents a slight inconsistency in methodology from Chapter 2.1, in that the bootstrap method assumes each observation to be independent rather than clustered by health center. However, because relatively little intra-cluster correlation was observed (Table 2.2), this concern is more theoretical than practical. The results of the imputed and weighted analysis are shown in Table 2.6.

The intervention effects seen among mothers with strong ties to the health center are attenuated and no longer statistically significant (compare: Figure 2.3, Table 2.3). Of note, if the IPCW analysis is performed without multiple imputation (i.e. excluding individuals lacking measured values for the subgroups variables), the results corroborate the findings from the unweighted complete-case analysis of Chapter 2.1 (data not shown). For example, the relative risk of S-ECC among those naming the health center as the main source of guidance would be 0.51 (95% CI: 0.25, 0.92). This small shift in the results could be due to the weighting, the imputation, selection bias, or a combination of these. The IPCW analysis places a relatively large weight on the individuals with imputed values for the health center connections variables that defined the subgroups (Table 2.7).

These individual were weighted heavily because those *missing* values for the subgroup variables, but *having* measured dental outcomes, tended to be those who were not collected at the 12-month assessment but recovered at the 36-month assessment. Because being missing at 12 months was a strong predictor of missing dental data, anyone recovered was given a large weight to "stand in" for the observations missing at both time points (mean weight = 2.93). Among these influential observations, there was a higher prevalence of S-ECC in the intervention group, at least among those frequently assigned a positive value for same health center or health center guidance (Table 2.8).

Is it appropriate to include missingness status at the 12-month assessment as part of the mechanism that predicts censoring probability? Those observations up-weighted the most in the analysis (missing at 12 months but later recovered) might not be representative of those lost to follow-up (Table 2.9). For many characteristics, the "recovered" population stands out as unlike other individuals with missing data. For example, these individuals were, on average, most likely to be mothers of more than one child and most likely to have a low household income. Notably, 60% of these women were from the two southernmost regions of the city. Most likely, this is a result of factors unrelated to the participants - a fieldworker assigned to this region "lost" a number of completed 12-month questionnaires.

To address these concerns, the weighted and imputed analysis was repeated, but whether or not a participant was present at the 12-month assessment no longer was included as a predictor of having dental data. As expected, this reduced the average weights assigned to those lacking responses for subgroup variables (Table 2.10). The effect measure modification seen in the complete-case analysis was mirrored closely (Table 2.11). There also appeared to be an efficiency gain, as confidence intervals were slightly narrowed.

The estimates obtained from the complete case analysis and those obtained following imputation and weighting under two different protocols differ subtly. Which of these represents the "best" estimate of the impact of the intervention depends on the relative merits and limitations of the un-verifiable assumptions supporting each. The complete cases analysis was limited to a specific subset of the data (those with measured values on the outcome *and* subgroup variables). It is quite plausible that excluded observations differ systematically from those observed, violating the MCAR assumption. In the imputed and weighted analyses, we assume that missingness and the subgroup variables can be predicted accurately from measured variables, and that there are no additional unmeasured predictors (i.e. missingness is random within strata of the measured predictors). We assume, in part, that certain individuals in the observed data set are better proxies for those missing than others, and that by up-weighting these observations, we can reasonably approximate the results we would have obtained had the entire initial cohort been measured. We can question whether this assumption is well supported. On average, the prediction method used for imputation estimated a 38.4% probability of changing health centers for those who actually did and a 30.5% probability of changing health centers for those who did not - not greatly different from the marginal probability of 33.1%. Without strong predictors, the imputed values have a high probability of resulting in misclassification, which could dilute any effect modification in the imputed data set.

These missing data approaches do not account for empirical confounding that might arise in a cluster-randomized trial, given the coarse nature of the randomization scheme. One of the theoretical benefits of a randomized trial is that the intervention is allocated independent of measured and unmeasured characteristics of the participants. In practice, this is not certain in samples of limited size (e.g. 20 clusters). However, we did not observe a large imbalance of any individual baseline characteristics by allocation status (Table 2.1).

Summary:

There is a theoretical concern that the subgroup effects reported in Chapter 2.1 could be the result of selection bias originating from losses to follow-up. However, estimates from an imputed and weighted analysis were consistent with the complete case findings. The imputed and weighted analysis yielded results much closer to the complete case finding if presence at the 12-month assessment was excluded as a potential predictor of having an observed outcome. In each of these analyses, validity is dependent on assumptions about the causal mechanisms responsible for the missing observations that are not testable from data. While considerable caution is required in the interpretation of any secondary analysis, it does not appear likely that selection bias due to missing data is driving the apparent caries protective effect of the Porto Alegre Ten Steps intervention among mothers with strong connections to their local health centers.

Chapter 2. Section 2.
Secondary and Exploratory Analyses Related to the Porto Alegre Ten Steps Trial

Part C.
Non-Differential Loss to Follow-up with Respect to Treatment Can Result in a Biased Estimate of the Risk Difference

Here, I explore an additional, more general concern related to missing data. If the parameter of interest is not the relative risk (RR), but the risk difference (RD), the latter can be biased even in situations in which the former is not. The risk difference changes with the risk[2] of the outcome in the untreated (control) group, even if the RR is constant. In most studies, a difference in baseline risk in the observed and missing populations is likely. Thus, even if loss to follow-up is non-differential with respect to treatment (exposure) status, the RD estimated from a complete case analysis will be biased under plausible circumstances. I demonstrate this by means of an example.

Compare Figures 2.9 and 2.11. When loss to follow-up is non-differential (Figure 2.11), the treatment, A, is not a cause of having an observed outcome, D. Even if the analysis is conditional on D=1 (i.e. D=1 when the outcome, Y, is observed), the observed A-Y relationship is not expected to be biased with respect to the unconditional analysis, because no back door paths connect A and Y.

In the following hypothetical example, the complete-case analysis does not lead to a biased estimate of the RR.

Let: The probability of A = 0.5; The probability of W = 0.5
The probability of missingness (D = 0) increases by 1.5-fold when W = 1.
A and W are marginally independent, but both A and W affect Y:

If A=0 & W=0, the probability of Y = 0.16	If A=1 & W=0, the probability of Y = 0.12
If A=0 & W=1, the probability of Y = 0.24	If A=1 & W=1, the probability of Y = 0.18

Tables 2.13 and 2.14 show the results obtained under this scenario if the overall probability of missingness is 0.25 in a theoretical population (n = 10,000). Table 2.13 shows the "full" population, where outcomes are known for all individual, regardless of D. Here, the "true" RR and RD can be calculated: RR = 0.75 and RD = -5%.[3] If the analysis is restricted to those strata where D = 1 (Table 2.14), the observed RR is not changed (0.75), but the RD is biased (-4.933%).

This small bias in the RD is more pronounced as the probability of missingness increases (Figure 2.12) and as strength of the W-D relationship increases (Figure 2.13). The bias also slightly increases as the occurrence of Y rises in the untreated group (Figure 2.14). The bias

[2] Here, "risk" refers generically to the proportion of the outcome in a given population; the bias-inducing situation shown here applies to rates, the prevalence, and other similar measures.
[3] Note: While W is not an effect modifier of the RR of A on Y, the RD does differ by strata of W (-4% when W=0 and -6% when W=1). I assume that the marginal (i.e. population-level) RD is the parameter of interest.

grows in magnitude in absolute terms[4] as the strength of the A-Y relationship intensifies (Figure 2.15).

Would this small bias in the RD be of any practical significance in relation to the sampling variability present in most epidemiologic studies? See the example below:

As before, let: The probability of A = 0.5; The probability of W = 0.5
The probability of missingness (D = 0) increases by 1.5-fold when W = 1.
A and W are marginally independent, but both A and W affect Y:

If A=0 & W=0, the probability of Y = 0.16 If A=1 & W=0, the probability of Y = 0.12
If A=0 & W=1, the probability of Y = 0.24 If A=1 & W=1, the probability of Y = 0.18
Now, let the overall probability of missingness be **60%**

This would lead to a "true" RD of -5%, but a biased RD of -4.5% when the analysis is restricted to only observed individuals. Due to sampling variability, however, any given sample can differ from these expected parameters.

In 1,000,000 simulated sample populations of size 1000 under the data generating equations above (expected missingness = 60%), the mean RD estimated from a complete case analysis (restricted to D = 1 observations) in these populations was -4.50368%. This observed (biased) RD estimate differed from the "true" (unbiased) RD of -5% by more than 1/2 a percentage point in either direction in 892,038 of the 1,000,000 simulations (89.2%). However, in 1,000,000 simulated sample populations of size 400 under the same data generating equations but with no missingness (mean RD = -5.00006%), the observed RD estimate differed from the "true" RD of -5% by more than 1/2 a percentage point in either direction in 894,580 of the 1,000,000 simulations (89.5%): an even greater difference than when data were missing. However, the analysis with missing observations yielded a RD estimate ≥0% in 10.9% of the simulations, which occurred in only 9.5% of the simulations with no missingness. Therefore, it is somewhat more likely that the missing data scenario would result in incorrectly failing to reject a false null hypothesis.

This small bias is more pronounced when the magnitude of the RD is greater:

As before, let: The probability of A = 0.5; The probability of W = 0.5
The probability of missingness (D = 0) increases by 1.5-fold when W = 1.
The overall probability of missingness is 60%
A and W are marginally independent.
Both A and W affect Y, but let the effect of A on Y be **greater**:

If A=0 & W=0, the probability of Y = 0.16 If A=1 & W=0, the probability of Y = **0.48**
If A=0 & W=1, the probability of Y = 0.24 If A=1 & W=1, the probability of Y = **0.72**

Here, the "true" causal RR of A on Y is 3.0, and the "true" causal RD is 40%.[5] Restricted to complete cases, the observed RR would not differ (3.0), but the observed RD would be reduced to 36%. In 1,000,000 simulated sample populations, the mean RD estimated from a complete case analysis (restricted to D = 1 observations) differed from the "true" (unbiased) RD of 40% by

[4] However, the bias does not increase in relative terms.

[5] Note: As before, while W is not an effect modifier of the RR of A on Y, the RD does differ by strata of W (32% when W=0 and 48% when W=1). I assume that the marginal RD is the parameter of interest.

more than 4 percentage points in either direction in 534,295 of the 1,000,000 simulations (53.4%). In 1,000,000 simulated sample populations of size 400 under the same data generating equations, but with no missingness, the observed RD estimate differed from the "true" (unbiased) RD of 40% by more than 4 percentage points in either direction in 374,014 of the 1,000,000 simulations (37.4%). The analysis with missing observations yielded a RD estimate <30% in 13.7% of the simulations, which occurred in 9.0% of the simulations with no missingness.

These examples show that while, in theory, the RD will be biased in some situations where there is no bias of the RR, the magnitude of this bias is unlikely to be of practical significance unless the RD is large in magnitude and missingness is common. In fact, based on a simulation exercise, if the RD was small and the study sample size moderate, this bias was of little importance relative to sampling variability. Even when the RD was large, sampling variability frequently resulted in a deviation from the true RD equal in magnitude to the bias due to missing data.

Chapter 2 Figures and Tables

Figure 2.1. Flow diagram of the health center clusters and individual participants from enrollment to assessment

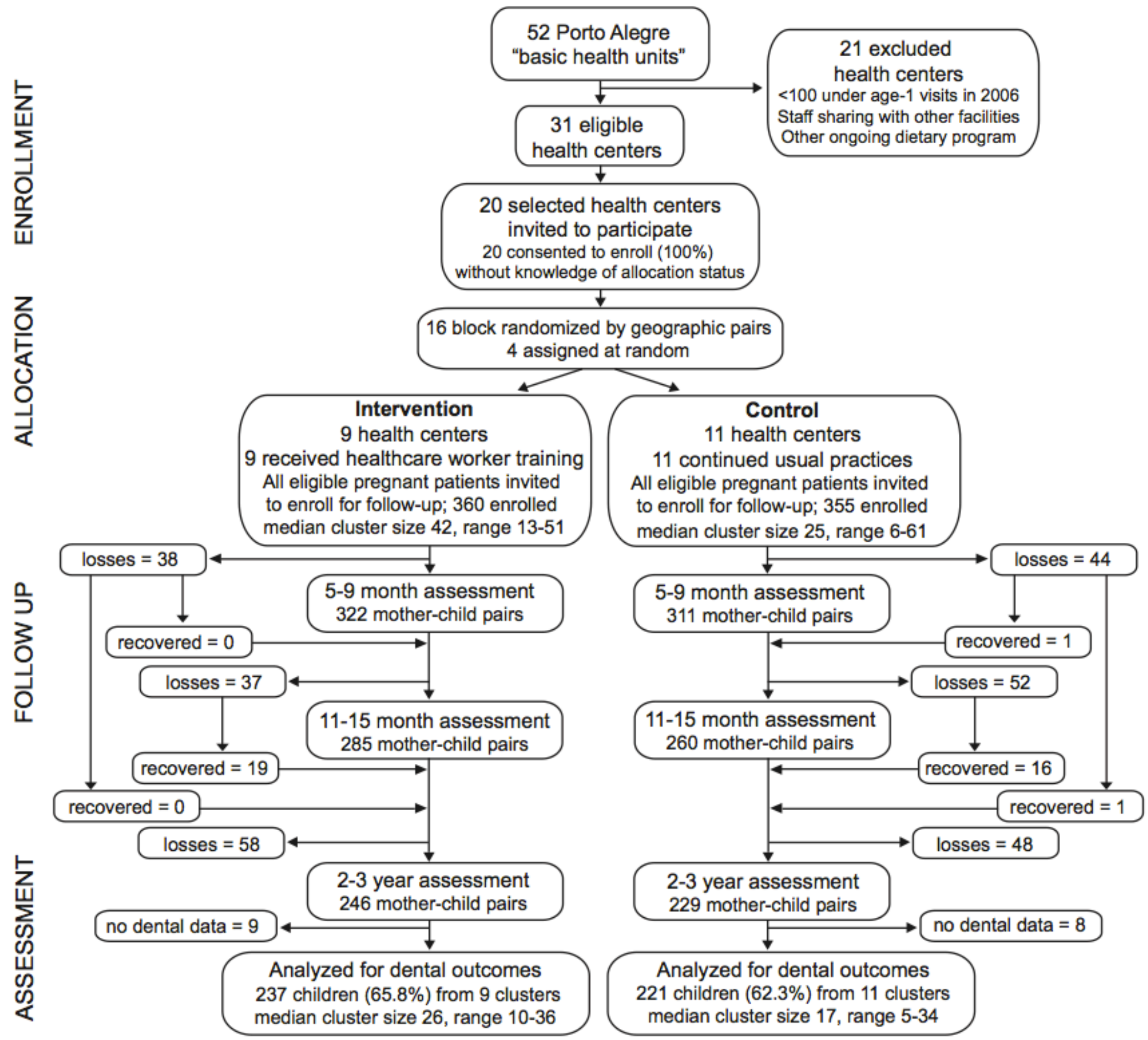


Table 2.1. Descriptive characteristics at baseline of individual participants with dental data

Characteristic	Intervention (n = 237)	Control (n = 221)
Maternal age at delivery, mean (SD) [range], years	27.1 (6.7) [15.5-43.9]	25.7 (6.6) [12.3-44.3]
Child age at dental exam, mean (SD) [range], years	3.2 (0.2) [2.6-3.8]	3.2 (0.2) [2.8-3.8]
Male child, No. (%)	119 (50.2)	114 (51.6)
Household members, mean (SD) [range]	4.1 (1.9) [1-11]	4.3 (2.3) [1-16]
Mother has previous children, No. (%)	140 (59.1)	119 (53.9)
Mother is literate, No. (%)	235 (99.2)	219 (99.1)
Mother has ≤ 8 y of formal education, No. (%)	111 (46.8)	103 (46.6)
Father has ≤ 8 y of formal education, No. (%) (n = 439)[a]	112 (49.8)	107 (50.0)
Household monthly income, mean (SD) [range], BRL (n = 444)[a]	1109 (707) [0-4100]	1061 (681) [0-3800]
Household income ≤ 3 times minimum monthly salary,[b] No. (%) (n = 444)[a]	181 (79.4)	178 (82.4)
Social class by ABIPEME index,[c] No. (%) (n = 457)[a]		
A1	0 (0)	0 (0)
A2	0 (0)	0 (0)
B1	8 (3.4)	7 (3.2)
B2	46 (19.5)	38 (17.2)
C	137 (58.1)	135 (61.1)
D	40 (17.0)	39 (17.7)
E	5 (2.1)	2 (0.9)
Self-identified maternal race, No. (%)		
white	144 (60.8)	112 (50.7)
black, mixed, or other	93 (39.2)	109 (49.3)
Maternal smoking status in pregnancy, No. (%)		
Never	134 (56.5)	111 (50.2)
Current	42 (17.7)	45 (20.4)
Former	61 (25.7)	65 (29.4)
Maternal BMI < 18.5 by self-reported pre-pregnancy weight, No. (%) (n = 443)[a]	15 (6.6)	15 (6.9)
Maternal BMI ≥ 25 by self-reported pre-pregnancy weight, No. (%) (n = 443)[a]	66 (29.1)	75 (34.7)

Abbreviations: ABIPEME, Brazilian Association of Economic Research Institutes; BMI, body mass index; BRL, Brazilian real
[a]Sample size < 458 for some variables due to missing data at baseline.
[b]Equivalent to approximately 900 US dollars in 2008 currency.
[c]Socioeconomic classification scale based on material possessions and education, A = highest status, E = lowest status.

Figure 2.2 Process outcomes by health center allocation status, as reported by mothers

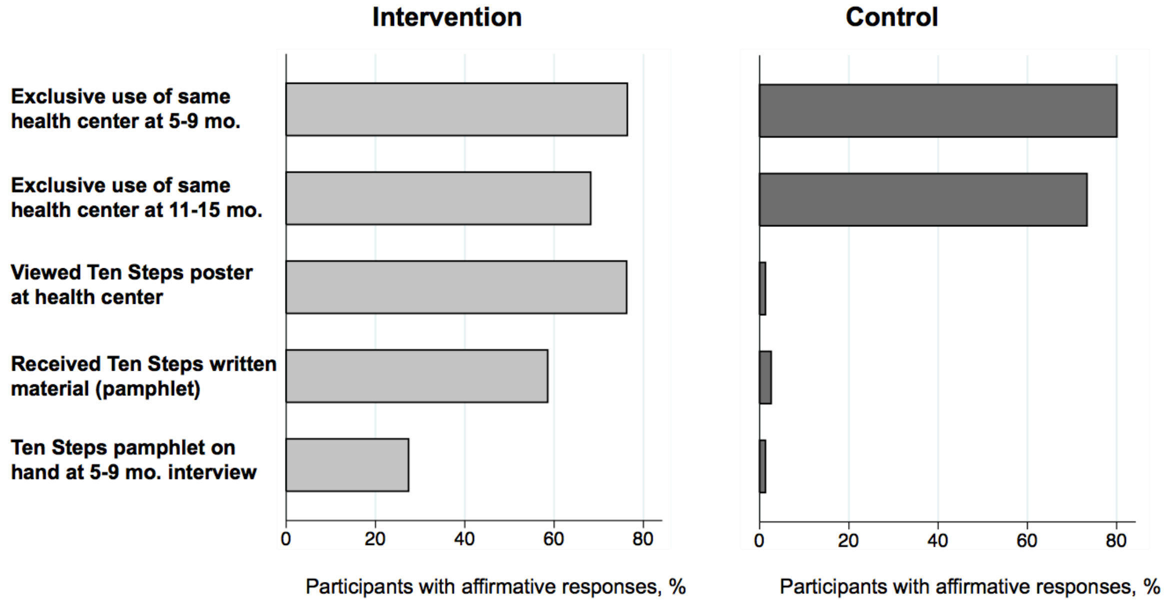


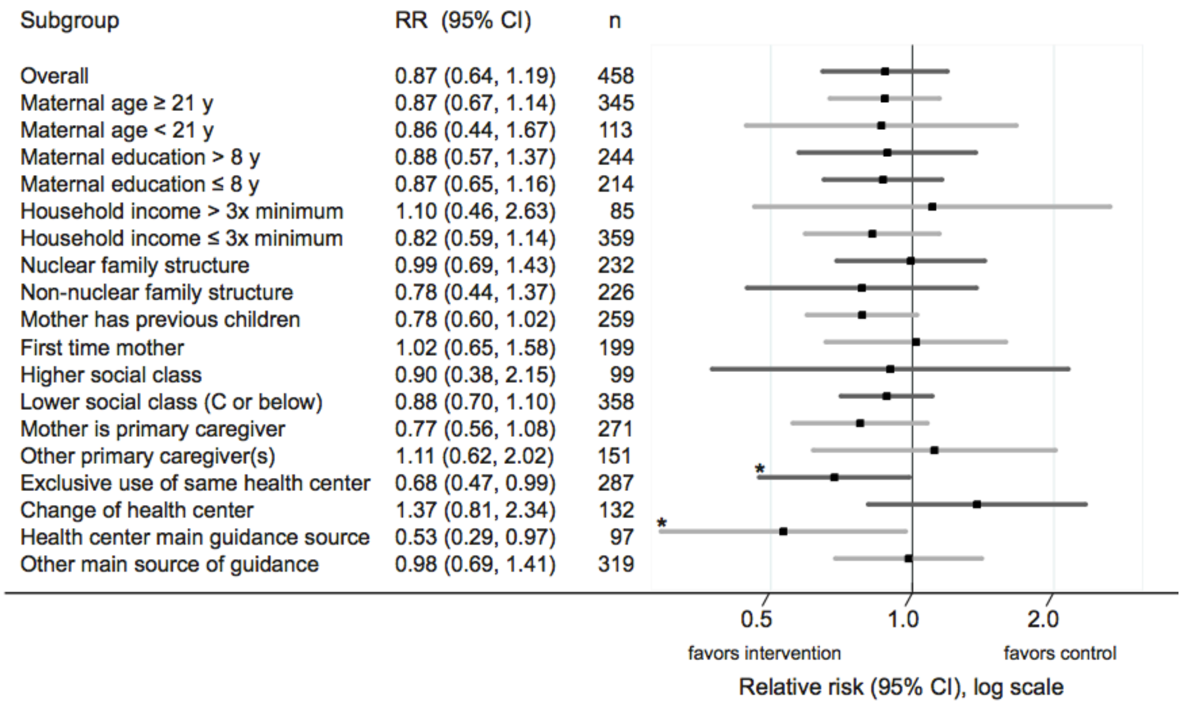
Figure shows the percentage of participating mothers reporting experience with process components of the intervention (e.g. health center attendance, interaction with printed materials) by allocation status of the health center from which they were recruited.

Table 2.2 Dental caries outcomes by allocation status

DENTAL CARIES OUTCOMES (binary)	Intervention	Control	ICC	Relative risk (95% CI)	Risk difference (95% CI)
Early childhood caries	124/237	126/221	0.014	0.92 (0.75, 1.12)	-5% (-16, 6)
Cavitated decay	88/237	93/221	0.020	0.88 (0.66, 1.17)	-5% (-16, 7)
Severe early childhood caries	76/237	81/221	0.015	0.87 (0.64, 1.19)	-5% (-15, 6)
DENTAL CARIES OUTCOMES (count)	Intervention	Control	ICC	dmfs ratio[a] (95% CI)	Mean difference (95% CI)
dmfs, mean (SD), any decay	2.8 (5.4)	3.6 (6.9)	0.010	0.78 (0.53, 1.15)	-0.8 (-2.2, 0.6)
dmfs, mean (SD), cavitated decay only	2.1 (5.0)	3.0 (6.8)	0.010	0.70 (0.44, 1.12)	-0.9 (-2.2, 0.4)

Abbreviations: CI, confidence interval; dmfs, decayed missed filled surfaces index; ICC, inter-cluster correlation coefficient; SD, standard deviation.
[a]dmfs ratio = dmfs intervention / dmfs control.

Figure 2.3. Impact of the intervention on severe early childhood caries by subgroups



Relative risk estimates for S-ECC across demographic and behavioral subgroups are shown. Squares represent point estimates; widths of bars show confidence intervals; shading denotes pairs of related strata. Some pairs total less than 458 due to missing data. * = p<0.05
Abbreviations: RR, relative risk; CI, confidence interval

Table 2.3 Subgroup results for severe-ECC, adjusted for empirical confounding

Group	n	Unadjusted RR	95% CI	n	Adjusted [a] RR	95% CI
Overall	458	0.87	0.64, 1.19	429	0.88	0.65, 1.18
Exclusive use of same health center	287	0.68*	0.47, 0.99	266	0.72	0.49, 1.06
Change of health center	132	1.37	0.81, 2.34	126	1.31	0.75, 2.29
Health center main source of feeding guidance	97	0.53*	0.29, 0.97	91	0.53*	0.29, 0.99
Other main source of feeding guidance	319	0.98	0.69, 1.41	298	0.95	0.66, 1.38

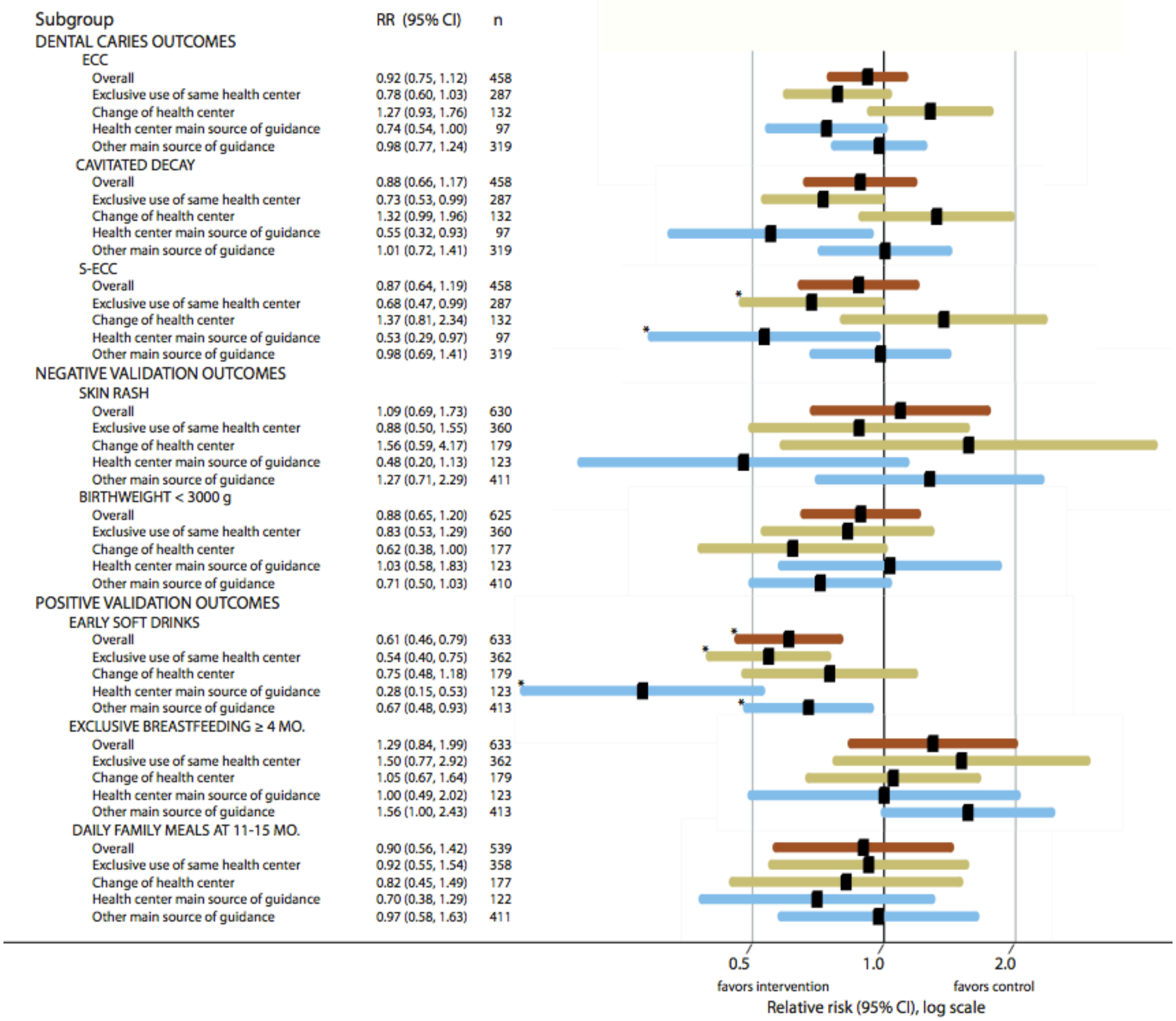
[a] Adjusted for: maternal education, maternal smoking in pregnancy, child gender, household income, maternal pre-pregnancy BMI<18.5, and child age at dental assessment
* = p<0.05

Table 2.4 Validation outcomes by allocation status

	Intervention	Control	ICC	Relative risk (95% CI)	Risk difference (95% CI)
NEGATIVE VALIDATION OUTCOMES (binary)					
Skin rash	45/320	40/310	0.009	1.09 (0.69, 1.73)	1% (-5, 8)
Birth weight < 3000 g	77/320	83/305	0.006	0.88 (0.65, 1.20)	-3% (-11, 5)
POSITIVE VALIDATION OUTCOMES (binary)					
Early soft drinks (< 6mo)	74/322	118/311	0.006	0.61* (0.46, 0.79)	-15%* (-23, -7)
Exclusive breastfeeding ≥4mo	87/322	65/311	0.036	1.29 (0.83, 1.99)	6% (-4, 16)
Daily Family Meals (11-15mo)	61/282	62/257	0.036	0.90 (0.56, 1.42)	-2% (-13, 8)

Abbreviations: CI, confidence interval; ICC, inter-cluster correlation coefficient. * = p<0.05

Figure 2.4. Impact of the intervention on selected health outcomes by subgroups related to maternal connections to health center



Relative risk estimates for dental caries, negative validation, and positive validation outcomes by subgroups. Squares represent point estimates; widths of bars show confidence intervals; color denotes pairs of related strata. Some strata vary in number due to missing data. * = p<0.05   Abbreviations: RR, relative risk; CI, confidence interval

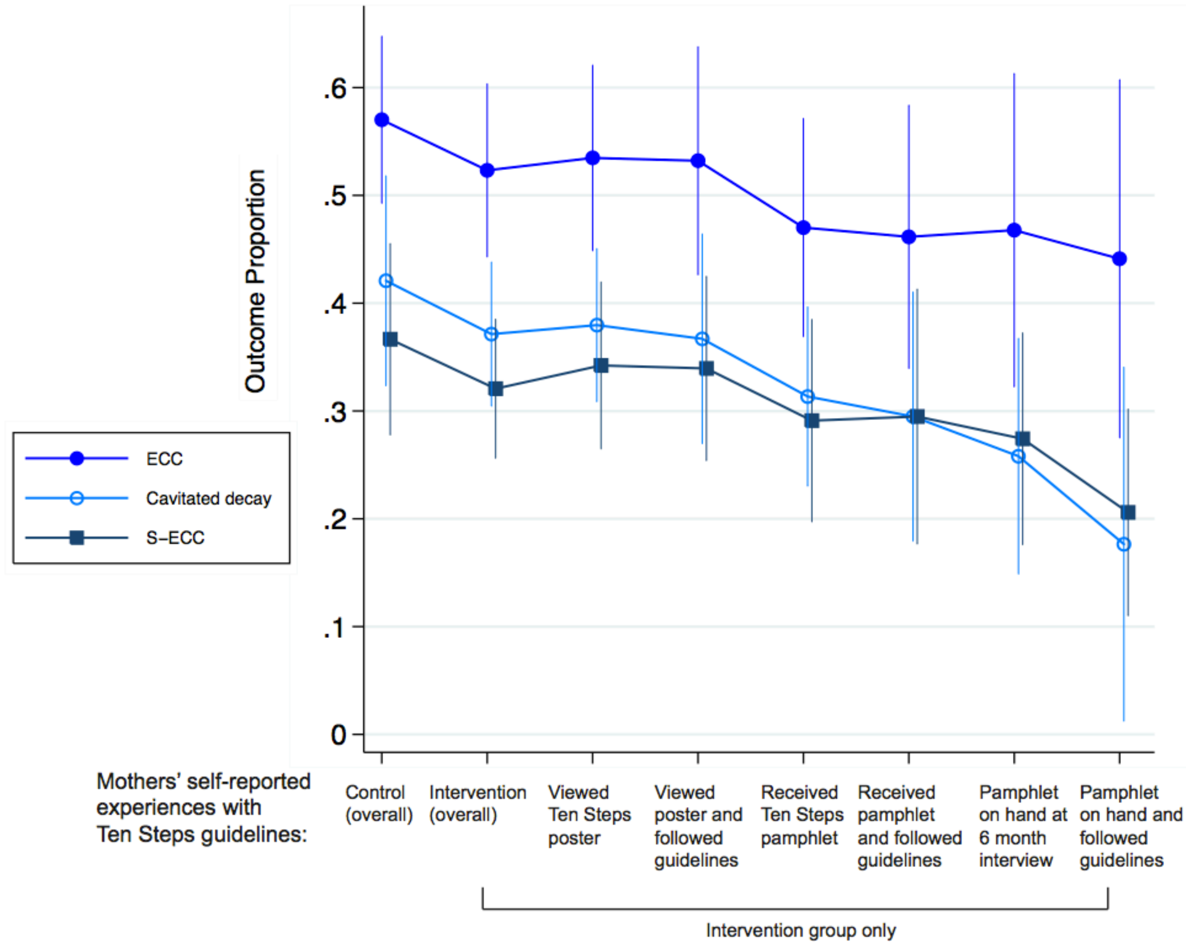
Figure 2.5. A logic model of generic program components
Inputs → Activities → Outputs → Short-Term Outcomes → Intermediate-Term Outcomes

Table 2.5. Selected process components from the Porto Alegre Ten Steps Intervention

INPUTS			
Process Component	Variable to Measure	Issues and Challenges	Available Data
Training and education for healthcare workers (the intervention)	Training content	Few: documented and well-described	Presenter notes, slides
	Sites receiving training	None: all intervention sites trained	Site visits documented
	Staff attendance at the training	Those attending the session might not reflect those seeing patients if staff turnover frequent	Not documented
Posters and material given to health centers (the intervention)	Number of posters and pamphlets delivered	Few: easy to count	Not documented
ACTIVITIES			
Process Component	Variable to Measure	Issues and Challenges	Available Data
Infant feeding recommendations included in patient consultations	Number and duration of counseling sessions focusing on feeding practices	Places burden on healthcare providers to collect this information; Charts not accessible to study team; If collected from mothers, recall may be unreliable	Mothers asked: "Who is your most important source of infant feeding advice?" At best, this is indirect measure influenced by other factors, as well
Posters seen by mothers attending the clinics	Posters in visible place and noticed	Could have gone to clinics to check for posters; Mothers' recall may be unreliable; Seeing posters ≠ reading	Did not document whether health centers put up posters; Did ask mothers whether they saw them
Informational Material Given to Mothers	Number of pamphlets distributed; Number received by mothers	Mothers' recall may be unreliable; Having pamphlets ≠ reading; Distribution ≠ distribution to target patients	Mothers asked whether were given pamphlet and whether currently have it physically on-hand
OUTPUTS			
Process Component	Variable to Measure	Issues and Challenges	Available Data
Increase in knowledge and self-efficacy of healthcare workers	Difficult to quantify	Very difficult to measure; healthcare workers not made available for interviews or surveys in this study	Not documented
Increase in knowledge and self-efficacy of mothers	Difficult to quantify	Very difficult to measure; planned focus-group interviewers were postponed and later cancelled	Mothers asked whether they followed the guidance they were given (regardless of source)

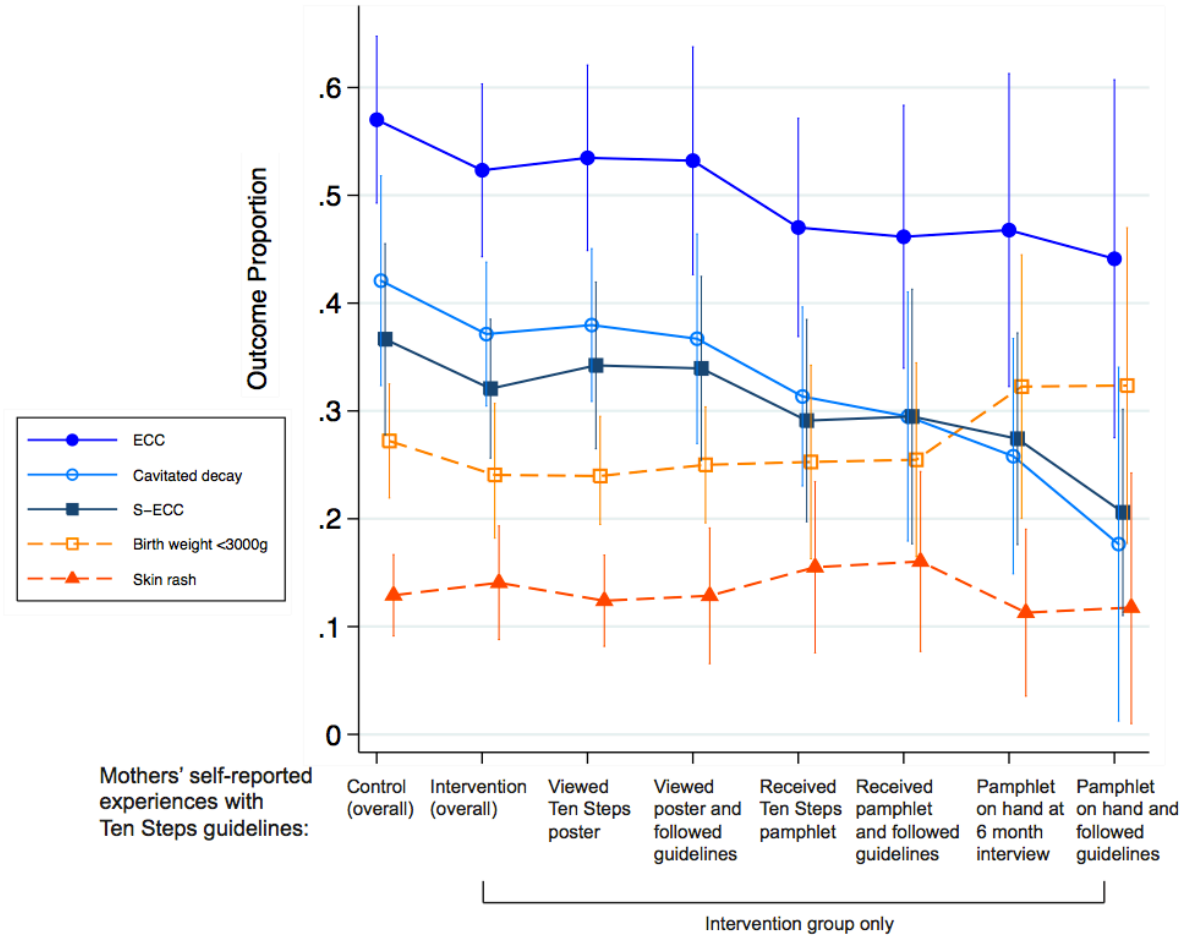
OUTPUTS (CONTINUED)			
Behavior change in infant feeding practices	Infant feeding habits	Common challenges in measuring routine diet; food frequency questionnaires not appropriate in this population; Social desirability bias; Mothers' recall may be unreliable	Many variables collected from survey and 24-hour recalls
OUTCOMES (SHORT-TERM)			
Process Component	Variable to Measure	Issues and Challenges	Available Data
Various diet quality, growth, and general health outcomes in infancy	Many: dietary intakes, anthropometry, and reported symptoms and medications	Usual measurement issues related to recall and questionnaire interpretation	Many variables collected from baseline through 2-3 years on up to 715 participants
OUTCOMES (INTERMEDIATE-TERM)			
Process Component	Variable to Measure	Issues and Challenges	Available Data
Dental caries experience at age 2-3	Affected tooth surfaces	Have strict protocol and case-definitions, though clinical diagnosis not 100% reliable	Dental health assessments available for 458 children

Figure 2.6. Outcome trends by increasing exposure to process components of the intervention: dental caries outcomes



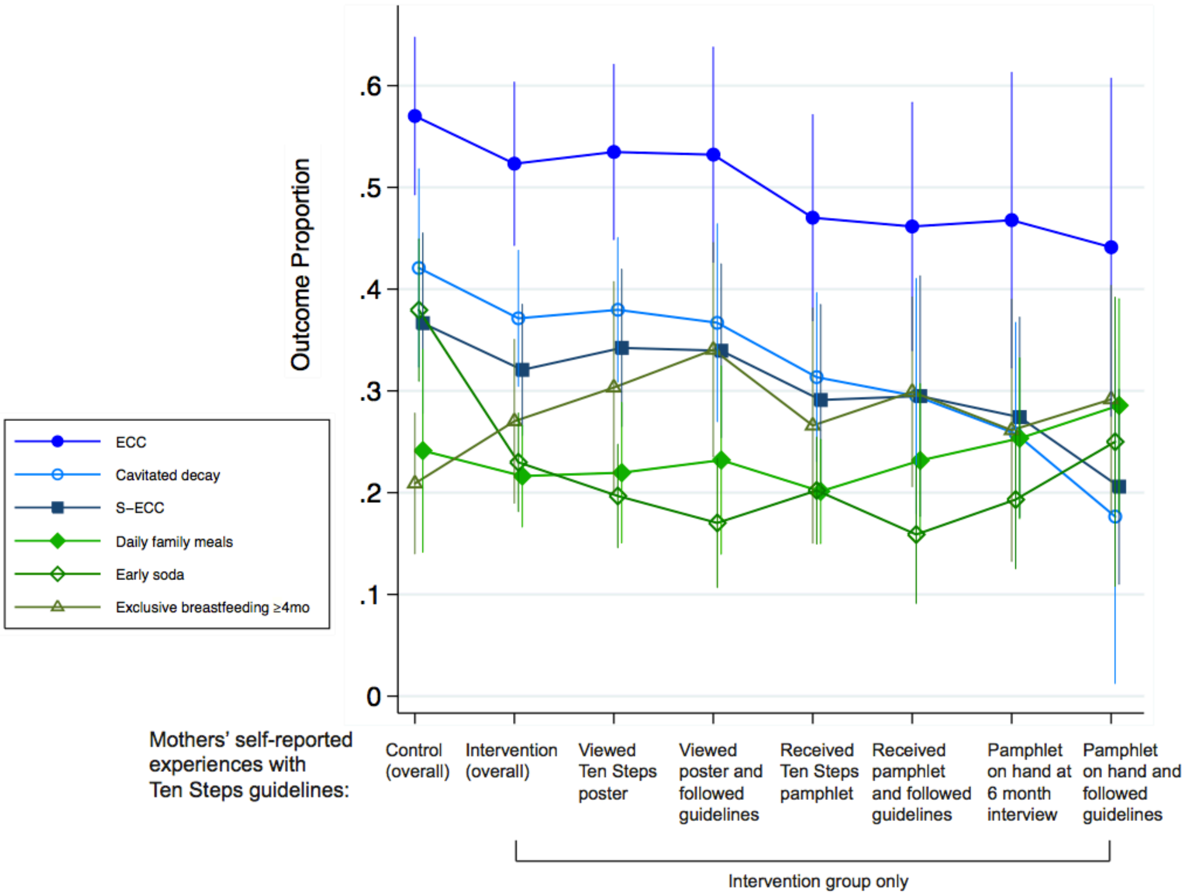
Proportions of children with the indicated health conditions are shown across increasing levels of experience and/or interaction with the process components of the intervention. Vertical lines represent 95% confidence intervals. Abbreviations: ECC, early childhood caries; S-ECC, severe early childhood caries

Figure 2.7. Outcome trends by increasing exposure to process components of the intervention: dental caries and negative validation outcomes



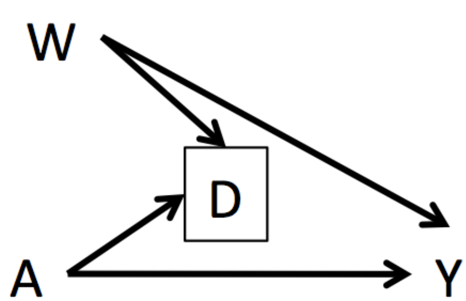
Proportions of children with the indicated health conditions are shown across increasing levels of experience and/or interaction with the process components of the intervention. Vertical lines represent 95% confidence intervals. Abbreviations: ECC, early childhood caries; S-ECC, severe early childhood caries

Figure 2.8. Outcome trends by increasing exposure to process components of the intervention: dental caries and positive outcomes



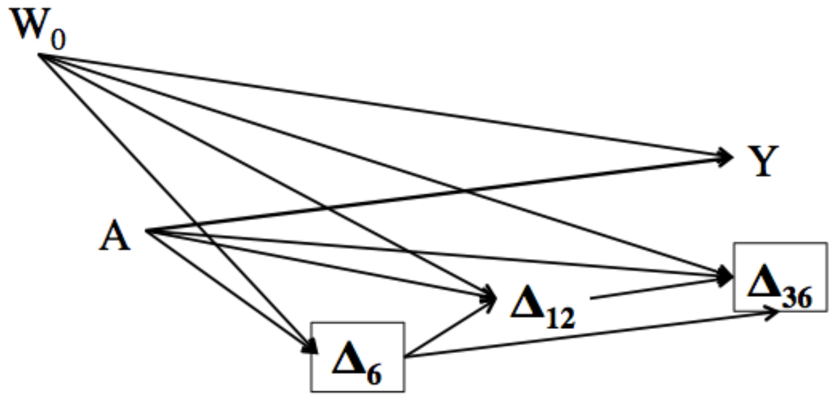
Proportions of children with the indicated health conditions are shown across increasing levels of experience and/or interaction with the process components of the intervention. Vertical lines represent 95% confidence intervals. Abbreviations: ECC, early childhood caries; S-ECC, severe early childhood caries

Figure 2.9. The complete-case analysis can lead to a biased effect estimate



The effect of treatment, A, on the outcome, Y, will be estimated with bias when conditional on having the outcome measured, D, under this plausible scenario.

Figure 2.10. The complete-case analysis can lead to a biased effect estimate



The complete case analysis of the Porto Alegre trial is conditional on being present at both 6 and 36 months. This could lead to a biased estimate of the impact of the intervention.

Table 2.6. Impact of the Porto Alegre Ten Steps intervention on dental caries, overall and by subgroup, IPCW weighted and subgroup status imputed (if missing)

	Relative Risk	95% CI	Risk Difference	95% CI
Overall				
ECC	0.95	0.78, 1.15	-2.9%	-13.8%, 7.4%
Cavitation	0.91	0.70, 1.16	-3.6%	-13.3%, 5.8%
S-ECC	0.90	0.69, 1.20	-3.4%	-12.7%, 6.1%
Exclusive use of same health center				
ECC	0.84	0.66, 1.04	-9.5%	-21.1%, 2.2%
Cavitation	0.79	0.59, 1.06	-9.4%	-20.3%, 2.3%
S-ECC	0.76	0.54, 1.05	-9.5%	-20.7%, 1.8%
Change of health center				
ECC	1.26	0.90, 1.83	12.0%	-5.5%, 29.4%
Cavitation	1.29	0.82, 2.21	9.4%	-7.3%, 27.1%
S-ECC	1.38	0.85, 2.48	10.3%	-5.1%, 26.5%
Health center main source of guidance				
ECC	0.79	0.53, 1.13	-13.9%	-35.0%, 7.2%
Cavitation	0.61	0.35, 1.01	-19.6%	-38.6%, 0.6%
S-ECC	0.63	0.29, 1.18	-14.2%	-34.3%, 5.3%
Other main source of guidance				
ECC	1.01	0.80, 1.25	0.7%	-11.6%, 11.5%
Cavitation	1.03	0.78, 1.34	1.2%	-9.6%, 11.7%
S-ECC	0.99	0.71, 1.37	-0.4%	-11.1%, 10.7%

Table 2.7. Inverse probability censoring weights assigned according to measurement status (missing vs. observed) of subgroup variables

	Missing Change of Health Center (but have dental data)	Missing Feeding Guidance Source (but have dental data)	All Observations with Dental Data
Mean weight	2.78	2.68	1.57
Median weight	2.49	2.43	1.45
Range	1.35 - 7.18	1.17 - 7.18	1.11 - 7.18
% of total weight	15.1%	15.7%	100%
% of observations	5.5%	5.8%	64.1%

Note: Weights averaged over 100 multiple imputations

Table 2.8. Weighted S-ECC occurrence if missing subgroup variables but having dental data

Subgroup	n	n x weight	partially weighted[1] S-ECC prevalence	fully weighted [2] S-ECC prevalence
Exclusive use of same health center				
Intervention	12.8	32.0	0.406	0.378
Control	13.7	43.7	0.323	0.265
Health center main source of guidance				
Intervention	3.5	8.2	0.384	0.387
Control	4.8	17.0	0.175	0.141

Note: All values averaged over 100 multiple imputations

[1] $\sum(\text{S-ECC}_i \times \text{mean}(n)_i) / \sum(\text{mean}(n)_i)$

[2] $\sum(\text{S-ECC}_i \times \text{mean}(n)_i \times \text{weight}_i) / \sum(\text{mean}(n)_i \times \text{weight}_i)$

Table 2.9. Baseline characteristics by missingness category

Characteristic	Missing at 6, 12, and 36-month assessment n = 80	Missing after 6-month assessment and not recovered n = 177	Missing after 6-month assessment but recovered n = 35	Never missing n = 422
Mother has ≤ 8 years of formal education	43 (54%)	83 (47%)	16 (46%)	198 (47%)
Social class C or below	67 (85%)	144 (81%)	29 (83%)	329 (78%)
Mother pre-pregnancy BMI ≤ 18.5	9 (11%)	24 (14%)	6 (18%)	67 (16%)
Mother has one or more previous child	45 (56%)	93 (53%)	25 (71%)	234 (55%)
Income below 3-times minimum salary	61 (82%)	145 (84%)	31 (89%)	327 (80%)
Mother smoking during pregnancy	20 (25%)	35 (20%)	9 (26%)	78 (18%)
Resides in southern region of the city	24 (30%)	39 (22%)	21 (60%)	87 (21%)
S-ECC	*missing*	*missing*	11 (31%)	146 (35%)

Table 2.10. Weighted S-ECC occurrence if missing subgroup variables but having dental data; presence at the 12-month assessment not included in missingness mechanism

Subgroup	n	n x weight	partially weighted[1] S-ECC prevalence	fully weighted[2] S-ECC prevalence
Exclusive use of same health center				
Intervention	13.1	20.3	0.412	0.421
Control	13.7	22.8	0.315	0.305
Health center main source of guidance				
Intervention	3.5	5.3	0.362	0.352
Control	4.8	8.0	0.193	0.188

Note: All values averaged over 100 multiple imputations

[1] $\sum(\text{S-ECC}_i \times \text{mean}(n)_i) / \sum(\text{mean}(n)_i)$

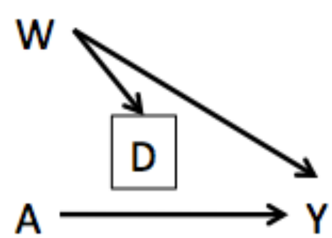
[2] $\sum(\text{S-ECC}_i \times \text{mean}(n)_i \times \text{weight}_i) / \sum(\text{mean}(n)_i \times \text{weight}_i)$

Table 2.11. Impact of the Porto Alegre Ten Steps intervention on dental caries, overall and by subgroup, IPCW weighted and subgroup status imputed (if missing); presence at the 12-month assessment not included in missingness mechanism

	RR	95% CI	RD	95% CI
Overall				
ECC	0.92	0.78, 1,10	-4.3%	-13.3%, 5.4%
Cavitation	0.87	0.70, 1.08	-5.5%	-14.8%, 3.0%
S-ECC	0.87	0.66, 1.11	-4.9%	-13.8%, 3.5%
Exclusive use of same health center				
ECC	0.81*	0.65, 0.99	-11.5%	-22.7%, 0.6%
Cavitation	0.75*	0.54, 0.97	-11.8%*	-24.5%, -1.2%
S-ECC	0.72*	0.51, 0.97	-11.4%*	-22.2%, -1.3%
Change of health center				
ECC	1.25	0.91, 1.79	12.0%	-4.7%, 28.7%
Cavitation	1.27	0.83, 2.05	9.1%	-7.4%, 25.7%
S-ECC	1.35	0.83, 2.19	9.8%	-6.2%, 25.0%
Health center main source of guidance				
ECC	0.74	0.52, 1.00	-18.1%	-36.5%, 0.3%
Cavitation	0.52*	0.30, 0.82	-26.8%*	-45.0%, -8.7%
S-ECC	0.54*	0.26, 0.93	-19.3%*	-36.3%, -2.5%
Other main source of guidance				
ECC	1.00	0.82, 1.22	0.1%	-10.5%, 10.6%
Cavitation	1.03	0.77, 1.31	1.0%	-10.2%, 10.6 %
S-ECC	0.98	0.72, 1.30	-0.8%	-11.5%, 8.8%

* = p<0.05

Figure 2.11. Non-differential loss to follow-up with respect to treatment status



The estimated effect of treatment, A, on the outcome, Y, can be estimated without bias from the complete case analysis (e.g. conditional on D=1) if A is not a cause of D.

Table 2.13. Contingency table for the "full" population (independent of D)

	Y = 1	Y = 0
A = 1	750	4250
A = 0	1000	4000

Table 2.13. Contingency table for the observed population (restricted to D=1 observations)

	Y = 1, D = 1	Y = 0, D = 1
A = 1, D = 1	555	3195
A = 0, D = 1	740	3010

Figure 2.12. The RD becomes more biased as the probability of missingness increases

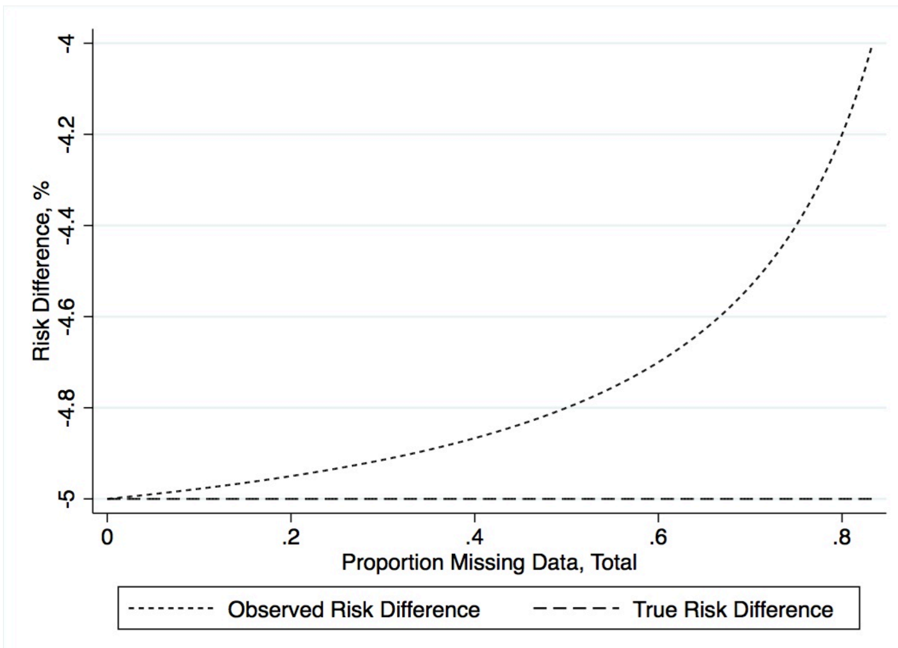


Figure 2.13. The RD becomes more biased as the strength of the W-D relationship increases

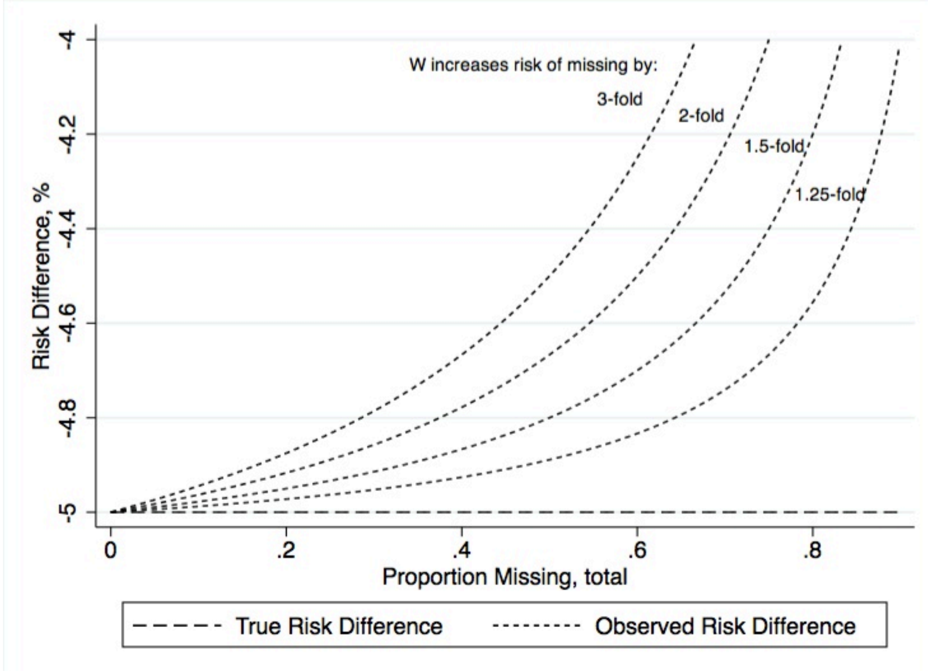


Figure 2.14. The RD becomes more biased as the baseline risk of Y increases

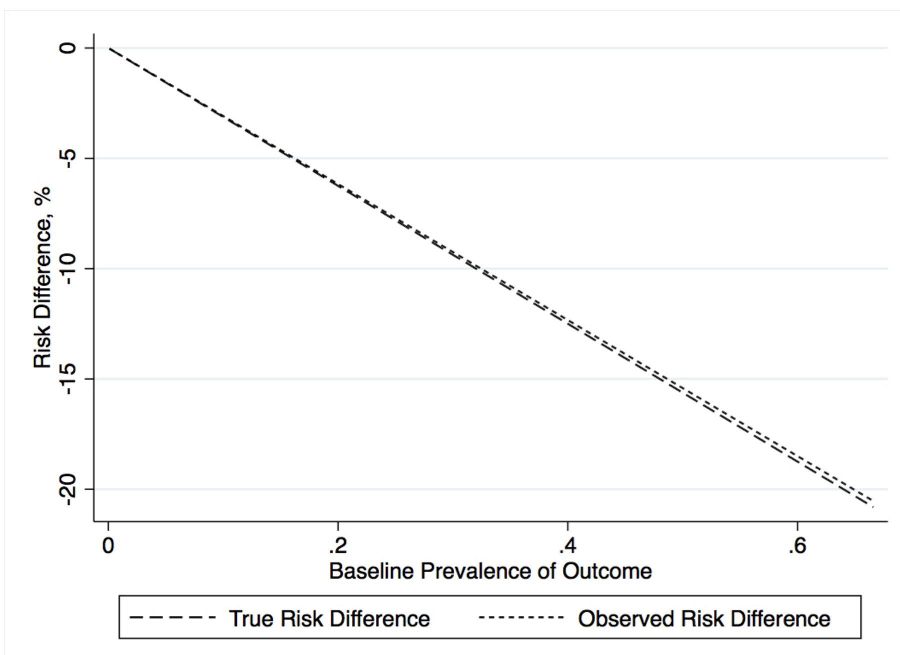
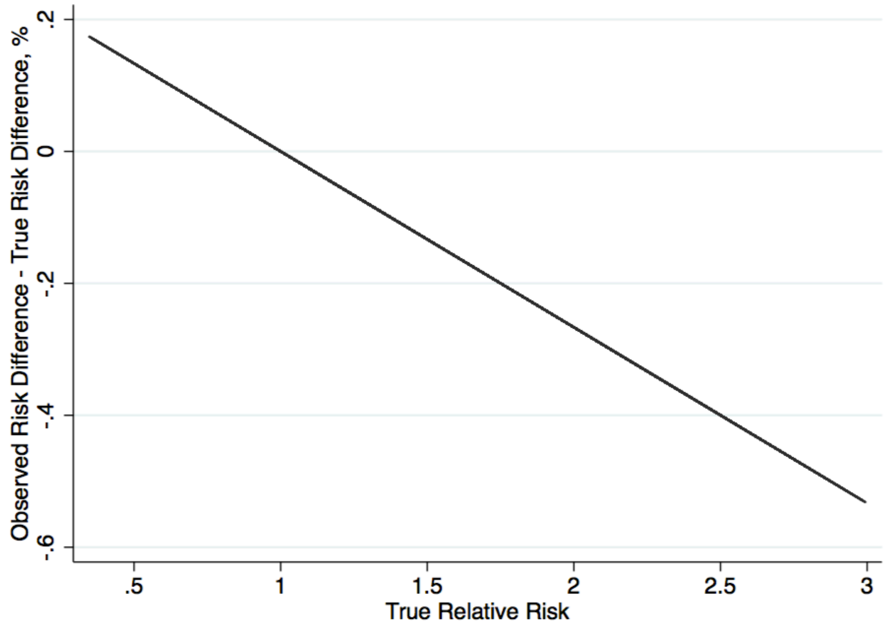


Figure 2.15. The RD becomes more biased as the strength of the A-Y relationship increases



Chapter 3. Estimation of the Association of Long-Duration Breastfeeding and Severe Early Childhood Caries Using Marginal Structural Models[1]

1. A preliminary report was presented at the International Association for Dental Research / American Association for Dental Research General Session and Exhibition, Seattle, USA: Chaffee BW, Feldens CA, and Vítolo MR. 2013. Extended Breastfeeding-Caries Association Differs by Nursing Frequency: a Prospective Study. *J Dent Res.* 92(Spec Iss A): 2882

Abstract

Breastfeeding offers significant health benefits. However, previous studies conflict regarding long-duration breastfeeding and early childhood caries (ECC). This study aimed to estimate the association between breastfeeding ≥24 months and severe-ECC in preschoolers. A birth cohort (n=715) from low-income families in Porto Alegre, Brazil (2008-2012) yielded prospective data. Severe-ECC prevalence was compared over categories of breastfeeding duration using marginal structural models to account for time-dependent confounding by other feeding habits. Stratified analyses assessed whether daily breastfeeding frequency modified the association of breastfeeding duration and severe-ECC. Multiple imputation and censoring weights were used to account for incomplete covariate information and missing outcomes, respectively. Credibility intervals (CI) were estimated using bootstrap re-sampling. Breastfeeding ≥24 months was associated with the highest adjusted population-average severe-ECC prevalence (0.46, 95% CI: 0.38, 0.54) in comparison with breastfeeding <6 months (0.22, 95% CI: 0.15, 0.28), 6-11 months (0.39, 95% CI: 0.26, 0.53), or 12-23 months (0.36, 95% CI: 0.21, 0.54). High frequency breastfeeding enhanced the association between long-duration breastfeeding and caries (excess prevalence due to interaction: 0.16, 95% CI: -0.10, 0.41). In this population, breastfeeding ≥24 months, particularly if frequent, was associated with severe-ECC.

Introduction

Early childhood caries (ECC) is the multi-factorial disease responsible for tooth decay in the primary teeth (1). It causes pain, impairs oral function, reduces quality of life (2, 3), and heightens the risk for decay in the adult dentition (4, 5). Despite advances in preventive modalities (6), 60-90% of children are affected worldwide (7), often with untreated decay. Better characterization of modifiable early-life risk factors would inform future interventions.

Breastfeeding offers numerous, substantial health benefits for both mother and child (8, 9). The World Health Organization (WHO) recommends that women in developing countries practice on-demand breastfeeding to age two years or beyond (10). However, the nature of the relationship between the age to which a child is breastfed and dental caries remains uncertain. Laboratory models suggest that human milk can cause caries (11, 12), particularly in combination with added sugars (13). However, the epidemiological literature linking breastfeeding and ECC has been highly heterogeneous (14). Some recent studies support a positive association between long-duration breastfeeding and ECC (15-18), while others do not (19-20).

The timing of breastfeeding behaviors in relation to other early-life feeding habits complicates studies of the association between breastfeeding duration and ECC. Early cessation of breastfeeding might accelerate the introduction of particular foods (21, 22), and the foods consumed early in life are likely to play a role in caries development (23-25). In turn, a child's early-life food experiences might also influence the duration to which a breastfeeding child continues nursing (21). Regression modeling is problematic in the presence of such time-dependent confounding, in which a variable (e.g. early-life food experiences) can be part of a causal pathway between an earlier aspect of exposure (e.g. early breastfeeding) and the outcome, while simultaneously operating as confounder with respect to a later aspect of exposure (e.g. continued breastfeeding) and the outcome (Figure 3.1).

Marginal structural models (MSMs), in contrast, have been used to make causal inference in the presence of time-varying covariates (26-29). When MSMs are estimated using inverse probability (IP) weighting, observations are up-weighted that, based on covariates, were less likely to obtain their observed exposure status. The weighting yields a "pseudo-population" in which there is balance across the exposed and unexposed populations with respect to the confounding variables included in estimation of the weights. This allows for an un-confounded estimate of the exposure-outcome relationship, provided that assumptions hold for consistency, positivity, exchangeability, and correct specification of the treatment models used to generate the weights (27, 28, 30).

We aimed to estimate the association between extended breastfeeding (≥24 months) and the occurrence of severe-ECC (S-ECC) in a birth cohort of urban, low-income Brazilian children. Further, as duration represents a single exposure dimension, we secondarily examined whether the association between long-duration breastfeeding and S-ECC is stronger with more frequent daily breastfeeding episodes.

Materials and Methods

Participants

This study follows a birth cohort nested in a cluster-randomized trial in Porto Alegre, Brazil. The community water supply is fluoridated to 0.7 ppm (31), and 52 public healthcare centers provide primary medical services to a predominantly low-income population. A stratified random sample (n = 20 health centers) was selected from 31 eligible municipal clinics for participation in the original trial of healthcare worker training (32, 33).

In 2008, 715 of 736 eligible pregnant women (97.1%) with scheduled appointments at participating clinics agreed to enroll their children in a cohort to track health outcomes. The trial had provided intervention clinics with healthcare worker training that promoted healthful complementary feeding for incorporation into counseling with new and expectant mothers. After three years, the intervention did not extend the total duration of breastfeeding (hazard ratio for breastfeeding cessation: 0.94, 95% credibility interval: 0.79, 1.11), although the mean duration of exclusive breastfeeding had been greater in the intervention group (32). S-ECC was not reduced significantly among children born to intervention group clinic attendees (33).

Measurement of baseline variables

Teams of trained fieldworkers collected socio-demographic information for each mother at baseline (during pregnancy) via structured questionnaires. Collected information included maternal birth date, household size, maternal education (≤8 years), maternal smoking (current vs. never or former smoker), indoor bathroom (yes/no), region of the city (indicators for 8 geo-administrative districts), parity (first child yes/no), maternal partner status (married or with partner vs. single, separated, or widowed), household income (monthly income ≤1500 Brazilian reais; approximately 900 US dollars in 2008), outside income source (e.g. government program support), social class (Brazilian Association of Economic Research Institutes classification C or lower), low body mass index (BMI) (≤18, based on measured height and self-reported pre-pregnancy weight). Child gender and birth date were collected at age 5-9 months.

Measurement of feeding habits

Infant feeding habits were recorded at each of three home visits, corresponding to mean ages of 6 months (range: 5-9), 12 months (range: 11-15), and 38 months (range: 31-46). At each visit, mothers were asked whether they had ever breastfed and whether they were currently breastfeeding their child. Breastfeeding duration was considered the age to which any breastfeeding continued, regardless of the consumption of complementary foods. Those currently breastfeeding were asked how frequently they nursed during the day and night (0, 1, 2-3, or “many times,” separately for day and night). Those no longer breastfeeding were asked at what age (in months) breastfeeding ceased. Breastfeeding duration was considered censored if a child had been breastfeeding at the time of one home visit but the age of cessation was not later recorded (e.g. due to loss to follow-up).

At the 6-month assessment, mothers completed a 24-hour recall of all foods and beverages consumed by her child the preceding day. The number of infant feeding bottles was recorded (later categorized as 0, 1-3, ≥4). A child was classified as receiving sugar in the bottle if contents were mixed with added sugar. Questionnaire items addressed the age of introduction of 32 specific foods (e.g. fruits, beans, soft drinks, candies). At the 12-month assessment, the questionnaire posed whether 29 specific food items were consumed in the previous month. Two feeding indices were created as measures of dietary patterns in order to account for foods consumed in combination and to increase the efficiency of the analysis (34). The first, referred to here as the food introduction index, was a count of low nutrient-density and/or presumably cariogenic foods introduced before age 6 months: added sugar, candy, chips, chocolate, chocolate milk, cookies, fried foods, fruit-flavored drink, gelatin, honey, ice cream, soft drinks, and sweet biscuits. The second, termed the first-year feeding index, was the sum of the food introduction index and the count of the following foods consumed in the month preceding the 12-month assessment: added sugar in a drink, candy, cake, chips, chocolate, chocolate milk, cookies, creamed caramel, fried foods, fruit-flavored drink, gelatin, honey, ice cream, other confection, soft drinks, and sweet biscuits. These indices were created specifically for this analysis due to a lack of existing diet quality indices specific to cariogenic feeding behaviors in comparable populations.

Mothers additionally completed two 24-hour recalls of child intakes at the 38-month assessment. However, these variables were included only as a sensitivity check, because our threshold for defining long-duration breastfeeding (≥24 months) temporally preceded these measures.

Measurement of dental caries

From August 2011 to June 2012, oral health status was evaluated at the tooth surface level following WHO protocol (35), with non-cavitated (white-spot) lesions also recorded. Assessments took place in participants' homes, aided by a lighted intraoral mirror. Teeth were brushed and then dried with gauze. Following National Institutes of Health case definitions (36), S-ECC was defined by the presence of one or more affected maxillary anterior tooth or at least four decayed, missing due to caries, or filled tooth surfaces (dmfs ≥4). For children <36 months, one or more affected smooth surface also qualified as S-ECC (36). A single dentist-examiner (Author 1) completed 94.7% (434/458) of the evaluations. A second calibrated dentist completed the remaining following identical protocol. The estimation of inter-rater (unweighted kappa=0.75) and intra-rater (unweighted kappa=0.83 for both examiners) reliabilities is described elsewhere (33).

Statistical methods

In the primary analysis, the proportion of children with S-ECC was compared across four categories of breastfeeding duration: <6 months, 6-11 months, 6-23 months, and ≥24 months. Three marginal structural models were fit. The weights used in estimating unadjusted models incorporated only clinic allocation status to account for the nested study design. Adjusted models additionally accounted for selected baseline socio-demographic variables: maternal age, education, parity, pre-pregnancy BMI, smoking status, social class, and child age and gender. Fully-adjusted models included these variables, as well as selected bottle use variables and feeding habits (see below).

In estimating MSMs, IP weighting was used to generate a "pseudo-population" representative of a hypothetical population in which breastfeeding duration (A) had been allocated independently of confounding variables. Weights were assigned inversely to the predicted probability of observed exposure, given baseline characteristics (W) and longitudinally recorded feeding habits (L_t), giving the greatest weight to observations with exposure and confounder combinations least represented in the sample, relative to what would have been observed under random exposure allocation. Predicted probabilities of exposure were estimated using Super Learner, a data-adaptive machine-learning tool that uses a library of algorithms for prediction (37).

Breastfeeding duration reflects a mother's cumulative behavior regarding breastfeeding cessation or continuation over time. Thus, the four exposure categories reflect maternal behaviors to continue breastfeeding beyond 6, 12, or 24 months, given breastfeeding in the prior interval. Therefore, in generating weights, we estimated treatment models to predict three probabilities: the probability of breastfeeding at 6 months ($\Pr[A_6=1]$); the probability of breastfeeding at 12 months, given breastfeeding at 6 months ($\Pr[A_{12}=1 \mid A_6=1]$); and the probability of breastfeeding at 24 months, given breastfeeding at 12 months ($\Pr[A_{24}=1 \mid A_{12}=1]$). Each treatment model was estimated while incorporating temporally appropriate putative confounders: in fully-adjusted models, the 6-month treatment model included clinic allocation status and baseline socio-demographic variables only; the 12-month treatment model included these variables and added the 6-month bottle use variables and the food introduction index; the 24-month treatment model replaced the food introduction index with the first-year feeding index. To stabilize the weights and reduce the variability in estimates, weights were multiplied by the marginal probability of the observed exposure category (27).

The stabilized treatment weights were

$$SW^T = \frac{\Pr[A < 6months]\ I[A < 6months]}{\Pr[A_6 = 1 \mid W]}$$

$$+ \frac{\Pr[A = 6\text{-}11months]\ I[A = 6\text{-}11months]}{\Pr[A_6 = 1 \mid W]\ \Pr[A_{12} = 1 \mid A_6 = 1, W, L_6]}$$

$$+ \frac{\Pr[A = 12\text{-}23months]\ I[A = 12\text{-}23months]}{\Pr[A_6 = 1 \mid W]\ \Pr[A_{12} = 1 \mid A_6 = 1, W, L_6]\ \Pr[A_{24} = 0 \mid A_{12} = 1, W, L_6, L_{12}]}$$

$$+ \frac{\Pr[A \geq 24months]\ I[A \geq 24months]}{\Pr[A_6 = 1 \mid W]\ \Pr[A_{12} = 1 \mid A_6 = 0, W, L_6]\ \Pr[A_{24} = 1 \mid A_{12} = 1, W, L_6, L_{12}]},$$

where indicators (I) take a value of 1 when the exposure category was observed and 0 otherwise.

For each model, missing baseline variables and missing or censored breastfeeding durations were multiply imputed from probabilities estimated using Super Learner. IP censoring weights were estimated to up-weight observations most resembling those with missing outcomes based on covariates and exposure status. The probability of having an observed outcome (Pr[C=0]) was estimated using Super Learner and predictor variables clinic allocation status, maternal age, education, partner status, parity, smoking status in pregnancy, and pre-pregnancy BMI, household income, indoor bathroom, number of members, outside source of income, region of the city, and social class, and child breastfeeding duration category, gender, and first-year feeding index. Censoring weights were equal to the inverse probability of having an observed outcome, given exposure and covariates. The numerator of the stabilized censoring weights was the product of the probability of having outcome data, given breastfeeding duration category, and a 1/0 indicator for having outcome data:

$$SW^C = \frac{\Pr[C = 0 \mid A]\ I[C = 0]}{\Pr[C = 0 \mid A, W, L_t]}$$

The final MSM weights (action weights) were the product of the stabilized treatment weights and the stabilized censoring weights: $SW^A = SW^T \times SW^C$.

For each model, point estimates (prevalence ratio, PR, and prevalence difference, PD) were averaged over 200 multiple imputations to account for differences in imputed values and subsequent changes in weights. Percentile-based 95% credibility intervals (CI) were estimated as the 2.5 and 97.5 quantiles from 5000 bootstrap iterations to account for variance contributions from sampling, imputation, and weighting. For comparison, an analogous complete-case regression analysis was completed using log-linear models and robust variance. Analyses were completed in R version 2.15.2 (http://www.r-project.org).

As a secondary analysis, we examined whether frequent daytime breastfeeding (≥4 daily episodes) intensified the association of breastfeeding duration and S-ECC, restricting the analysis to children breastfed ≥6 months, the starting age at which frequency data were collected. A binary high frequency daytime breastfeeding variable and a long duration-high frequency interaction term were included in the MSM, and high frequency daytime breastfeeding was added to both the treatment models and stabilized weight numerators (38). We defined the excess prevalence due to interaction (EPI) as a departure from additivity, following the example proposed by Rothman for the relative risk (39). If D and F represent the presence of long-duration and high frequency breastfeeding, respectively, and $\overline{D}$ and $\overline{F}$ the absence of these two factors, then the EPI = PD(DF) – PD($D\overline{F}$) – PD($\overline{D}F$). As a sensitivity check, we also estimated models in which frequency strata were defined by high frequency breastfeeding in either the day or night, versus high frequency breastfeeding in neither. Nighttime breastfeeding was not used alone to define strata, because high frequency daytime breastfeeding remained common (>50%) when nighttime high frequency breastfeeding was absent. Because tests for statistical interaction may have low power (40), 80% credibility intervals were additionally provided.

Ethics

This study received approval from the Ethics Committee in Human Research at the Federal University of Health Sciences of Porto Alegre and the Committee for the Protection of Human Subjects at the University of California Berkeley Children with caries or suspected anemia, under-nutrition, or overweight status were referred to their local health center.

Results

Table 3.1 demonstrates selected characteristics of the study population. Breastfeeding was common, with 98.9% (627/633) of mothers interviewed at the 6-month assessment reporting having ever initiated breastfeeding, and 47.2% (282/598) of interviewed mothers continuing to breastfeed to at least 12 months. The consumption of sweetened foods and beverages at an early age was also common (Table 3.1). A nursing bottle was used by 61.1% (375/614) and 75.7% (345/456) of children at the 6-month and 38-month assessments, respectively.

At the 38-month assessment, 54.6% (250/458) of children had at least one tooth surface affected by caries; S-ECC was observed among 34.3% (157/458). These conditions were most common among children breastfed for ≥24 months (Figure 3.2).

The highest prevalence of S-ECC was associated with breastfeeding ≥24 months in all marginal structural models (Table 3.2). However, the models did not indicate an elevated prevalence of S-ECC comparing breastfeeding to 12-23 months versus breastfeeding to 6-11 months (fully-adjusted PR: 0.91, 95% CI: 0.49, 1.63). As a sensitivity check, adding bottle-feeding variables collected at age 38-months to the fully adjusted model did not appreciably alter the estimates; for example, the prevalence ratio comparing breastfeeding durations ≥24 months to <6mo changed to 2.10 (95% CI: 1.52, 3.06) from 2.08 (95% CI: 1.52, 3.04).

Compared to breastfeeding 6-23 months, breastfeeding ≥24 was associated with an elevated prevalence of S-ECC that did not reach statistical significance (unadjusted PR: 1.31, 95% CI: 0.97, 1.79; adjusted PR: 1.22, 95% CI: 0.89, 1.66; fully-adjusted PR: 1.21, 95% CI: 0.86, 1.70). When stratified by frequent daytime breastfeeding, however, breastfeeding ≥24 months was associated with S-ECC only when nursing was frequent (fully-adjusted PR: 1.41, 95% CI: 0.98, 2.16) (Table 3.3). The EPI was 0.16 (95% CI: -0.10, 0.41, 80% CI: -0.01, 0.32), suggesting elevated S-ECC when breastfeeding is both frequent during the day and ≥24 months in duration. Results were similar when stratification was based on high frequency breastfeeding in either the day or night versus neither day or night (fully-adjusted PR with frequent breastfeeding: 1.44, 95% CI: 1.01, 2.16); the EPI increased to 0.25 (95% CI: -0.05, 0.51, 80% CI: 0.06, 0.42).

There were quantitative differences in the estimates obtained from the complete-case regression analysis (Table 3.4). However, findings were qualitatively consistent with the MSM results, in support of a positive association between S-ECC and breastfeeding to ≥24 months.

Discussion

In this population of urban, low-income Brazilian families, we estimated an increase in severe early childhood caries prevalence at age 38 months with breastfeeding to 24 months or beyond. More frequent breastfeeding episodes strengthened this association, suggesting that duration itself might not be the only dimension of breastfeeding exposure relevant for caries occurrence. This possible caries risk should be examined further and weighed against any general health benefits of breastfeeding beyond two years, for which evidence is not extensive (41).

These findings are consistent with studies to consider the potential cariogenicity of long-duration breastfeeding in populations where later weaning is relatively common. Several investigations have reported positive associations when examining breastfeeding durations ≥18-24 months (15-17, 42, 43). Studies that have reported no association between caries and breastfeeding have generally considered earlier cut points to define long duration breastfeeding (i.e. ≥8-13 months) and have featured populations in which breastfeeding to age 2 years was rare, such as in Germany (44), Italy (19), and the Unites States (20). A large trial of hospital-based breastfeeding promotion in Belarus did not affect caries prevalence at age 6 years (45), where <20% of children in either trial arm were breastfed beyond 12 months. Considering that we estimated a similar prevalence of S-ECC with breastfeeding to 12-23 months as with durations to 6-11 months, our results are not necessarily in conflict with these studies to examine earlier cut points in which few participants were exposed to very extended breastfeeding.

The frequency at which breastfeeding occurs appears to be an important element in determining cariogenicity. Daily breastfeeding frequency was associated with S-ECC in a previous study of Brazilian preschoolers, in which breastfeeding frequency, but not duration ≥12 months, maintained statistical significance in multi-variable models (23). A combined measure of breastfeeding duration and frequency was strongly associated with ECC in Mayanmar (46), and a measure of nighttime breastfeeding burden, which was based on frequency, was positively associated with ECC in Iran (47), although not statistically significant. The relationships of breastfeeding duration and frequency are somewhat analogous to previous research into caries and sucrose consumption. The evidence supporting a biological role for consumed sugars in the caries process is substantial (48), yet a systematic review of the sucrose-caries relationship found a greater number of studies reporting a positive association with the frequency of sugar consumption than the quantity consumed (49).

While our stated objective was to estimate the association between long-duration breastfeeding and caries, we additionally found that breastfeeding <6 months was associated with the lowest S-ECC prevalence of the four breastfeeding duration categories we studied. This result is inconsistent with a number of studies to report elevated caries risk with short duration breastfeeding (17, 50, 51). Coupling the weaker prior expectation of observing this result with the strong outside evidence of breastfeeding benefits in this age range, this is less relevant for future interventions or policy.

This study features longitudinally and prospectively collected infant feeding information, an important strength in contrast to previous cross-sectional studies. By using IP weighting estimators, our analysis respects the temporal sequence of exposure and covariate information, accounting for time-dependent confounding by early-life feeding habits (52). These methods have not been broadly used in oral health epidemiology.

Some limitations warrant consideration. Data were not extensive regarding oral health behaviors (e.g. fluoride exposure, preventive dental visits). However, these are unlikely to be important confounders unless such factors are strongly associated with breastfeeding practices, which was not the case in this study population. Estimates of the excess prevalence due to interaction were imprecise: a smaller number of children breastfed infrequently, particularly to long durations, reduced the statistical power available to detect effect measure modification.

As a whole, our results do not imply that some aspect of human milk itself is inherently harmful to the primary dentition. On the contrary, the health benefits of breastfeeding are considerable. However, the public health implication of this work is to add to the evidence that, as with any other carbohydrate potentially fermentable by caries-causing oral bacteria, extended and frequent exposure to breast milk following tooth eruption represents a potential risk for dental decay. Professional dental organizations have recognized the importance of supporting a mother's decision to breastfeed (53-55). The suggestions of some such groups to avoiding *ad libitum* breastfeeding after tooth eruption (53) and to follow good oral hygiene practices after nursing (54) are not unlike more general recommendations to practice moderation in carbohydrate consumption for optimal oral and general health.

Some caution should be taken in the interpretation of our findings. This study population, notable for a relatively high prevalence of both breastfeeding and nursing bottle use into the fourth year of life, might not be representative of the breastfeeding-caries relationship worldwide. Given the known health benefits of breastfeeding, women in low-resource settings should not be discouraged from breastfeeding from a standpoint of preventing tooth decay in their children. However, our results are congruent with a more nuanced approach to breastfeeding guidelines, in which breastfeeding itself need not be discouraged but that frequent or at-will feeding are limited for children at later ages.

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Chapter 3 Figures and Tables

Figure 3.1. Conceptual Diagram of the Temporal Relationships Between Breastfeeding Duration, Early Childhood Caries and Other Infant Feeding Practices

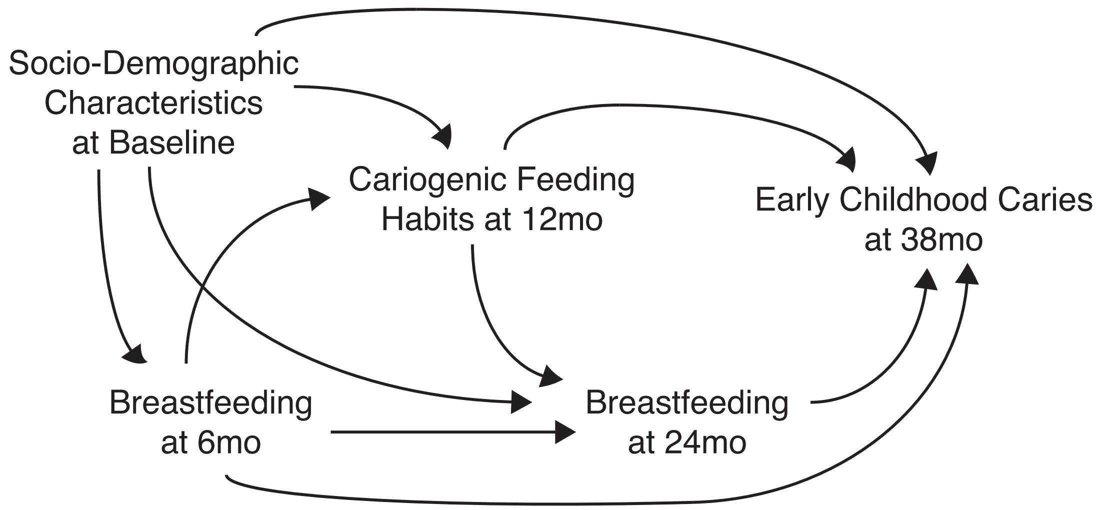

Figure 3.1. In relation to the outcome early childhood caries at age 38 months, early-life feeding practices (e.g. cariogenic feeding habits at age 12 months) might served as both a mediator of an earlier aspect of exposure history (e.g. breastfeeding at 6 months) and a confounder of a later aspect of exposure history (e.g. breastfeeding at 24 months).

Table 3.1. Characteristics of Preschoolers Enrolled From Municipal Health Centers in Porto Alegre, Brazil, 2008-2012

Characteristic		Number of observations[1]
Socio-demographic characteristics		
Maternal age at expected delivery date, mean (SD), years	26.0 (6.7)	715
Mother has ≤ 8 y of formal education, n (%)	340 (47.6)	715
Household income ≤ 3 times minimum salary[1], n (%)	565 (81.9)	690
Social class by ABIPEME index[2], n (%)		713
A1	0 (0)	
A2	5 (0.7)	
B1	25 (3.5)	
B2	114 (16.0)	
C	416 (58.6)	
D	142 (19.9)	
E	11 (1.5)	
Self-identified maternal race, n (%)		715
white	395 (55.2)	
black, mixed, or other	320 (44.8)	
Male child, n (%)	333 (52.4)	635
Feeding Habits		
Introduced to soft drinks before age 6 months, n (%)	192 (30.3)	633
Introduced to any sweets before age 6 months, n (%)	557 (90.8)	613
Soft drinks in prior month at age 11-15 months, n (%)	413 (76.6)	539
Ever initiated breastfeeding	627 (98.9)	633
Exclusive breastfeeding to age ≥4 months, n (%)	152 (24.0)	633
Breastfeeding duration to age <6 months, n (%)	216 (34.1)	633
Breastfeeding duration to age 6-11 months, n (%)	100 (16.7)	598
Breastfeeding duration to age 12-23 months, n (%)	65 (12.1)	537
Breastfeeding duration to age ≥24 months, n (%)	156 (29.1)	537
Sweet substances in bottle at age 5-9 months, n (%)	198 (32.3)	614
Consuming sweet substances in bottle at age 2-3 years, n (%)	312 (68.4)	456
Dental Caries Experience at age 2-3 years		
Any affected tooth, n (%)	250 (54.6)	458
S-ECC, n (%)	157 (34.3)	458
dmfs (any decay), mean (SD)	3.2 (6.1)	458
dmfs (cavitated decay only), mean (SD)	2.6 (5.9)	458

Abbreviations: SD, standard deviation; ABIPEME, Brazilian Association of Economic Research Institutes; S-ECC, severe early childhood caries; dmfs, decayed missing filled surfaces index
[1]Number of observations differ for some variables due to missing data and/or losses to follow-up
[2]Monthly income of ≤1500 Brazilian reais; approximately 800 US dollars in 2008
[3]Socioeconomic classification scale based on material possessions and education, A = highest status, E = lowest status

Figure 3.2. The Prevalence of Severe Early Childhood Caries at 38-Months by Categories of Breastfeeding Duration in the Observed Sample

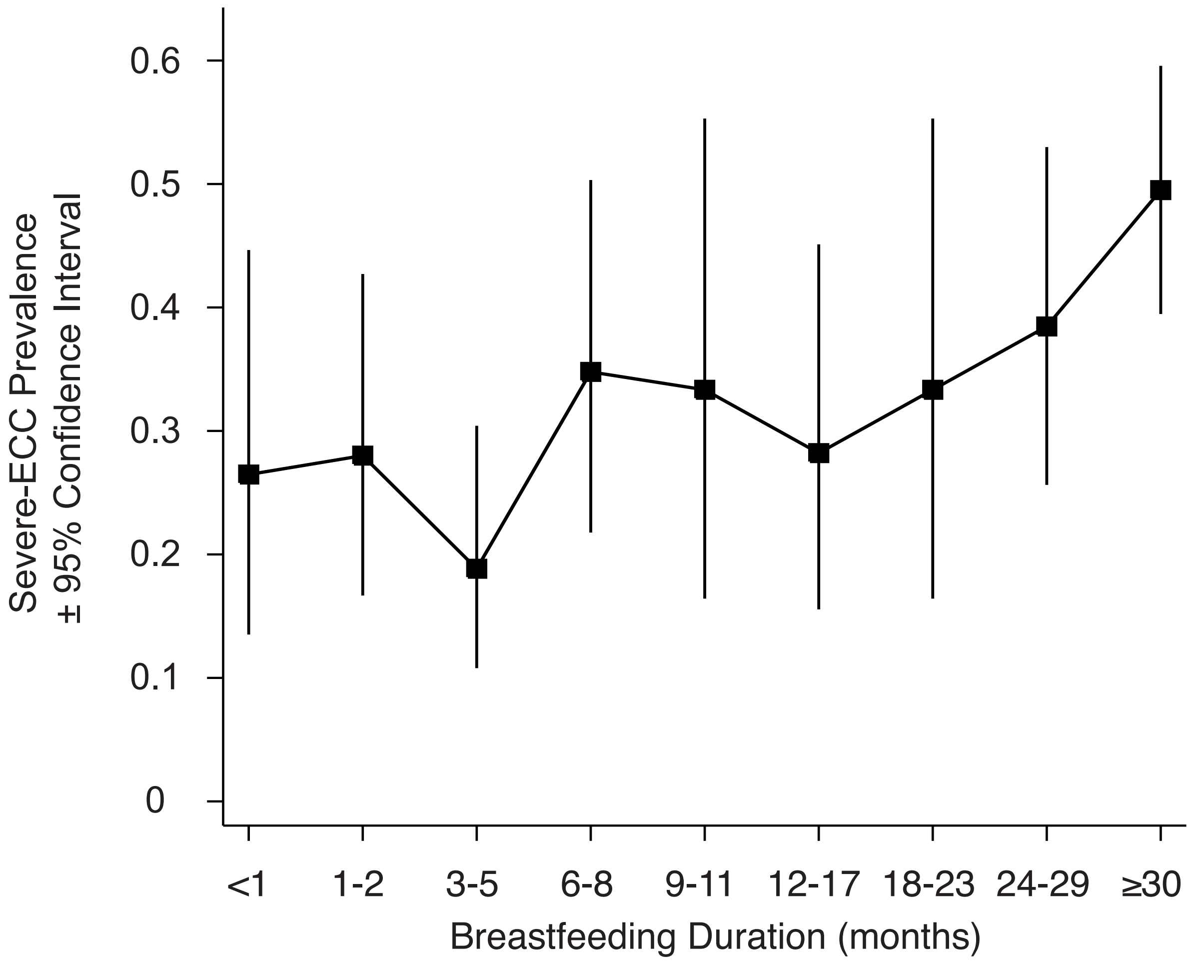

Figure 3.2. The unadjusted (crude) prevalence of severe early childhood caries by categories of breastfeeding duration is shown for the 439 children with observed data for both breastfeeding duration and dental caries.

Table 3.2. Unadjusted and Adjusted Associations of Breastfeeding Durations and Severe Early Childhood Caries in Preschoolers From Porto Alegre, Brazil, 2008-2012

	Marginal Prevalence[1]	95% CI	Prevalence Ratio	95% CI	Prevalence Difference	95% CI
Breastfeeding Duration						
Unadjusted[1] Model						
<6 months (reference)	0.23	0.16, 0.30	1		0	
6-11 months	0.38	0.27, 0.50	1.66	1.06, 2.57	0.15	0.02, 0.29
12-23 months	0.31	0.20, 0.43	1.35	0.81, 2.16	0.08	-0.05, 0.22
≥24 months	0.45	0.38, 0.53	1.98	1.44, 2.87	0.22	0.12, 0.33
Adjusted[2] Model						
<6 months (reference)	0.22	0.15, 0.28	1		0	
6-11 months	0.39	0.27, 0.53	1.79	1.13, 2.80	0.17	0.03, 0.32
12-23 months	0.34	0.22, 0.49	1.57	0.97, 2.65	0.12	-0.01, 0.29
≥24 months	0.45	0.38, 0.53	2.06	1.51, 3.00	0.23	0.14, 0.34
Fully-Adjusted[3] Model						
<6 months (reference)	0.22	0.15, 0.28	1		0	
6-11 months	0.39	0.26, 0.53	1.80	1.10, 2.83	0.17	0.02, 0.33
12-23 months	0.36	0.21, 0.54	1.62	0.90, 2.84	0.14	-0.02, 0.34
≥24 months	0.46	0.38, 0.54	2.08	1.52, 3.04	0.24	0.14, 0.34

Abbreviation: CI, credibility interval

[1]Population-average prevalence of severe early childhood caries at given categories of breastfeeding duration, as estimated from marginal structural models

[2]Includes allocation status from nesting intervention study only

[3]Includes allocation status from nesting intervention study and maternal age, education, parity, pre-pregnancy BMI, smoking status, social class, and child age and gender

[4]Includes all adjusted model variables and time-varying bottle use variables and feeding habits

Table 3.3. Unadjusted and Adjusted Associations of Breastfeeding ≥24 Months and Severe Early Childhood Caries, Stratified by Frequency of Daytime Breastfeeding, in Preschoolers From Porto Alegre, Brazil, 2008-2012

	Marginal Prevalence[1]	95% CI	Prevalence Ratio	95% CI	EPI	95% CI
Breastfeeding Duration and Frequency						
Unadjusted[1] Model						
Duration 6-23 months and low frequency	0.38	0.25, 0.51	1			
Duration ≥24 months and low frequency	0.37	0.22, 0.52	0.97	0.53, 1.68		
Duration 6-23 months and high frequency	0.31	0.22, 0.42	1			
Duration ≥24 months and high frequency	0.48	0.39, 0.57	1.53	1.06, 2.30	0.18	-0.07, 0.43
Adjusted[2] Model						
Duration 6-23 months and low frequency	0.38	0.25, 0.51	1			
Duration ≥24 months and low frequency	0.36	0.20, 0.53	0.94	0.51, 1.67		
Duration 6-23 months and high frequency	0.33	0.23, 0.44	1			
Duration ≥24 months and high frequency	0.47	0.38, 0.56	1.42	0.99, 2.12	0.16	-0.10, 0.41
Fully-Adjusted[3] Model						
Duration 6-23 months and low frequency	0.38	0.25, 0.52	1			
Duration ≥24 months and low frequency	0.36	0.20, 0.54	0.95	0.49, 1.70		
Duration 6-23 months and high frequency	0.33	0.23, 0.45	1			
Duration ≥24 months and high frequency	0.47	0.37, 0.57	1.41	0.98, 2.16	0.16	-0.10, 0.41

Abbreviations: CI, credibility interval; EPI, excess prevalence due to interaction
[1]Population-average prevalence of severe early childhood caries at given categories of breastfeeding duration, as estimated from marginal structural models
[2]Includes allocation status from nesting intervention study only
[3]Includes allocation status from nesting intervention study and maternal age, education, parity, pre-pregnancy BMI, smoking status, social class, and child age and gender
[4]Includes all adjusted model variables and time-varying bottle use variables and feeding habits

Table 3.4. Unadjusted and Adjusted Associations from Regression Models of Breastfeeding Durations and Severe Early Childhood Caries in Preschoolers From Porto Alegre, Brazil, 2008-2012

	Unadjusted Model n = 439		Adjusted Model n = 422		Fully Adjusted Model n = 338	
Model Variables	Prevalence Ratio	95% CI	Prevalence Ratio	95% CI	Prevalence Ratio	95% CI
Breastfeeding <6 months (reference)	1.00		1.00		1.00	
Breastfeeding 6-11 months	1.45	0.93, 2.23	1.52	0.99, 2.33	1.82	1.12, 2.97
Breastfeeding 12-23 months	1.28	0.80, 2.05	1.44	0.89, 2.32	1.51	0.82, 2.77
Breastfeeding ≥24 months	1.96	1.40, 2.73	2.04	1.45, 2.85	1.99	1.24, 3.20
Clinic allocation (intervention)	0.85	0.65, 1.09	0.89	0.69, 1.15	0.91	0.69, 1.20
Maternal age (years)			0.98	0.96, 1.01	0.99	0.96, 1.01
Maternal education (≤8 years)			1.24	0.93, 1.65	1.23	0.90, 1.67
Maternal smoking (current)			1.49	1.13, 1.95	1.15	0.84, 1.58
Parity (has previous child)			1.18	0.85, 1.63	1.21	0.87, 1.70
Social class (C or lower)			1.09	0.77, 1.54	1.04	0.74, 1.47
Pre-pregnancy BMI ≤18			1.43	1.06, 1.93	1.47	1.09, 1.99
Child age at dental assessment (years)			1.25	0.64, 2.42	1.11	0.55, 2.23
Child gender (male)			1.18	0.91, 1.53	1.24	0.94, 1.63
First-year feeding index					1.05	1.01, 1.09
Daily bottles at 5-9 months (1-3)					0.61	0.38, 0.98
Daily bottles at 5-9 months (≥ 4)					0.88	0.51, 1.51
Added sugar in bottle at 5-9 months					1.45	0.94, 2.25

Abbreviation: CI, credibility interval; BMI, body mass index

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